FOOD COMPOSITION AND ADDITIVES

Determination of Walnut Protein in Processed Foods by Enzyme-Linked Immunosorbent Assay: Interlaboratory Study

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Because food allergens from tree nuts, including walnuts, are a frequent cause of adverse food reactions for allergic patients, the labeling of foods containing ingredients derived from tree nuts is required in numerous countries. According to Japanese regulations, the labeling of food products containing walnuts is recommended. To ensure proper labeling, a novel sandwich ELISA kit for the determination of walnut protein in processed foods (Walnut Protein [2S-Albumin] Kit; Morinaga Institute of Biological Science, Inc.; "walnut kit") has been developed. We prepared seven types of incurred samples (model processed foods: biscuits, bread, sponge cake, orange juice, jelly, chicken meatballs, and rice gruel) containing 10 g walnut soluble protein/g of food for use in interlaboratory evaluations of the walnut kit. The walnut kit displayed sufficient reproducibility relative standard deviations (interlaboratory precision: 5.8–9.9% RSD_R) and a high level of recovery (81–119%) for all the incurred samples. All the repeatability relative standard deviation (RSD_r) values for the incurred samples that were examined were less than 6.0%. The results of this interlaboratory evaluation suggested that the walnut kit could be used as a precise and reliable tool for determination of walnut protein in processed foods.

Over recent decades, food allergies have emerged as an important public health concern in industrialized countries. Up to 8% of young children and 2% of adults are estimated to have food allergies, and these percentages appear to be increasing (1–5). The clinical manifestations of food allergies vary from mild symptoms, such as oral allergy syndrome or mild urticaria, to severe anaphylactic reactions with fatal consequences. The most effective means of preventing allergic reactions to food is to avoid foods that contain allergens; therefore, patients with food allergies must be able to obtain accurate information regarding the presence of food allergens in processed foods. Nevertheless, various studies have shown that severe allergenic reactions can be induced by the accidental intake of food products containing allergenic materials (6, 7). Accordingly, information regarding potentially allergenic ingredients in food products is necessary.

The issue of a labeling system for allergenic ingredients in food products has been discussed by international organizations such as the Codex Alimentarius Commission of the World Health Organization (WHO) and the Food and Agriculture Organization (FAO). In 1999, the Joint FAO/WHO Codex Alimentary Commission Session agreed to label eight kinds of foods containing known allergens, including tree nuts and nut products (8, 9). Tree nuts are regarded as one of the "big eight" allergenic foods believed to be responsible for 90% of all food allergies (10). Subsequently, various countries or regions have considered the labeling list established by the Codex guidelines and have developed their own lists of the most commonly allergenic foods. In response to the FAO/WHO recommendation, walnut labeling has become mandatory in the United States, European Union, Canada, and Australia/New Zealand.

Since April 2002, the Ministry of Health, Labour, and Welfare (MHLW) of Japan has enforced a labeling system for allergenic food materials to ensure that information on these foods is available to allergic consumers. According to Japanese regulations, the labeling of food products containing eggs, milk, wheat, buckwheat, peanuts, shrimp, and crab is mandatory and is recommended for 18 other food materials, including walnut, in light of the number of allergic patients and the degree of the seriousness of their allergic reactions.

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In a notification issued in 2002 (11), the MHLW announced the official Japanese methods for detecting allergens requiring mandatory labeling and the threshold value for labeling [10 g/g (allergenic ingredient soluble protein weight/food weight)]. The MHLW also described the criteria for interlaboratory validation protocols in their official guidelines, which were issued in 2006 (12). Briefly, the interlaboratory validation protocols must meet the following criteria: number of laboratories _8, number of incurred samples _5, number of dose levels _1 [including 10 g/g (allergenic ingredient soluble protein weight/food weight)], recovery of 50–150%, and reproducibility relative standard deviation (RSD_R) _25%. The reference material, the initial extract solution, and the allergen extraction procedure were also specified and standardized in the guidelines.

Tree nuts are regarded as one of the most potent allergenic ingredients among all known allergenic foods and often cause food anaphylaxis and anaphylaxis death (13–16). Walnut allergy is the most common tree nut allergy and occurs in all age groups (17, 18). In addition, walnut allergy is extremely severe, inducing life-threatening allergic reactions similar to those induced by peanut allergy (19–21). In Japan, epidemiological investigations have revealed that the number of patients with walnut allergy is increasing (5, 22). Furthermore, despite labeling precautions, walnut protein remains quite dangerous, as it is often present in commercial foods as a hidden allergen arising from cross-contamination during food processing. In most factories, many different products are manufactured from various ingredients, and sometimes these products are even manufactured on the same production line. Therefore, a reliable method of detecting walnut protein is needed to monitor and ensure accurate labeling. We have developed a highly sensitive ELISA kit capable of detecting walnut protein [Walnut Protein (2S-Albumin) Kit; hereafter referred to as "walnut kit"] (23). In the present paper, we describe the results of an interlaboratory evaluation of the performance of this ELISA kit.

Experimental

Materials and Methods

(**a**) *Preparation of the defatted walnut powder.*—The walnuts (Chandler) were kindly provided by Tabata, Inc. (Chiba, Japan). They were ground in a mill, and the walnut powder was collected. After removing the fats using acetone, the defatted walnut powder was dried for 16 h. The walnut soluble protein (WP) was extracted from the defatted walnut powder using buffer A [120 mM Tris-HCl (pH 7.4), 0.1% (w/v) bovine serum albumin, and 0.05% (v/v) Tween 20] containing 0.5% (w/v) sodium dodecyl sulfate (SDS) and 2% (v/v) 2-mercaptoethanol (2-ME). The WP content was then calculated using a 2-D Quant Kit (GE Healthcare, Buckinghamshire, UK). One gram of defatted walnut powder was obtained from 3.99 g raw walnuts, and the amount of WP/g of defatted walnut powder was approximately 556 mg (23).

(**b**) *Preparation of incurred samples.*—To obtain a final walnut protein concentration of 10 g/g (WP weight/sample weight) in the incurred samples, the amount of defatted walnut powder to be spiked in the incurred samples at the ingredient stage was calculated, taking into account the protein content of the defatted walnut powder and the change in weight of the incurred samples during preparation. Because the protein amount/g defatted walnut powder was approximately 556 mg, we spiked 18.0 mg defatted walnut powder to make 1 kg of each incurred sample [final concentration of 10 g/g (WP weight/sample weight)], as the ratio of the WP weight to the defatted walnut powder weight was estimated to be 55.6%.

All the incurred samples were prepared using the general procedures used by food manufacturers. The detailed procedures are described below.

The biscuits were made from wheat flower, sugar, shortening, salt, bicarbonate, dihydroxysuccinic acid, lecithin, and proteinase. All the raw materials and the defatted walnut powder were kneaded, molded, and baked at 240 C for 8.5 min. The cooked biscuits were stored at –40 C before use.

The bread was made from wheat flower, sugar, shortening, yeast, salt, skim milk, and water. All the raw materials and the defatted walnut powder were kneaded, leavened, and baked at 200 C for 20 min. The cooked bread was stored at –40 C before use.

The sponge cake was made from wheat flower, sugar, whole egg, emulsifiable fat, and water. All the raw materials and the defatted walnut powder were kneaded and baked at 180 C for 30 min. The cooked sponge cake was stored at –40 C before use.

The orange juice was made from orange concentrate, sugar, citric acid, ascorbic acid, and water. Orange concentrate, sugar, water, and the defatted walnut powder were mixed and homogenized. After the homogenate was buffered to pH 4.5 with citric acid, it was divided into cans and heated at 90 C for 10 min. The cooked orange juice was stored at –40 C before use.

The jelly was made from sugar, agar, citric acid, sodium citrate, muscat flavoring, and water. The raw materials and the defatted walnut powder were mixed. The mixture was heated to 90 C and divided into cans. After the canned jelly had been hardened at 15 C for 3 h, it was stored at –40 C before use.

The chicken meatballs were made from white chicken meat, lard, potato starch, and sugar. Lard, potato starch, sugar, and the defatted walnut powder were added to ground white chicken meat and thoroughly mixed. The mixture was ground using a small cutter, and the kneaded mixture was manually inserted into casings. The chicken meatballs were stored at –20 C before use.

The rice gruel was made from rice and water. The rice and the defatted walnut powder were cooked in a rice cooker and stored at –20 C before use.

Each type of incurred sample was also prepared as a blank sample that did not contain the defatted walnut powder to examine potential contamination, false-positive, and matrix effects. All the values for the blank samples of the model processed foods that were determined using the walnut kit were less than the LOD values determined by an in-house study (data not shown).

(**c**) *Homogeneity tests of the incurred samples.*—The homogeneity of the incurred samples was verified by the coordinator prior to distribution according to the procedure described in the International Harmonized Protocol for Proficiency Testing of (Chemical) Analytical Laboratories (24); the only modification to this procedure was that the number of homogeneity test materials was six. Twelve test portions of each incurred sample were analyzed using the walnut kit. The resulting WP concentrations were then analyzed using a one-way analysis of variance (ANOVA).

(**d**) *Test materials for interlaboratory study.*—Seven incurred samples (model processed foods: biscuits, bread, sponge cake, orange juice, jelly, chicken meatballs, and rice gruel) containing WP were prepared and used as test materials. The defatted walnut powder was spiked at the ingredient stage before processing to establish a final level of 10 g/g (WP weight/sample weight). The prepared incurred samples were homogenized with a food processor (DLC-XG; Cuisinart, Stamford, CT) and sent to the participating laboratories as test materials.

Walnut Protein (2S-Albumin) ELISA Kit (Walnut Kit; Ref. 23)

Polyclonal antibodies to 2S-albumin, the allergenic protein in walnuts, were used in the walnut kit, and a WP solution was utilized for calibration. Diluted standard solutions and sample solutions were added to a polyclonal antibody-coated module and incubated for 1 h at room temperature. After the module was washed, anti-2S-albumin antibody labeled with horseradish peroxidase was added and allowed to stand for 30 min at room temperature. After the final washing, a solution of 3,3 ,5,5 -tetramethylbenzidine was added, and the module was allowed to stand at 25 C for exactly 10 min. The reaction was stopped by the addition of 0.5 M sulfuric acid, and the absorbances were measured at 450 nm with 620 nm as the reference wavelength.

Information about the walnut kit including specificity was described earlier (23). The walnut kit has a high specificity for walnut, although it has slight cross-reactivity to pecans and hazelnuts at levels greater than the LOD.

The value of 134 mg WP/g was obtained when raw Chandler walnut was examined using the walnut kit (23). This value is in good agreement with the value described above in the *Preparation of the defatted walnut powder* section (556 mg of WP was obtained from 3.99 g raw Chandler walnuts, i.e., the content of WP in the raw walnuts was approximately 13.9%). This result shows the high reliability of the walnut kit to detect raw, not defatted, walnut. In addition, the activity of five raw or roasted walnut varieties (Chandler and Howard grown in California, Miette grown in France, Chinese grown in China, and Shinano grown in Japan) ranged from 82.1 to 125% (ratio to raw Chandler walnut), indicating that the walnut kit has similar reactivities among these walnut varieties (23).

Extraction

The incurred samples were homogenized using a food processor. A 1.0 g portion of the test material was extracted using 19 mL buffer A containing 0.5% (w/v) SDS and 2% (v/v) 2-ME. The mixture was shaken horizontally overnight (16 h) at room temperature, then centrifuged at 3000 *g* for 20 min after adjusting the pH to 6.0–8.0. The supernatant was filtered, if necessary, diluted 20 times with buffer A, and subjected to ELISA.

Calibration Standard Solution

The calibration standard solution was prepared according to the official Japanese guidelines and the method used in previous reports in Japan (25–27). The initial extract for the calibration standard solution was prepared from defatted walnut powder as follows: a 0.2 g sample defatted walnut powder was added to 20 mL buffer A containing 0.5% (w/v) SDS and 2% (v/v) 2-ME. The mixture was then shaken for 16 h at room temperature prior to extraction. After extraction, the sample was centrifuged at 10 000 *g* for 30 min, and the supernatant filtered through a 0.8 m micro-filter paper (DISMIC 25cs; Advantec, Tokyo, Japan) to obtain the extract. The protein content of the initial extract was assayed using a 2-D Quant Kit. The initial extract was diluted with buffer A to prepare the calibration standard solution (50 ng/mL extracted protein) for the walnut kit.

Interlaboratory Study

Twelve laboratories participated in the interlaboratory evaluation, which was organized by the National Institute of Health Sciences (Tokyo, Japan). The participants included manufacturing companies, public research institutes, local public inspection institutes, and private inspection institutes. The organizer sent each laboratory the seven test materials (3 g each), the ELISA kit, the extraction solution, and the calibration standard solution. The participants took two portions from each test material, extracted the protein using the extraction procedure, and assayed the extract using the ELISA kit. The calibration standard solution was diluted and assayed simultaneously with the incurred sample extracts. Each sample extract was analyzed in triplicate (three wells/sample extraction), and the average absorbance of three wells was used for the calculation. The resulting absorbance data for the calibration solutions and test materials were reported to the coordinator.

The organizer calculated a four-parameter logistic calibration curve based on the absorbance data of the calibration standard solution and calculated the concentrations of WP in the incurred samples using the calibration curve. Each set of samples was extrapolated from the standard curve run on the same plate.

Statistical Analysis

Twenty-four data items (two portions evaluated at 12 laboratories) were used in the calculations. The Cochran and Grubbs tests were used to remove outlying data (P =

Table 1. Homogeneity test results of the incurred samples

Sample	Mean	RSD, %[a]	*n*	F-ratio	*F*crit[b]
Biscuit	9.1	4.2	6	1.8	4.4
Bread	13.2	2.9	6	3.5	4.4
Sponge cake	10.2	2.6	6	1.2	4.4
Orange juice	10.6	4.7	6	0.6	4.4
Jelly	10.3	1.8	6	1.4	4.4
Chicken meatball	10.5	8.3	6	0.3	4.4
Rice gruel	12.1	3.2	6	0.3	4.4

[a] RSD, %, calculated from s_s (SD of sampling) and s_a (SD of analysis).
[b] *F*crit = Critical *F* value.

2.5%). The Cochran test was used to remove data from laboratories reporting a significantly large variation between the results of the two portions taken from the test material. The Grubbs test was used to remove data from laboratories reporting a mean of the results for the two portions that was significantly different from those of the other laboratories. The use of these statistical tests to identify outliers was in accordance with the AOAC protocol (28). The recovery, repeatability, and reproducibility were then calculated using data generated by a one-way ANOVA applied to the data after the removal of outliers.

Results and Discussion

Homogeneity of the Test Materials

Table 1 shows the average concentration, the RSD percentages calculated from s_s (SD of sampling), and s_a (SD of analysis), the number of test materials, the *F*-ratio, and the critical *F* value. The s_s and s_a values were calculated using data generated by a one-way ANOVA. The resultant *F*-ratios of the homogeneity test for the biscuit, bread, sponge cake, orange juice, jelly, chicken meatball, and rice gruel samples were <3.5. The *F*-ratios for all the incurred samples were below the critical *F* value (4.4). For most of the test materials, the RSD values between the portions were <8.3%, which was smaller than the required RSD_R values (_25%). We, therefore, concluded that the homogeneity of the test materials was acceptable for the purposes of this study.

Calibration Curve

Figure 1 shows the calibration curve for the determination of WP using the walnut kit. The calibration curve was obtained using the four-parameter logistic model. This curve showed an excellent correlation between the protein concentration and the optical density in the range of 0.78 to 50 ng/mL (correlation coefficient >0.999), and a concentration of 25 ng/mL, which corresponds to a WP dose level of 10 g/g in the test materials, fell within the dynamic range of the curve. We determined the LOD and LOQ according to the guidelines issued by the International Standards Organization and the International Union of Pure and Applied Chemistry (29) in an in-house study. The LOD was calculated as three times the SD of the mean value of the dilution buffer after eight experiments. The LOQ was calculated as 10 times the SD of the mean value of the dilution buffer after eight experiments. The LOD and LOQ of the

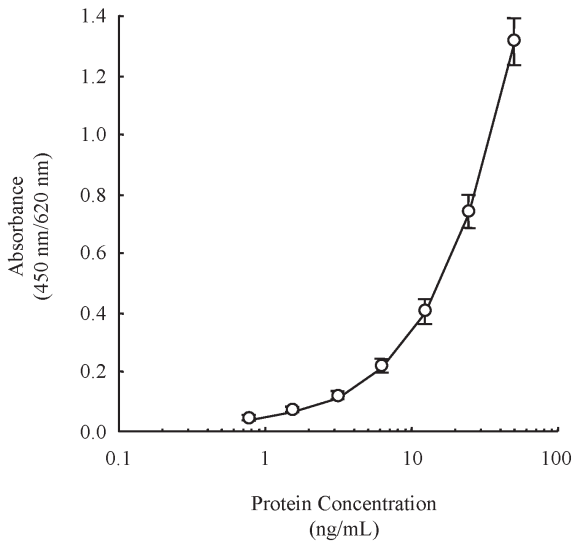

Figure 1. Walnut protein calibration curve for the walnut kit. The calibration curve was obtained using a four-parameter logistic method from the mean value after eight experiments. Concentration of calibration standard solution (calculated WP weight/food weight) = 0.78 ng/mL (0.31 g/g), 1.56 ng/mL (0.62 g/g), 3.13 ng/mL (1.25 g/g), 6.25 ng/mL (2.5 g/g), 12.5 ng/mL (5 g/g), 25 ng/mL (10 g/g), and 50 ng/mL (20.0 g/g). The equation of the calibration curve is *y* = [(A – D)/(1 + (*x*/C)^B)) + D] (*x*, protein concentration; *y*, optical density; A = 0.014; B = 1.006; C = 187.327; D = 6.172). The correlation coefficient (r) between the protein concentration and the optical density was found to be >0.999.

Table 2. Results of the interlaboratory study for the walnut kit protein recovery content[a]

	Biscuit		Bread		Sponge cake		Orange juice		Jelly		Chicken meatball		Rice gruel	
Lab	Mean, g/g[b]	Recovery, %[c]	Mean, g/g	Recovery, %	Mean, g/g	Recovery, %	Mean, g/g	Recovery, %	Mean, g/g	Recovery, %	Mean, g/g	Recovery, %	Mean, g/g	Recovery, %
A	7.8	78	11.1	111	9.5	95	9.0	90	9.2	92	9.0	90	11.3	113
B	8.3[d]	83	10.8	108	9.7	97	9.8	98	10.3	103	9.1	91	11.3	113
C	8.0	80	11.7	117	9.0	90	8.5	85	9.4	94	10.5	105	11.2	112
D	7.8	78	11.1	111	10.2[d]	102	10.0	100	10.2	102	10.1	101	11.2	112
E	7.9	79	12.2	122	9.8	98	9.9	99	10.8	108	11.1	111	12.4	124
F	9.0	90	13.1	131	10.8	108	11.2	112	11.2	112	12.3	123	13.4	134
G	8.1	81	12.3	123	9.8	98	9.5	95	10.2	102	11.1	111	12.0	120
H	9.3	93	10.0	100	9.6	96	10.0	100	10.7	107	10.6	106	12.9	129
I	7.6	76	10.8	108	9.0	90	10.3	103	9.7[d]	97	10.5	105	11.2	112
J	8.1	81	12.0	120	9.9	99	10.6	106	11.0	110	11.6	116	13.0	130
K	8.2	82	11.6	116	9.6	96	10.6	106	10.8	108	10.8	108	12.6	126
L	7.8	78	11.3	113	9.0	90	9.3	93	9.6	96	10.4	104	10.8	108

[a] The incurred samples contained WP of approximately 10 g/g.
[b] Mean = average concentration of WP (WP weight/food weight).
[c] Recovery = mean/10 (g/g), %.
[d] Values removed after Cochran test.

walnut kit, as determined using the dilution buffer, were 0.39 ng/mL (equivalent to 0.16 g/g of food sample) and 0.78 ng/mL (equivalent to 0.31 g/g of food sample), respectively. Consequently, the practical determination range was between 0.78 and 50 ng/mL.

Recovery, Repeatability, and Reproducibility

The values reported by the participants are summarized in Table 2. As shown in this table, the data for biscuits from "Laboratory B," the data for sponge cake from "Laboratory D," and the data for jelly from "Laboratory I" were detected as outliers using the Cochran test. The number of remaining laboratories after removing the outliers, the average concentration (g/g), s_r (repeatability SD, g/g), s_R (reproducibility SD, g/g), recovery (%), repeatability (RSD_r, %), and reproducibility (RSD_R, %) calculated using an ANOVA are shown in Table 3.

The recoveries of WP from the seven types of test materials using the walnut kit ranged from 81 to 119%. In spite of the high degree of processing, these values indicate a high degree of recovery for all the test materials, especially for the sponge cake, orange juice, and jelly samples (96, 99, and 103%, respectively). The recoveries from bread and rice gruel were slightly high (115 and 119%, respectively), while that of biscuits was slightly low (81%). However, these recoveries tended to follow the mean values of each incurred sample in the homogeneity test (Table 1). Therefore, the recoveries, the average values from each participant, are considered to be reliable. The variations in these recoveries might be affected by the food processing conditions and/or the physical

Table 3. Recovery, repeatability (RSD_r), and reproducibility (RSD_R) values of the walnut kit for WP

Sample[a]	No. of laboratories	Mean, g/g[b]	s_r, g/g	s_R, g/g	Recovery, %[c]	RSD_r, %	RSD_R, %
Biscuit	11	8.1	0.4	0.6	81	4.4	7.1
Bread	12	11.5	0.3	0.9	115	3.0	7.5
Sponge cake	11	9.6	0.3	0.6	96	3.1	5.8
Orange juice	12	9.9	0.6	0.8	99	5.6	8.5
Jelly	11	10.3	0.4	0.7	103	4.3	7.3
Chicken meatball	12	10.6	0.6	1.0	106	6.0	9.9
Rice gruel	12	11.9	0.6	1.0	119	5.0	8.3

[a] The incurred samples contained WP of approximately 10 g/g.
[b] Mean = average concentration of WP (WP weight/food weight).
[c] Recovery = mean/10 (g/g), %.

properties of the food matrixes containing the WP. In any event, the recoveries of all incurred samples satisfied the criteria for the interlaboratory validation protocol (50–150%).

Repeatability is a measure of the variation arising from the entire analytical procedure in a particular laboratory. In the intralaboratory evaluations, all the RSD_r values were found to be <6.0%. According to Horwitz's theory, the RSD_r value is generally likely to be less than 2/3 of the RSD_R value (30). In this study, all the RSD_r values for the walnut kit were less than 2/3 of the corresponding RSD_R value. Thus, these satisfactory RSD_r values were considered to guarantee the reliability of this analytical method.

The reproducibilities, expressed by the RSD_R values of the WP content from the seven types of test materials using the walnut kit, ranged between 5.8 and 9.9%. The reproducibilities of all the incurred samples also satisfied the criteria for the interlaboratory validation protocol (_25%). Thus, the walnut kit displayed a relatively high level of reproducibility in the interlaboratory evaluation results.

The organizer of this study surveyed the instruments (the shaker used for extraction and the microplate reader used for the determination) used by the participants in the interlaboratory evaluation. These instruments were not uniform, but the equipment differences did not affect the data obtained in this study. Therefore, we concluded that this kit would be a very robust tool as a general method for monitoring allergen ingredients to ensure correct labeling.

We previously reported the results of interlaboratory studies examining 13 ELISA kits for the determination of egg, milk, wheat, buckwheat, peanut, crustacean, and soybean proteins in processed foods (25–27). In the case of one buckwheat kit, the results of the recovery, repeatability, and reproducibility for the detection of buckwheat proteins in the five model processed foods were 58–136%, 6–13% RSD_r, and 10–25% RSD_R, respectively. In addition, the recovery of one milk kit was 89–137%, the reproducibility of one crustacean kit was 18–21% RSD_R, and the reproducibility of one soybean kit was 9–13% RSD_R. Thus, the recovery, repeatability, and reproducibility results of the interlaboratory validation of the walnut kit were excellent compared with the results of previous interlaboratory studies examining other ELISA kits.

The Japanese MHLW established an interlaboratory validation protocol in its official guidelines, which were issued in 2006 (12). Briefly, the following criteria for the interlaboratory validation protocol were defined: number of laboratories _8, number of incurred samples _5, number of dose levels _1 [including 10 g/g (allergenic ingredient soluble protein weight/food weight)], recovery of 50–150%, and RSD_R _25%. These criteria were based on the ISO5725 (JIS Z8402) guidelines, which are almost the same as those of the AOAC INTERNATIONAL (28). In the official guidelines, the initial extract solution and the allergen extraction procedure are also specified and standardized.

The present study suggested that the walnut kit is a reliable and precise method of determining the WP content, and that the performance of the walnut kit satisfies the validation criteria described in the official guidelines published by the Japanese government. Because food allergies can induce severe symptoms, the accuracy of the method is crucial. The data obtained in the interlaboratory validation clearly show that the walnut kit can quantify the WP content of the incurred samples very accurately. Moreover, the walnut kit produces good repeatability and reproducibility measures because of the high precision of the assay performance. The present interlaboratory evaluation was performed using seven incurred samples, including highly processed foods, such as biscuits, sponge cake, and rice gruel, as it is necessary to ensure that the kit is able to detect WP in commercial foods to guarantee accurate labeling. The present results demonstrated that the walnut kit is capable of detecting WP in processed foods and could be applicable to monitoring of the food labeling system, in accordance with Japanese regulations. Furthermore, we have already developed a method for detecting walnuts using conventional PCR and electrophoresis (31). By combining these methods, the risk of false-negative and/or false-positive results in inspections for walnut contamination could be minimized.

Conclusions

In conclusion, the present ELISA kit is a rapid, precise, and reliable tool for the analysis of WP in processed foods. The proposed system is capable of accurately monitoring labeling systems in a reliable manner and may be useful for inspections performed in accordance with Japanese regulations.

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