body-associated protein in soybean, has been identified as one of the major allergenic proteins and named Gly m Bd 30K. Our ELISA method is highly specific for this soybean protein, with the LOD of 0.47 ng/mL (equivalent to 0.19 μg/g food) and limit of quantification (LOQ) of 0.94 ng/mL (equivalent to 0.38 μg/g food). Recovery ranged from 87.7% to 98.7%, while the intra- and interassay coefficients of variation were less than 4.2% and 7.5%, respectively. These results show that this ELISA method is specific, precise, and reliable for quantitative analysis of the soybean protein in processed foods. Five types of incurred samples (model processed foods: rice gruel, sausage, sweet adzuki-bean soup, sweet potato cake, and tomato sauce) containing 10 μg soybean soluble protein/g food were prepared for use in interlaboratory evaluations of the soybean ELISA kit (Sakai *et al.*, 2009). The kit displayed a sufficient RSD_r value (interlaboratory precision: 9.3–13.4% RSD_r) and a high recovery (97–114%) for all incurred samples. The RSD_r value for the incurred samples was mostly $<4.8\%$. The results of this interlaboratory evaluation suggest that the soybean kit can be used as a precise and reliable tool for determination of soybean proteins in processed foods.

A sensitive qualitative detection method for soybeans in foods using PCR was also developed (Yamakawa *et al.*, 2007b). For specific detection of soybeans with high specificity, the primer pair was designed using the gene encoding the *Glycine max* repetitive sequence. Trace amounts of soybeans in commercial food products could be qualitatively detected by this method.

2. Walnut

Tree nuts are regarded as one of the most potent of all known food allergens and are often attributed as the cause of severe food anaphylaxis and death. Walnut (*Juglans regia*) is the most common allergenic tree nut and this allergy can be observed in all age groups (Bock *et al.*, 2001). In addition, the walnut allergy is extremely potent, inducing life threatening allergic reactions similar to peanut allergy (Clark and Ewan, 2003; Pumphrey, 2000; Pumphrey and Roberts, 2000). According to Japanese regulations, the labeling of food products containing walnut is recommended. To ensure proper labeling, a novel sandwich ELISA kit for the determination of walnut protein in processed foods has been developed (Doi *et al.*, 2008). The sandwich ELISA method is highly specific for walnut soluble proteins. The recovery ranged from 83.4% to 123%, while the intra- and interassay coefficients of variation were less than 8.8% and 7.2%, respectively. We prepared seven types of incurred samples (model processed foods: biscuit, bread, sponge cake, orange juice, jelly, chicken meatball, and rice gruel) containing 10 μg walnut soluble protein/g food for use in interlaboratory evaluations of the walnut ELISA method (Sakai *et al.*, 2010a). The walnut kit displayed a sufficient RSD_r

(interlaboratory precision: 5.8–9.9% RSD_r) and a high level of recovery (81–119%) for all the incurred samples. All RSD_r values for the incurred samples examined were less than 6.0%. The results of this interlaboratory evaluation suggest that the walnut ELISA method can be used as a precise and reliable tool for determination of walnut proteins in processed foods.

A sensitive qualitative detection method for walnut using PCR was also developed (Yano *et al.*, 2007). For detection of walnuts with high specificity, the primer pair was designed based on walnut *matK* genes. Trace amounts of walnuts in commercial food products can be qualitatively detected using this method.

3. Kiwifruit

Kiwifruit (*Actinidia deliciosa* and *A. chinensis*) is a major fruit allergen that produces severe symptoms and is responsible for a large number of clinical cases worldwide (Lucas *et al.*, 2003; Lucas *et al.*, 2004; Möller *et al.*, 1998a). Under Japanese regulations, it is recommended for labeling as much as possible. To develop PCR-based methods for detection of trace amounts of kiwifruit in foods, we designed two primer pairs targeting the ITS-1 region of the *Actinidia* spp. using PCR simulation software (Taguchi *et al.*, 2007). On the basis of the known distribution of a major kiwifruit allergen (actinidin) within the *Actinidia* spp., in addition to reports on clinical and immunological cross-reactivities, one of the primer pairs was designed to detect all *Actinidia* spp. and the other to detect commercially grown *Actinidia* spp. (i.e., *A. arguta* and is interspecific hybrids) except for *A. polygama*. The specificity of these methods using designed primer pairs was verified by PCR on eight *Actinidia* spp. and 26 other plants, including fruits. The methods were considered to be specific enough to yield products of the target-size only from *Actinidia* spp. and sensitive enough to detect 5–50 fg of *Actinidia* spp. DNA spiked in 50 ng salmon testis DNA used as a carrier (1–10 ppm of kiwifruit DNA) and 1700 ppm (wt/wt) of fresh kiwifruit puree spiked in a commercial plain yogurt (corresponded to ca. 10 ppm of kiwifruit protein). These methods are expected to be useful in the detection of unidentified kiwifruit and its related species in processed foods.

4. Banana

Banana contains food allergens that are common to those in latex or pollens (Ito *et al.*, 2006; Sanchez-Monge *et al.*, 1999). Many clinical studies have reported cross-reactivity of banana and latex, referred to as the latex-fruit syndrome (Blanco *et al.*, 1999; Ikezawa and Osuna, 2002; Möller *et al.*, 1998b). These studies monitored the number of patients with food allergy in Japan and found that patients with banana allergy comprised the second largest population (below only kiwifruit allergy) among those with fruit allergies. We developed specific PCR methods for detection of

banana residue in processed foods. For high banana specificity, the primer pair was designed based on the large subunit of ribulose-1, 5-bisphosphate carboxylase (*rbcL*) genes of chloroplasts and used to obtain amplified products specific to banana by both conventional and real-time PCR (Sakai *et al.*, 2010b). To confirm the specificity of these methods, genomic DNA samples from 31 other species were examined; no amplification products were detected. Subsequently, eight kinds of processed foods containing banana were investigated using these methods to confirm the presence of banana DNA. Conventional PCR had a detection limit of 1 ppm (wt/wt) banana DNA spiked in 50 ng of salmon testis DNA, while SYBR Green I real-time semi-quantitative PCR had a detection limit as low as 10 ppm banana DNA. Thus, both methods show high sensitivity and may be applicable as specific tools for the detection of trace amounts of banana in commercial food products.

5. Pork, beef, chicken, mutton, and horseflesh

As the modern diet often comprises processed foods, especially minced meats, manufacturers are obligated to properly label raw materials. Hence, a rapid method of detecting meat ingredients in processed foods is needed to verify proper labeling. A rapid real-time quantitative PCR method to detect trace amounts of pork, beef, chicken, mutton, and horse meat in foods was developed (Tanabe *et al.*, 2007). The primers and TaqMan minor groove binder (MGB) probes were designed using the gene encoding cytochrome *b* for specific detection of each species. The LOQ of this method was 100 fg/μL of each mitochondrial DNA in 10 ng/μL of wheat mitochondrial DNA matrix. The calculated R^2 values of the standard curves for the five species ranged between 0.994 and 0.999. This method is particularly useful in the detection of unidentified minced meat in processed foods for verification of food labeling.

IV. PATIENT EVALUATION OF ALLERGY FOOD LABELING

To clarify the usefulness and reliability of the food-labeling system, food allergy patients (or their parents) at Sagamihara National Hospital were asked to evaluate it by questionnaire. We received responses from 169 patients. As shown in Table 4.20, patients' profiles were an average age of 49.3 ± 35.6 months, age of the first onset of symptoms of 10.1 ± 14.1 months, and average of 2.9 ± 2.5 eliminated foods. Eliminated foods included hen's eggs (135), cow's milk (79), and wheat (47), as well as peanuts and fish eggs. Of these patients, 44.2% had a past history of anaphylaxis, and 80.2% had experienced symptoms following exposure to even extremely small amounts of the causative foods.

TABLE 4.20 Characteristics of surveyed subjects

169 parents of food allergy patients at Sagamihara National Hospital	
Age of patients	49.3 ± 35.6 months M/F = 1.9
Age of first onset of symptom	10.1 ± 14.1 months
Number of eliminated foods	2.9 ± 2.5
Eliminated foods	
Hen's eggs	135
Cow's milk	79
Wheat	47
Peanuts	51
Fish eggs	28
Past history of anaphylaxis	44.2%
Incidence of symptom by extremely small amount	80.2%

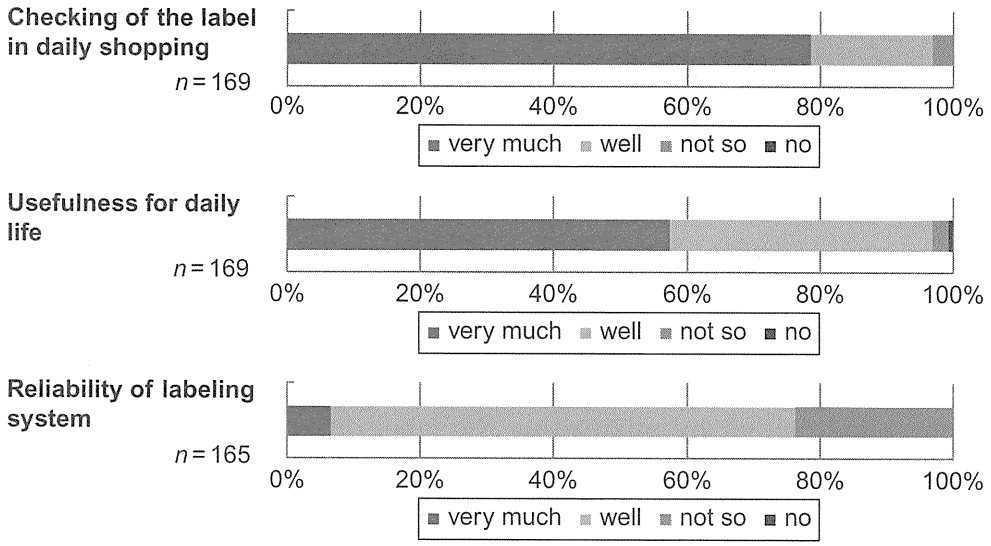

FIGURE 4.7 Evaluation of allergy food labeling.

As shown in Fig. 4.7, 97% of patients routinely checked the allergy food label during daily shopping, and 97% evaluated the allergy food labeling as "very useful" or "useful." In addition, 76.4% of the respondents relied on the allergy food-labeling system, and 79.3% had a correct understanding of the food-labeling system based on self-evaluation. On the other hand, 48.8% of respondents answered that the labeling system was "very easy" or "easy" to understand (Fig. 4.8). Patients who had experienced accidental intake by misreading a label or by mislabeling comprised 30.9% and 13.9%, respectively (Fig. 4.9).

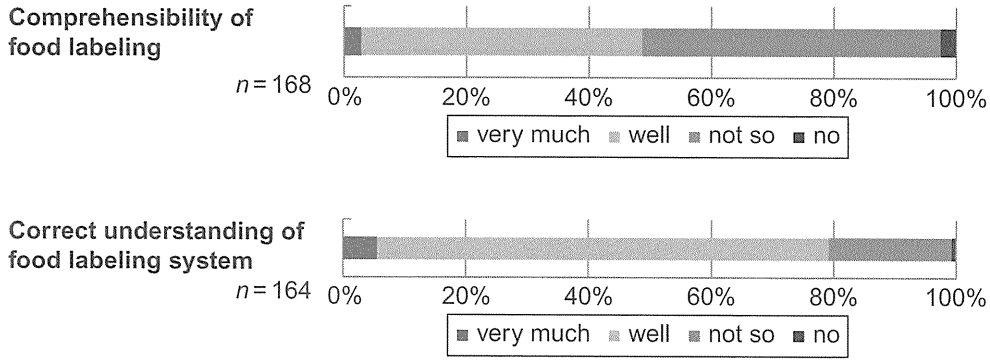

FIGURE 4.8 Comprehension and understanding of allergy food labeling.

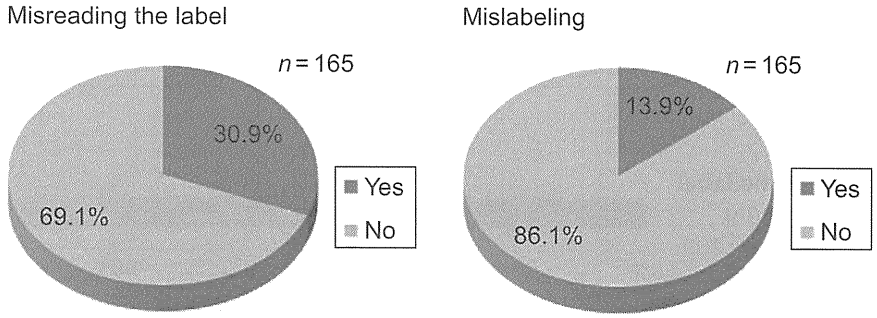

FIGURE 4.9 Incidences of accidental intake by misreading and mislabeling of food labels.

Overall, the Japanese food allergy-labeling system was highly evaluated by food allergy patients and parents. Almost all patients felt that the food-labeling system was very useful, although there were cases of accidental intake either by misreading the label or by mislabeling by food companies.

ACKNOWLEDGMENTS

The authors acknowledge the help and advice of Ms. Chizuko Sugizaki and Ms. Noriko Hayashi, and the advice and support from Professor Atsuo Urisu, Dr. Reiko Teshima, Dr. Reiko Adachi, and the staff of Division of Novel Foods and Immunochemistry, National Institute of Health Sciences. This study was supported by Health and Labor Science Research Grants for Research from the Ministry of Health, Labor, and Welfare of Japan.

REFERENCES

Blanco, C., Diaz-Perales, A., Collada, C., Sánchez-Monge, R., Aragoncillo, C., Castillo, R., Ortega, N., Alvarez, M., Carrillo, T., and Salcedo, G. (1999). Class I chitinases as potential panallergens involved in the latex-fruit syndrome. *J. Allergy Clin. Immunol.* **103**, 507–513.

Bock, S. A. and Atkins, F. M. (1990). Patterns of food hypersensitivity during sixteen years of double-blind, placebo-controlled food challenges. *J. Pediatr.* **117**, 561–567.

Bock, S. A., Munoz-Furlong, A., and Sampson, H. A. (2001). Fatalities due to anaphylaxis reaction to foods. *J. Allergy Clin. Immunol.* **107**, 191–193.

Clark, A. T. and Ewan, P. W. (2003). Interpretation of tests for nut allergy in one thousand patients, in relation to allergy or tolerance. *Clin. Exp. Allergy* **33**, 1041–1045.

Doi, H., Shibata, H., Shoji, M., Sakai, S., and Akiyama, H. (2008). A reliable enzyme linked immuno-sorbent assay for the determination of walnut proteins in processed foods. *J. Agric. Food Chem.* **56**, 7625–7630.

Ebisawa, M., Ikematsu, K., Imai, T., and Tachimoto, H. (2003). *J. World Allergy Organiz.* **15**, 214–217.

Food and Agriculture Organization of the United Nations, World Health Organization (FAO (1995). Report of FAO technical consultation on food allergens. Rome 1995, November, 13–14.

Foucard, T. and Malmheden Yman, I. (1999). A study on severe food reactions in Sweden—Is soy protein an underestimated cause of food anaphylaxis? *Allergy* **54**, 261–265.

Hefle, S. L., Nordlee, J. A., and Taylor, S. L. (1996). Allergenic foods. *Crit. Rev. Food Sci. Nutr. Allergy Clin. Immunol.* **36**(Suppl.), 69–89.

Ikezawa, Z. and Osuna, H. (2002). Latex-fruits syndrome. *Arerugi* **51**, 591–604 (in Japanese).

Ito, A., Ito, K., Morishita, M., and Sakamoto, T. (2006). A banana-allergic infant with IgE reactivity to avocado, but not to latex. *Pediatr. Int.* **48**, 321–323.

Lucas, J. S. A., Lewis, S. A., and Hourihane, J. O. (2003). Kiwi fruit allergy: A review. *Pediatr. Allergy Immunol.* **14**, 420–428.

Lucas, J. S. A., Grimshaw, K. E. C., Collins, K., Warner, J. O., and Hourihane, J. O. (2004). Kiwi fruit is a significant allergen and is associated with differing patterns of reactivity in children and adults. *Clin. Exp. Allergy* **34**, 1115–1121.

Matsuda, R., Yoshioka, Y., Akiyama, H., Aburatani, K., Watanabe, Y., Matsumoto, T., Morishita, N., Sato, H., Mishima, T., Gamo, R., Kihira, Y., and Maitani, T. (2006). Inter-laboratory evaluation of two kinds of ELISA kits for the detection of egg, milk, wheat, buckwheat, and peanut in foods. *J. AOAC Int.* **89**, 1600–1608.

Möller, M., Kayma, M., Vieluf, D., Paschke, A., and Steinhart, H. (1998). Determination and characterization of cross-reacting allergens in latex, avocado, banana, and kiwi fruit. *Allergy* **53**, 289–296.

Morishita, N., Kamiya, K., Matsumoto, T., Sakai, S., Teshima, R., Urisu, A., Moriyama, T., Ogawa, T., Akiyama, H., and Morimatsu, F. (2008). A reliable enzyme-linked immuno-sorbent assay for determination of soybean proteins in processed foods. *J. Agric. Food Chem.* **56**, 6818–6824.

Notification No. 1106001 of November 6, 2002, and the revised Notification No. 0622003 of June 22, 2006, Department of Food Safety, the Ministry of Health, Labour and Welfare of Japan.

Pumphrey, R. S. H. (2000). Lessons for management of anaphylaxis from a study of fatal reaction. *Clin. Exp. Allergy* **30**, 1144–1150.

Pumphrey, R. S. H. and Roberts, I. S. D. (2000). Postmortem findings after fatal anaphylactic reactions. *J. Allergy Clin. Pathol.* **53**, 273–276.

Sakai, S., Matsuda, R., Adachi, R., Akiyama, H., Maitani, T., Ohno, Y., Oka, M., Abe, A., Seiki, K., Oda, H., Shiomi, K., and Urisu, A. (2008). Interlaboratory evaluation of two enzyme-linked immunosorbent assay kits for the determination of crustacean protein in processed foods. *J. AOAC Int.* **91**, 123–129.

Sakai, S., Adachi, R., Akiyama, H., Teshima, R., Morishita, N., Matsumoto, T., and Urisu, A. (2009). Interlaboratory evaluation of an enzyme-linked immunosorbent assay kit for the determination of soybean protein in processed foods. *J. AOAC Int.* **93**, 243–248.

Sakai, S., Adachi, R., Akiyama, H., Teshima, R., Doi, H., and Shibata, H. (2010a). Determination of walnut protein in processed foods by enzyme-linked immunosorbent assay interlaboratory study. *J. AOAC Int.* **93**, 1255–1261.

Sakai, Y., Ishihata, K., Nakano, S., Yamada, T., Yano, T., Uchida, K., Nakao, Y., Urisu, A., Adachi, R., Teshima, R., Akiyama, H., Sakai, Y., *et al.* (2010b). Specific detection of banana residue in processed foods using polymerase chain reaction. *J. Agric. Food Chem.* **58**, 8145–8151.

Sampson, H. A. (2001). Utility of food-specific IgE concentrations in predicting symptomatic food allergy. *J. Allergy Clin. Immunol.* **107**, 891–896.

Sanchez-Monge, R., Blanco, C., Diaz-Perales, A., Collada, C., Carrillo, T., Aragoncillo, C., and Salcedo, G. (1999). Isolation and characterization of major banana allergens: Identification as fruit class I chitinases. *Clin. Exp. Allergy* **29**, 673–680.

Seiki, K., Oda, H., Yoshioka, H., Sakai, S., Urisu, A., Akiyama, H., and Ohno, Y. (2007). A reliable and sensitive immunoassay for the determination of crustacean protein in processed foods. *J. Agric. Food Chem.* **55**, 9345–9350.

Shibahara, Y., Oka, M., Tominaga, K., Ii, T., Umeda, M., Uneo, N., Abe, A., Ohashi, E., Ushio, H., and Shiomi, K. (2007). Determination of crustacean allergen in food products by sandwich ELISA. *Nippon Shokuhin Kagaku Kogaku Kaishi* **54**, 280–286.

Sicherer, S. H., Sampson, H. A., and Burks, A. W. (2000). Peanut and soy allergy: A clinical and therapeutic dilemma. *Allergy* **55**, 515–521.

Taguchi, H., Watanabe, S., Hirao, T., Akiyama, H., Sakai, S., Watanabe, T., Matsuda, R., Urisu, A., and Maitani, T. (2007). Specific detection of potentially allergenic kiwifruit in foods using polymerase chain reaction. *J. Agric. Food Chem.* **55**, 1649–1655.

Tanabe, S., Hase, M., Yano, T., Sato, M., Fujimura, T., and Akiyama, H. (2007). Real-time quantitative PCR detection method for pork, chicken, beef, mutton, and horseflesh in foods. *Biosci. Biotechnol. Biochem.* **71**, 3131–3135.

Watanabe, K., Aburatani, T., Mizumura, M., Sakai, H., Muraoka, S., Mamegoshi, S., and Honjoh, T. (2005). Novel ELISA for the detection of raw and processed egg using extraction buffer containing a surfactant and a reducing agent. *J. Immunol. Methods* **300**, 115–123.

Watanabe, T., Akiyama, H., Yamakawa, H., Iijima, K., Yamazaki, F., Matsumoto, T., Futo, S., Arakawa, F., Watai, M., and Maitani, T. (2006). A specific qualitative detection method for peanut (*Arachis hypogaea*) in foods using polymerase chain reaction. *J. Food Biochem.* **30**, 215–233.

Yamakawa, H., Akiyama, H., Endo, Y., Miyatake, K., Sakata, K., Sakai, S., Toyoda, M., and Urisu, A. (2007a). Specific detection of wheat residues in processed foods by polymerase chain reaction. *Biosci. Biotechnol. Biochem.* **71**, 2561–2564.

Yamakawa, H., Akiyama, H., Endo, Y., Miyatake, K., Sakata, K., Sakai, S., Moriyama, T., Urisu, A., and Maitani, T. (2007b). A specific detection of soybean residues in processed foods using polymerase chain reaction. *Biosci. Biotechnol. Biochem.* **71**, 269–272.

Yamakawa, H., Akiyama, H., Endo, Y., Miyatake, K., Sakata, K., Sakai, S., Toyoda, M., and Urisu, A. (2008). Specific detection of buckwheat residues in Processed foods by polymerase chain reaction. *Biosci. Biotechnol. Biochem.* **72**, 2228–2231.

Yano, T., Sakai, Y., Uchida, K., Nakao, Y., Ishihata, K., Nakano, S., Yamada, T., Sakai, S., Urisu, A., Akiyama, H., and Maitani, T. (2007). Detection of walnut residues in processed foods by polymerase chain reaction. *Biosci. Biotechnol. Biochem.* **71**, 1793–1796.

Zarkadas, M., Scott, W. F., Salminen, J., and Pong, A. H. (1999). Common allergenic foods and their labelling in Canada. *Can. J. Allergy Clin. Immunol.* **4**, 118–141.

平成 23 年度厚生労働科学研究費補助金
免疫アレルギー疾患等予防・治療研究事業

「アレルギー疾患の全国全年齢有症率および
治療ガイドライン普及効果等疫学調査に基づく発症要因・
医療体制評価に関する研究」 研究報告書

発　行　　平成 24 年 3 月 31 日
発行者　　東京都立小児総合医療センター　アレルギー科
赤　澤　　晃
〒183-8561 東京都府中市武蔵台 2-8-29