



Note

Specific Detection of Buckwheat Residues in Processed Foods by Polymerase Chain Reaction

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A specific and qualitative detection method for buckwheat in foods using the polymerase chain reaction (PCR) was developed. Trace amounts of buckwheat in commercial food products were qualitatively detected by this method. It should be reliable for detecting buckwheat residues in processed foods and practical for monitoring the labeling system for allergenic food materials.

Key words: food allergy; buckwheat; *Fagopyrum esculentum*; *Fagopyrum tataricum*; polymerase chain reaction (PCR)

Buckwheat is known to cause immediate-type anaphylactic reactions through a specific IgE antibody.¹⁻³ To prevent possible life-threatening reactions, the most effective treatment is strictly to avoid the consumption of these allergenic foods. Hence, accurate information about potentially allergenic ingredients in a food product is necessary.⁴ In Japan, the Ministry of Health, Labour, and Welfare has enforced a labeling system for allergenic food materials since April 2002.⁵ Based on this system, the labeling of five food ingredients (egg, milk, wheat, buckwheat, and peanuts) is mandatory.

Buckwheat belongs to the polygonaceae family, and there are many species of it around the world. Among them, common buckwheat (*Fagopyrum esculentum*) is typically cultivated, and a little Tatarian buckwheat (*Fagopyrum tataricum*) is cultivated in some mountainous regions.⁶ In a previous study, Hirao *et al.* developed the PCR method to detect both cultivated and wild buckwheats,⁷ but this method can give a false positive if unintentional contamination of wild plant species occurs, during the harvesting of food materials.

In the present study, we developed a detection method for common buckwheat and Tatarian buckwheat with high specificity and sensitivity for monitoring of a labeling system for allergenic food materials. We concluded that the proposed method can be applied to processed food products, and that trace amounts of buckwheat contained in commercial food products can be detected by it.

Eight varieties of common buckwheat (*Fagopyrum esculentum*), including Mancan of three different origins (US, Canada, and China), two varieties of a Chinese cultivar (Utimouko and Keirin), three varieties of a Japanese cultivar (Kitawase, Shinano I, and Aizuzairai), and Tatarian buckwheat (*Fagopyrum tataricum*) were collected in our laboratory. Seeds of wild buckwheat (*Polygonum convolvulus*) and Polygonaceous plants, such as *Polygonum cuspidatum*, *Polygonum filiforme*, *Polygonum capitatum*, *Persicaria longiseta*, *Persicaria lapathifolia*, *Rumex acetosa*, *Rumex obtusifolius*, and *Rheum rhabarbarum*, and grain samples of common wheat, durum wheat, rye, barley, oats, rice, corn, peanuts, soybean, rapeseed, and foxtail millet were also purchased. Ten commercial food products with buckwheat as a listed ingredient were purchased from a local market in Saitama, Japan. In purchasing the commercial food products, we selected eight food products with buckwheat as a listed ingredient, and two food products with a remark of caution by the consumer, such as "this food product was made in a factory which manufactures food products containing buckwheat."

The seed materials of buckwheat, other crops, and commercial food products were ground with a Multi-Beads Shocker (Yasui Kikai, Osaka, Japan), and then the homogenous powders were used as test samples for the PCR. Wheat flour was used as the matrix to

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Table 1. List of Designed Primers

	Name	Sequence (5' → 3')	Specificity		Amplicon
A:	CP 03-5'	5'-CGG ACG AGA ATA AAG ATA GAG T-3'	Chloroplast DNA, sense	Plants	123 bp
	CP 03-3'	5'-TTT TGG GGA TAG AGG GAC TTG A-3'	Chloroplast DNA, antisense		
B:	FAG 19	5'-AAC GCC ATA ACC AGC CCG ATT-3'	Fagopyrum esculentum major allergenic storage protein gene, sense	Buckwheat	127 bp
	FAG 22	5'-CCT CCT GCC TCC CAT TCT TC-3'	Fagopyrum esculentum major allergenic storage protein gene, antisense		

A, To confirm the validity of DNA extracted from plants for the polymerase chain reaction.

B, For specific detection of buckwheat.

prepare mixed model samples. Wheat grains were ground to a fine powder with a grinder, and then 0, 0.0001, 0.001, 0.005, 0.01, 0.1, 1, and 100% buckwheat flour (Aizuzairai) were mixed according to the procedure described in a previous study, but with a slight modification.⁸⁾

Genomic DNA was extracted from each plant material, including the mixed model samples, using a silica-gel membrane-type kit (DNeasy Plant Mini, Qiagen, Hilden, Germany) according to the procedure described previously.⁹⁾ The genomic DNA was extracted from the commercial food products using an anion exchange-type kit (Genomic-tip 20/G, Qiagen, Hilden, Germany) according to the procedure described previously.¹⁰⁾ The extracted DNA was diluted with an appropriate volume of distilled water (DW) to a final concentration of 20 ng/μl and stored at −20 °C until needed. When the concentration of the extracted DNA was less than 20 ng/μl, an undiluted DNA extract was used in subsequent PCR analysis. The primers were synthesized and purified using a reversed-phase column (Operon Biotechnologies, Tokyo), diluted with an appropriate volume of DW to a final concentration of 50 μmol/l, and stored at −20 °C until needed.

The reaction mixture for the PCR was prepared in a PCR reaction tube. The reaction volume, of 25 μl, contained 50 ng of genomic DNA, 0.2 mM dNTP, 1.5 mM MgCl₂, 0.2 μM of the 5' and 3' primers, and 0.625 units of AmpliTaq Gold (Applied Biosystems, Foster City, CA), topped up with DW. When the concentration of the extracted DNA was less than 20 ng/μl, 2.5 μl of an undiluted DNA extract was added to the PCR reaction tube. The reaction was buffered with PCR buffer II (Applied Biosystems), and amplified in a thermal cycler (PTC-220 DNA Engine DYAD, Bio-Rad Laboratories, Hercules, CA). The PCR step-cycle conditions were as follows: pre-incubation at 95 °C for 10 min, 40 cycles consisting of denaturation at 95 °C for 0.5 min, annealing at 60 °C for 0.5 min, and extension at 72 °C for 0.5 min, followed by a final extension at 72 °C for 7 min. After PCR amplification, agarose gel electro-

phoresis of the PCR product was carried out following previous studies.⁸⁾

The amount of buckwheat protein in the commercial food products was measured using a sandwich ELISA kit for buckwheat (Morinaga, Yokohama, Japan) according to the procedure described in a previous study.¹¹⁾ Nair *et al.* reported the nucleotide sequence of the gene encoding the Fagopyrum esculentum major allergenic storage protein, with a molecular mass of 22 kDa,¹²⁾ so we chose this gene as a buckwheat-specific gene and designed the primer pair FAG 19/FAG 22 by referring to GenBank Accession no. AF152003. The primer pair FAG 19/FAG 22 generates a 127-bp amplified fragment. In addition, the primer pair CP 03-5'/CP 03-3', for universal detection of DNA derived from plants, was used to verify the extracted DNAs.¹⁰⁾ This primer pair generated a 123-bp amplified fragment. The sequences of the designed oligonucleotides in this study are listed in Table 1.

As shown in Fig. 1A, a fragment (127 bp) amplified using primer pair FAG 19/FAG 22 was specifically detected from common buckwheat and Tatarian buckwheat genomic DNA. In contrast, no amplified fragment was detected when DNA was extracted from 11 other plant species (common wheat, durum wheat, rye, barley, oats, rice, corn, peanuts, soybean, rapeseed, and foxtail millet) as the template DNA. In addition, no amplification of the 127-bp product occurred in *Polygonum convolvulus* or in the eight kinds of Polygonaceous plants other than buckwheat (Fig. 1B). Nucleotide sequence analysis of the PCR product obtained from common buckwheat and Tatarian buckwheat confirmed that the intended sequence of the Fagopyrum esculentum major allergenic storage protein gene had been amplified. These data suggest that common buckwheat and Tatarian buckwheat-genomic DNA can be specifically detected using the FAG 19/FAG 22 primer pair.

To assess the sensitivity of the proposed PCR method, we tested the mixed wheat flour samples containing 0, 0.0001, 0.001, 0.005, 0.01, 0.1, 1, and 100% of the

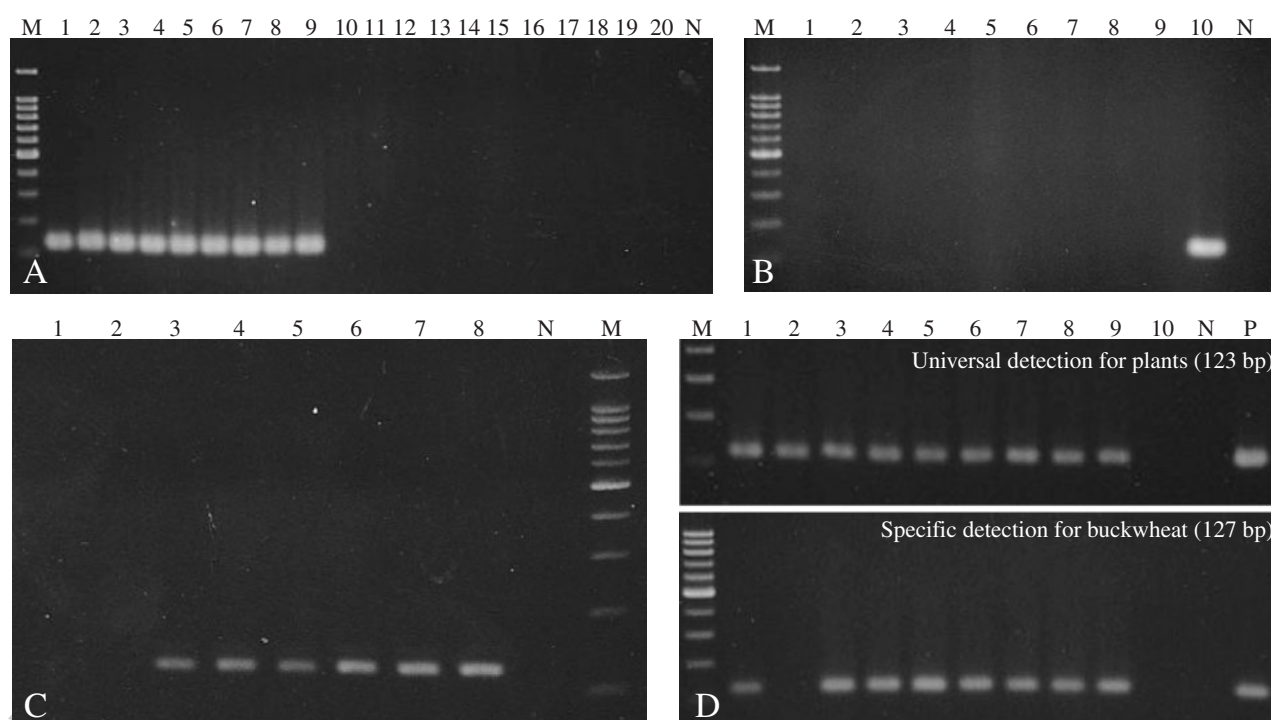


Fig. 1. Specific Detection of Buckwheat by PCR.

A, Specificity of the PCR method for buckwheat. M, 100-bp ladder size standard. Lanes 1–8, common buckwheat. Lane 1, U.S. Mancan; lane 2, Canadian Mancan; lane 3, Chinese Mancan; lanes 4–5, Chinese cultivars (lane 4, Utimouko; lane 5, Keirin); lanes 6–8, domestic cultivars (lane 6, Kitawase; lane 7, Shinano 1; lane 8, Aizuzairai); lane 9, Tatarian buckwheat; lane 10, common wheat; lane 11, durum wheat; lane 12, rye; lane 13, barley; lane 14, oats; lane 15, rice; lane 16, corn; lane 17, peanuts; lane 18, soybean; lane 19, rapeseed; lane 20, foxtail millet. N, no template control. B, Specificity of the PCR method for buckwheat. M, 100-bp ladder size standard. Lane 1, wild buckwheat; lane 2, *Polygonum cuspidatum*; lane 3, *Polygonum filiforme*; lane 4, *Polygonum capitatum*; lane 5, *Persicaria longiseta*; lane 6, *Persicaria lapathifolia*; lane 7, *Rumex acetosa*; lane 8, *Rumex obtusifolius*; lane 9, *Rheum rhabarbarum*; lane 10, common buckwheat (Aizuzairai). N, no template control. C, Sensitivity of the PCR method for buckwheat. Genomic DNAs extracted from eight mixing levels of buckwheat flour in wheat flour were used the template DNA. Lane 1, 0%; lane 2, 0.0001%; lane 3, 0.001%; lane 4, 0.005%; lane 5, 0.01%; lane 6, 0.1%; lane 7, 1%; lane 8, 100%. N, no template control. M, 100-bp ladder size standard. D, Investigation of commercial food products. Lane 1, wheat noodle #1; lane 2, wheat noodle #2; lane 3, buckwheat noodle; lane 4, rice cake; lane 5, dumpling; lane 6, cookies #1; lane 7, cookies #2; lane 8, fried snack; lane 9, snacks; lane 10, buckwheat tea. N, no template control. M, 100-bp ladder size standard.

buckwheat flour powder. Fifty ng of the genomic DNA extracted from the mixed samples was amplified in the PCR reaction. As expected, the target sequence for the buckwheat was clearly detected in the 0.001% to 100% mixed samples (Fig. 1C). This result suggests that buckwheat flour contamination as low as 0.001% can be detected in an unprocessed food.

To determine the applicability of the buckwheat DNA detection method to commercial food products, we purchased 10 food samples from a local market and tested them for the presence of buckwheat DNA using the proposed method. Eight of the purchased samples had buckwheat as a listed ingredient. The other two samples were wheat noodles with advisory remarks such as “this food product was made in a factory that manufactures food products containing buckwheat.” A sufficient amount (more than 20 ng/μl) of genomic DNA for the PCR was obtained from almost all the food products using the purification method described, except for the fried snack and buckwheat tea, using the purification method described. As shown in Fig. 1D, the universal primer pair CP 03-5'/CP 03-3' generated a

specific amplified fragment from all of the samples except for the buckwheat tea. In the eight food products, except for wheat noodle #2, buckwheat DNA was clearly detected by the PCR methods using the FAG 19/FAG 22 primer pair (Fig. 1D and Table 2). Based on these results, we postulate that the failure to detect the amplified PCR products from the buckwheat tea was probably due to DNA degradation during processing at a high temperature. The amount of buckwheat protein measured by the ELISA method for wheat noodle #1 and buckwheat tea was below 1 ppm, and that result appears to be consistent with that by the present PCR method (Table 2). These results suggest that the proposed PCR method using the FAG 19/FAG 22 primer pair is applicable in identifying buckwheat in processed food products except for those that are highly processed, and that the sensitivity of the PCR method is similar to that of the ELISA method.

In summary, a rapid PCR detection method was developed for specific detection of buckwheat. The methods reported in this study are simple, sensitive, and reliable in identifying trace amounts of buckwheat in

Table 2. Analyses of Buckwheat Protein and Buckwheat DNA in Commercial Food Products

No.	Food item*	Protein-ELISA method	PCR method
		Protein conc. (ppm)	Result
1	Wheat noodle #1	7.4	+
2	Wheat noodle #2	<1.0	—
3	Buckwheat noodle	>20	+
4	Rice cake	>20	+
5	Dumpling	>20	+
6	Cookies #1	>20	+
7	Cookies #2	>20	+
8	Fried snack	3.3	+
9	Snack	>20	+
10	Buckwheat tea	<1.0	—

ELISA, enzyme-linked immunosorbent assay; PCR, polymerase chain reaction

*Nos. 1 and 2 were wheat noodles by different manufacturers. These were labeled with advisory remarks such as “this food product was made in a factory that manufactures food products containing buckwheat.” Nos. 3 to 10 had buckwheat labeled in the list of ingredients.

processed foods. To minimize the risk of false negative and positive results, we believe that the PCR method can be used to complement the protein-based detection method.¹¹⁾

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References

- Wieslander, G., Review on buckwheat allergy. *Allergy*, **51**, 661–665 (1996).
- Horeish, A. J., Buckwheat sensitivity in children. *Ann. Allergy*, **30**, 685–689 (1972).
- Nakamura, S., Yamaguchi, M., Oishi, M., and Hayama, T., Studies on the buckwheat allergen report 1: on the cases with the buckwheat allergen. *Allerg. Immunol.* (Leipzig), **20**, 449–456 (1974/1975).
- Furlong, T. J., DeSimone, J., and Sicherer, S. H., Peanut and tree nut allergenic reactions in restaurants and other food establishments. *J. Allergy Clin. Immunol.*, **108**, 867–870 (2001).
- Ebisawa, M., Ikematsu, K., Imai, T., and Tachimoto, H., Food allergy in Japan. *J. World Allergy Org.*, **15**, 214–217 (2003).
- Si-quan, L., and Zhang, Q. H., Advances in the development of functional foods from buckwheat. *Crit. Rev. Food Sci. Nutr.*, **41**, 451–464 (2001).
- Hirao, T., Imai, S., Sawada, H., Shiomi, N., Hachimura, S., and Kato, H., PCR method for detecting trace amounts of buckwheat (*Fagopyrum* spp.) in food. *Biosci. Biotechnol. Biochem.*, **69**, 724–731 (2005).
- Watanabe, T., Kasama, K., Wakui, C., Shibuya, M., Matsuki, A., Akiyama, H., and Maitani, T., Laboratory-performance study of the notified methods to detect genetically modified maize (CBH351) and potato (NewLeaf Plus and NewLeaf Y). *Shokuhin Eiseigaku Zasshi* (in Japanese), **44**, 281–288 (2003).
- Yamakawa, H., Akiyama, H., Endo, Y., Miyatake, K., Sakata, K., Sakai, S., Toyoda, M., and Urisu, A., Specific detection of wheat residues in processed foods by polymerase chain reaction. *Biosci. Biotechnol. Biochem.*, **71**, 2561–2564 (2007).
- Watanabe, T., Akiyama, H., Maleki, S., Yamakawa, H., Iijima, K., Yamazaki, F., Matsumoto, T., Futo, S., Arakawa, F., Watai, M., and Maitani, T., A specific qualitative detection method for peanut (*Arachis hypogaea*) in foods using polymerase chain reaction. *J. Food Chem.*, **30**, 215–233 (2006).
- Akiyama, H., Nakamura, K., Harikai, N., Watanabe, H., Iijima, K., Yamakawa, H., Mizuguchi, Y., Yoshikawa, R., Yamamoto, M., Sato, H., Watai, M., Arakawa, F., Ogasawara, T., Nishihara, R., Kato, H., Yamauchi, A., Takahata, Y., Morimatsu, F., Mamegoshi, S., Muraoka, S., Honjyoh, T., Watanabe, T., Kozue, S., Imamura, T., Tiyoda, M., Matsuda, R., and Maitani, T., Inter-laboratory evaluation studies for establishment of notified ELISA methods for allergenic substances (buckwheat). *Shokuhin Eiseigaku Zasshi* (in Japanese), **45**, 313–318 (2004).
- Nair, A., Ohmoto, T., Woo, S. H., and Adachi, T., A molecular-genetic approach for hypoallergenic buckwheat. *Fagopyrum*, **16**, 29–36 (1999).