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

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A sensitive qualitative detection method for wheat in foods using polymerase chain reaction (PCR) was developed. Trace amounts of wheat in commercial food products could be qualitatively detected by this method. The sensitivity of the proposed PCR method appears to be similar to that of ELISA. The present method should be very useful for detecting wheat residues in processed foods.

Key words: food allergy; common wheat; *Triticum aestivum* L.; detection method; polymerase chain reaction (PCR)

Food allergies have become an important health problem in recent years in industrialized countries, and it is estimated that approximately 8% of children and 2% of adults have some type of food allergy.1–3) In particular, allergies to wheat have long been of importance, since wheat is consumed as a staple food in many countries.4–7) To prevent possible life-threatening reactions, hypoallergenic foods might be useful,8) but the most effective treatment is strictly to avoid the consumption of these allergic foods. Therefore, accurate information about potentially allergic ingredients in a food product is critical.9,10) In 1999, the Joint FAO/WHO Codex Alimentary Commission agreed to label eight kinds of food that contain ingredients known to be allergens, including wheat.11,12) In Japan, the Ministry of Health, Labor and Welfare has enforced a labeling system for allergenic food material since April, 2002.3) With this system, labeling of five food ingredients (egg, milk, wheat, buckwheat, and peanuts) is mandatory.

In the present study, we developed a detection method for wheat with high specificity and greater sensitivity than previously published methods.13,14) We show that the proposed method can be applied to processed food products, and that trace amounts of wheat contained in commercial food products can be detected by it.

Grain samples of common commercial wheat (*Triticum aestivum* L.) belonging to four classes, including Canada western red spring wheat, Australian standard wheat, Western white wheat, and domestic wheat (Norin 61), and Canadian amber durum wheat (*Triticum durum* L.) were collected in our laboratory. Grain samples of rye, barley, oats, rice, maize, soybean, foxtail millet, rapeseed, and buckwheat were also purchased. Nine different commercial food products with wheat in their lists of ingredients were purchased from a local market in Saitama.

The grain materials of wheat, 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 PCR. Maize was used as the matrix to prepare model mixed samples. Maize grains were ground to a fine powder with a grinder, and 0, 0.0001, 0.001, 0.005, 0.01, 0.1, 1, and 100% of wheat flour (Australian standard wheat) was then admixed according to the procedure described in a previous study, with a slight modification.15) Genomic DNA was extracted from each plant material, including model mixed samples, using a silica-gel membrane-type kit (DNeasy Plant Mini; Qiagen, Hilden, Germany). A 200-mg sample of ground sample was incubated for 15 min at 65 °C with the addition of 1.5 ml of buffer AP1 and 10 μl of RNase A, and then 400 μl of buffer AP2 was added to the mixture, which was cooled on ice for 5 min. Then the following steps were carried out according to previously described methods:16) Genomic DNA was extracted from commercial food products using an anion exchange-type kit (Genomic-tip...
20/G; Qiagen, Hilden, Germany) according to the procedure described in a previous study. 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 for subsequent PCR analysis. The primers were synthesized and purified in 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 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), all 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 condition was 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 eletrophoresis of the PCR product was carried out following previous studies. The amount of wheat protein in the commercial food products was measured using a sandwich ELISA kit for wheat (Morinaga, Yokohama, Japan) according to the procedure described in a previous study. To detect wheat specifically by PCR, we investigated wheat-specific genes such as the triticum aestivum triticin precursor gene and the triticum aestivum glutathione S-transferase gene. Based on these wheat-specific gene sequences, we designed the respective primer pairs and examined PCR amplification using them on the mixed samples.

Consequently, the triticum aestivum triticin precursor gene proved to be more sensitive and specific than the glutathione S-transferase gene and others for amplifying the gene sequence. Triticum aestivum triticin is the storage protein and is synthesized specifically during seed development. Hence, we chose the gene encoding the triticum aestivum triticin precursor as the wheat-specific gene and designed the primer pair Wtr 01-5'/Wtr 10-3' on the coding region of that gene by referring to GeneBank accession no. S62630. 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. This primer pair generated a 123-bp amplified fragment. The sequences of the designed oligonucleotides in this study are listed in Table 1.

Table 1. List of Designed Primers

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence (5’→3’)</th>
<th>Specificity</th>
<th>Amplicon</th>
</tr>
</thead>
<tbody>
<tr>
<td>A:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CP 03-5'</td>
<td>5’-CGG AGC AGA ATA AAG ATA GAG T-3'</td>
<td>Chloroplast DNA, sense</td>
<td>Plants</td>
</tr>
<tr>
<td>CP 03-3'</td>
<td>5’-TTT TGG GGA TAG AGG GAC TTG A-3'</td>
<td>Chloroplast DNA, antisense</td>
<td></td>
</tr>
<tr>
<td>B:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wtr 01-5'</td>
<td>5’-CAT CAC AATCAA CTT ATG GTG G-3'</td>
<td>Triticum aestivum triticin precursor, sense</td>
<td>Plants</td>
</tr>
<tr>
<td>Wtr 10-3'</td>
<td>5’-TTT GGG AGT TGA GAC GGG TTA-3'</td>
<td>Triticum aestivum triticin precursor, antisense</td>
<td>Wheat</td>
</tr>
</tbody>
</table>

A. To confirm the validity of DNA extracted from plants for the polymerase chain reaction.
B. For specific detection of wheat.

One to 32 μg genomic DNA from each 200 mg of grain samples was obtained by the purification method described above. As shown in Fig. 1A, a fragment (141 bp) amplified using the primer pair Wtr 01-5'/Wtr 10-3' was specifically detected from common wheat and durum wheat genomic DNA. By contrast, no amplified fragment was detected when DNA was extracted from nine other plant species (rye, barley, oats, rice, maize, soybean, foxtail millet, rapeseed, and buckwheat) as the template DNA. Nucleotide sequence analysis of the PCR product obtained from common wheat and durum wheat confirmed that the intended sequence of the triticum aestivum triticin precursor gene had been amplified. These data suggest that wheat-genomic DNA can be specifically detected using the Wtr 01-5'/Wtr 10-3' primer pair.

To assess the sensitivity of the proposed PCR method, we tested the mixed maize flour samples containing 0, 0.0001, 0.001, 0.005, 0.01, 0.1, 1, and 100% of wheat 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 wheat was clearly detected in the 0.005% to 100% mixed samples (Fig. 1B). This result suggests that wheat flour contamination of as low as 0.005% can be detected in an unprocessed food. To investigate the applicability of the wheat DNA detection method to commercial food products, we purchased nine food items with wheat in their lists of ingredients and tested them for the presence of wheat DNA using the proposed method. A sufficient amount (more than 20 ng/μl) of genomic DNA for PCR was obtained from all the food products, except for retort soup and sauce, using the described purification method. As shown in Fig. 1C, the universal primer pair CP 03-5'/CP 03-3' generated a specific amplified...
fragment from all of the samples. In the nine food products, except for the retort sauce, wheat DNA was clearly detected by the PCR method using the Wtr 01-5/Wtr 10-3 primer pair (Fig. 1C and Table 2). Based on these results, we postulate that the absence of amplified PCR products from the retort sauce was probably due to the limited amount of wheat-derived genomic DNA in it. The wheat protein in retort sauce was also not detected by the ELISA method, and that result appears to be consistent with that for the present PCR method (Table 2). These results suggest that the proposed PCR method using the Wtr 01-3/Wtr 10-5’ primer pair is applicable in identifying wheat in processed food products, except for those that are highly processed, and that the sensitivity of the PCR method appears to be similar to that of the ELISA method.

In summary, a rapid PCR detection method was

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

<table>
<thead>
<tr>
<th>No.</th>
<th>Food item</th>
<th>Protein-ELISA method</th>
<th>PCR method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Protein conc. (ppm)</td>
<td>Result</td>
</tr>
<tr>
<td>1</td>
<td>Fish meat paste</td>
<td>0.4</td>
<td>Positive</td>
</tr>
<tr>
<td>2</td>
<td>Pork sausage</td>
<td>10.4</td>
<td>Positive</td>
</tr>
<tr>
<td>3</td>
<td>Snacks</td>
<td>1.0</td>
<td>Positive</td>
</tr>
<tr>
<td>4</td>
<td>Chocolate</td>
<td>7.2</td>
<td>Positive</td>
</tr>
<tr>
<td>5</td>
<td>Cookies</td>
<td>&gt;12.8</td>
<td>Positive</td>
</tr>
<tr>
<td>6</td>
<td>Cracker</td>
<td>&gt;12.8</td>
<td>Positive</td>
</tr>
<tr>
<td>7</td>
<td>Instant Chinese noodle</td>
<td>&gt;12.8</td>
<td>Positive</td>
</tr>
<tr>
<td>8</td>
<td>Retort soup</td>
<td>8.5</td>
<td>Positive</td>
</tr>
<tr>
<td>9</td>
<td>Retort sauce</td>
<td>nd</td>
<td>Negative</td>
</tr>
</tbody>
</table>

nd, not detected; ELISA, enzyme-linked immunosorbent assay; PCR, polymerase chain reaction.
developed for specific detection of wheat. The methods reported in this study are simple, sensitive, and reliable in identifying trace amounts of wheat in processed foods. To minimize the risk of false negative or positive results, we believe that the PCR method can be used to complement the protein-based detection method.20)

Acknowledgments

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References