DNA Profiling of Fresh and Processed Fruits in Pear

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A DNA profiling method was developed for fresh and processed fruits in pear. Four different DNA extraction methods were examined to isolate genomic DNAs from fresh pear fruits, i.e., CTAB-based method and methods based on 3 kinds of DNA extraction kits (DNeasy Plant Mini Kit, G2 buffer & Genomic-tip20/G, Nucleon Phytopure Plant DNA Extraction Kit). All the methods enabled to recover genomic DNAs from samples of fresh fruits with such a high quality that cultivar identification could be performed based on SSR markers. Among them, the G2 buffer & Genomic-tip20/G method gave the best results for samples of fresh as well as processed fruits. Partially degraded genomic DNAs that were isolated from samples of dried fruits could be amplified by all the tested SSR markers. SSR analysis revealed that genotypes from dried fruits were identical with those of the European pear cultivar ‘Bartlett’, indicating that cultivar identification could be successfully performed. Severely degraded genomic DNAs less than 500 bp in size were recovered from samples of canned fruits and fruit juice. The amount and quality of the extracted DNAs were sufficient to enable amplification by primers corresponding to high copy rDNA sequences, whereas no bands were produced by primers of chloroplast DNA sequences. Out of 15 SSR loci, 9 SSRs with target sequences less than 150–160 bp could successfully amplify fragments for genomic DNAs from samples of canned fruits and fruit juice. In contrast, no amplified fragments were observed for the remaining 6 SSRs with longer target sequences. DNA profiling and cultivar identification were successfully performed by using SSR markers to amplify short target sequences less than 150 bp.

Key Words: cultivar identification, genomic DNA, processed fruits, SSR analysis.

Introduction

DNA markers have become powerful tools that are utilized for cultivar identification, parentage analysis, evaluation of genetic diversity and construction of genetic linkage maps. Several kinds of DNA markers have been developed for fruit tree species, i.e., restriction fragment length polymorphism (RFLP), randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), single nucleotide polymorphism (SNP) and simple sequence repeats (SSRs, also designated as microsatellites). Among them, SSRs have become the markers of choice in plant species because of their abundance, high degree of polymorphism, co-dominant inheritance and suitability for automation (Weber and May, 1989). A large number of SSR markers have been developed in pear (Yamamoto et al. 2002a, 2002b, 2002c) and major fruit trees such as apple (Gianfranceschi et al. 1998, Guilford et al. 1997, Liebhard et al. 2002), grapevine (Thomas and Scott 1993, Bowers et al. 1996), peach (Cipriani et al. 1999, Testolin et al. 2000, Yamamoto et al. 2002d), etc. DNA profiling has been conducted with genomic DNAs extracted from leaves in fruit tree species. However, there are very few applications for DNAs obtained from fresh and processed fruits.

Juicy fruits are mainly edible tissues for most fruit tree species. In addition to fresh fruits for table use, several kinds of processed fruits such as dried and canned fruits, pressed fruit juice, fruit jam and liquor are popularly consumed. It is considered that the identification of fresh and processed fruits is necessary because the customers have the right to be informed about the products they buy and consume. However, processed fruits usually do not retain sufficiently distinctive morphological features for identification. Therefore, it is necessary to identify the fruit cultivar used for processing by the application of DNA fingerprinting techniques.

It was reported that DNA analysis was sometimes associated with problems for processed foods such as low amount of extracted DNAs and severely degraded DNAs. Goda et al. (2001) described that a silica membrane type kit such as DNeasy plant mini kit (Qiagen) was not useful for DNA extraction from samples of canned papaya fruits. Although ion-exchange type kits provided sufficient amounts of purified DNAs from samples of canned papaya fruits, it was necessary to select suitable primer combinations to amplify the transgenes. Attempts were made to apply DNA profiling methods to identify grapevine cultivars in must and wine using SSR markers (Faria et al. 2000, Siret et al. 2000, Garcia-Beneytez et al. 2002). Cultivar identification was...
partially successful with genomic DNAs recovered from grape must and wine. However, there were some problems, such as low concentration of extracted DNAs, low reproducibility of DNA analysis, difficulty to identify cultivars in must mixtures and difficulty to analyze commercial wines. Similarly, authentication of canned seafood was sometimes difficult because DNAs were severely degraded during the canning process of fish muscle (Ram et al. 1996, Quinteiro et al. 1998). Combination of extraction methods and DNA markers has not yet been adequately developed for processed foods and further trials as well as improvements will be necessary.

In our previous studies, SSR markers were developed in pear and genetic linkage maps were constructed for Japanese and European pears (Yamamoto et al. 2002a, 2002b, 2002c). Genetic identification of Asian pear varieties was successfully evaluated by using the developed SSR markers (Kimura et al. 2002). Parentage of 14 pear cultivars was confirmed by using 20 SSR loci (Kimura et al. 2003a). Thus, DNA profiling systems, i.e., cultivar differentiation, cultivar identification and parentage analysis, have been developed using SSR markers in pear. It is considered that the developed DNA profiling methods could be applied to fresh and processed fruits in pear.

In the present study, we evaluated several DNA extraction methods for fresh pear fruits. And then DNA extraction and analysis were conducted using samples of processed fruits, i.e., canned and dried fruits, and fruit juice in pear. The relationship between the quality of the DNAs obtained and SSR analysis was examined.

Materials and Methods

Materials

Fresh fruits were used for 5 pear cultivars, including 3 Japanese pear (Pyrus pyrifolia Nakai) cultivars ‘Chojuro’, ‘Housui’ and ‘Okusankichi’, the Chinese pear (P. bretschneideri Rehd.) ‘Yali’ and the European pear (P. communis L.) ‘Bartlett’. All the samples were obtained from the National Institute of Fruit Tree Science (Ibaraki, Japan).

Samples of 3 types of processed pear fruits were used in the present study, i.e., canned pear fruits labeled as “Bartlett Pear” (Sembikiya Inc.), dried pear fruits labeled as “The Produce Stand” (Safeway Inc.) and pressed 100% juice labeled as “La France” (Katoh Bussan KS Inc.).

DNA extraction method

Fresh fruits of ‘Housui’ without skin were cut into small fragments of 0.1–5 g. These samples were stored at −30°C until use. The sliced fruit tissues were homogenized in liquid nitrogen. Genomic DNA was extracted from the resulting tissue powder by the following methods; modified CTAB method (abbreviated as CTAB, Yamamoto et al. 2001), G2 Buffer and Genomic-tip20/G method (abbreviated as Genomic-tip, Qiagen), DNeasy Plant Mini Kit method (abbreviated as DNeasy, Qiagen) and Nucleon Phytopure Plant DNA Extraction Kit method (abbreviated as Phyto-pure, Amersham Pharmacia Biotech.), according to the manufacturers’ instructions.

In the CTAB method, the homogenized tissue powder was suspended in 10 ml of extraction buffer (2% CTAB, 1.4 M NaCl, 20 mM EDTA and 0.1 M Tris-HCl, pH 8), 1 ml of lysis buffer (10% sodium N-lauroyl sarcosinate, 20 mM EDTA and 0.1 M Tris-HCl, pH 8), 0.2 ml of 2-mercaptoethanol and 100 mg of polyclar AT (insoluble polyvinyl pyrrolidone, GAF Chemicals), and then incubated at 60°C for 1 hour. The suspension was purified twice in a chloroform: isooamyl alcohol (24:1) solution and precipitated with ice-cold 2-propanol. The recovered DNA was dissolved in 100 µl of sterilized milli-Q water.

In the Genomic-tip method, the tissue powder was suspended in 10–15 ml of G2 extraction buffer (0.8 M guanidine-HCl, 30 mM EDTA, 30 mM Tris-HCl, pH 8, 5% Tween-20 and 0.5% Triton X-100), 4 µl of RNase solution (100 mg/ml), 0.2 ml of 2-mercaptoethanol and 100 mg of polyclar AT, and then incubated at 50°C for 2 hours. The suspension solution was added to Genomic-tip20/G and washed twice with 2 ml of QC buffer. Genomic DNA was eluted with 1 ml of QF buffer and precipitated with ice-cold 2-propanol. The recovered DNA was dissolved in 100 µl of sterilized milli-Q water.

In the DNeasy method, the tissue powder was suspended in 400 µl of AP1 lysis buffer, 4 µl of RNase solution (100 mg/ml), 8 µl of 2-mercaptoethanol and 4 mg of polyclar AT, and then incubated at 65°C for 10 minutes. AP2 buffer was added to the solution and the conjugate was applied to the QIA shredder spin column. After centrifugation, the flow-through fraction was recovered. AP3 buffer was added to the suspension and then applied to the DNeasy Mini Spin Column. After washing with AW buffer, genomic DNA was eluted with 50 µl of sterilized milli-Q water.

In the Phytopure method, the tissue powder was suspended in 600 µl of Reagent 1. After mixing, 200 µl of Reagent 2 was added to the suspension and incubated at 65°C. The solution was mixed with 500 µl of chloroform (~20°C) and 100 µl of extraction resin buffer. After centrifugation, the upper phase containing DNA was transferred to a new tube and an equal volume of ice-cold 2-propanol was added. The precipitated genomic DNA was recovered and dissolved in 50 µl of sterilized milli-Q water.

DNA extraction from samples of processed fruits

Canned pear fruits were cut into dice and lyophilized. Tissues (1–40 g of fresh weight) were homogenized in liquid nitrogen. Genomic DNA was extracted from the homogenized canned fruits by using the Genomic-tip method. Dried pear fruits (1–5 g) were cut into dice and homogenized in liquid nitrogen. Genomic DNA was also extracted by using the Genomic-tip method.

Pressed 100% fruit juice (5–90 ml) was centrifuged at 7,000 g for 20 min at room temperature. After centrifugation, the supernatant was transferred to a new tube and then
0.6 volume of 2-propanol was added. After gentle mixing, visible fibers were obtained. The precipitated DNA was recovered by centrifugation at 7,000 × g for 20 min at room temperature. Genomic DNA was extracted from the precipitate by using the Genomic-tip method.

**Evaluation of extracted DNAs**

Genomic DNAs extracted from samples of fresh fruits, canned fruits, dried fruits and fruit juice were evaluated by electrophoresis. The extracted DNAs were electrophoresed on 1–2% agarose gel and stained with ethidium bromide. Gel files were compared with lambda DNA under UV light. The amount of genomic DNAs was calculated based on absorbance at 260 and 280 nm using a spectrophotometer DU650 (Beckman).

Ribosomal DNA sequences of the NS5-NS6 region were used to evaluate the DNAs extracted from samples of dried fruits, canned fruits and fruit juice for PCR amplification (White et al. 1990). Two chloroplast DNA (hereafter referred to as cpDNA) regions were also used to evaluate DNAs for PCR amplification, i.e., atpB-rbcL (intergenic spacer between ATPase B subunit and ribulose 1,5-bisphosphate carboxylase/oxygenase large subunit genes) and trnL-trnF (intergenic spacer between tRNA-Leu and tRNA-Phe genes) (Taberlet et al. 1991; Small et al. 1998, Kimura et al. 2003b).

PCR amplification was performed in a 20 µl solution consisting of 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 0.01% gelatin, 0.2 mM each of dNTPs, 10 pmol of each primer and 0.5 unit of Taq polymerase (Invitrogen), and 1 µl of extracted DNA solution as template. PCR reaction was conducted to amplify the NS5-NS6, atpB-rbcL and trnL-F regions under the conditions described by Kimura et al. (2003b). PCR products were electrophoresed on 2–3% agarose gel.

**SSR analysis**

Fifteen SSR markers KA14, KA16, KU10, BGA35, BGT23b, NB103a, NH001c, NH002b, NH004a, NH005b, NH007b, NH009b, NH011b, NH014a and NH015a, were used to identify fresh and processed fruits (Yamamoto et al. 2002a, 2002b, 2002c). SSR-PCR amplification was performed in a 20 µl solution of 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 0.01% gelatin, 0.2 mM of each dNTP, 10 pmol of each forward primer labeled with a fluorescent chemical (Fam/Tet/Hex or Fam/Vic/Ned) and unlabelled reverse primer, 1 µl of extracted DNA solution as template, and 0.5 unit of Taq polymerase (Invitrogen). Amplification was conducted for the 15 SSR loci as described by Yamamoto et al. (2002a, 2002b, 2002c). The PCR products were separated and detected using a PRISM 377 DNA sequencer (PE Applied Biosystems). The size of the amplified bands was calculated based on an internal standard DNA (GeneScan-350TAMRA, PE Applied Biosystems) with GeneScan software (PE Applied Biosystems).

**Results**

**DNA extraction from samples of fresh fruits in pear**

Genomic DNAs were extracted from samples of fresh fruits of ‘Housui’ by using 4 different methods, i.e., CTAB, Genomic-tip, DNeasy and Phytopure (Fig. 1). DNA bands with a high molecular weight could be obtained by all the extraction methods. The amount of genomic DNAs was measured by absorbance at 260 nm and 4.7, 6.8, 1.6 and 3.2 µg of genomic DNAs were obtained from 1 g of fresh fruits by the CTAB, Genomic-tip, DNeasy and Phytopure methods, respectively (Table 1). The amount of genomic DNAs obtained by Genomic-tip was 1.5 to 4.5 times larger than that by the other extraction methods, whereas the amount was 0.04 times that of genomic DNAs extracted from young leaves.

Genomic DNAs extracted by Genomic-tip method from samples of fresh fruits of the other pear cultivars ‘Chojuro’, ‘Okusankichi’, ‘Yali’ and ‘Bartlett’, also showed a high molecular weight and the amount was approximately the same as that of ‘Housui’ (data not shown). Therefore, it was considered that the Genomic-tip method was the most efficient method to extract DNAs from samples of fresh fruits.

**DNA extraction from samples of processed fruits**

In our preliminary experiments, the use of the Genomic-tip method had enabled to recover a larger amount of

![Fig. 1. Genomic DNAs extracted from fresh fruit samples of pear cultivar ‘Housui’. Lanes 1–6 show the DNAs of the following samples: 1, 100 ng standard lambda DNA; 2, young leaves; 3, fresh fruits using the CTAB method; 4, fresh fruits using the DNeasy method; 5, fresh fruits using the Genomic-tip method; 6, fresh fruits using the Phytopure method.](image)

| Table 1. DNA extraction from samples of fresh and processed fruits in pear |
|-----------------------------|-----------------|----------------|
| Sample                      | DNA extraction method | Amount of extracted genomic DNA (µgDNA/g-sample) |
| Fresh fruits                | CTAB             | 4.7            |
|                            | DNeasy           | 1.6            |
|                            | Genomic-tip      | 6.8            |
|                            | Phytopure        | 3.2            |
| Dried fruits                | Genomic-tip      | 5.2            |
| Canned fruits               | Genomic-tip      | 1.9            |
| Pressed fruit juice         | Genomic-tip      | 1.6            |
| Leaves                      | Genomic-tip      | 164            |
genomic DNAs with a higher quality compared with the other methods for extraction from processed fruits (data not shown). The Genomic-tip method was used for further analysis of samples of dried, canned fruits and fruit juice. Smeared genomic DNAs, which seemed to be partially degraded (Fig. 2), were obtained from samples of dried fruits. High molecular weight DNAs more than 5 kbp in size could be observed, even if the ratio was very low. The amount of extracted DNAs measured with a spectrophotometer was 5.2 µg per 1 g of dried fruits.

In canned fruits, low molecular weight DNAs less than 500 bp in size, which appeared to be severely degraded (Fig. 2), were isolated from 5–40 g of samples. The amount of extracted DNAs was 1.9 µg per 1 g of canned fruits. The maximum value of absorbance was observed at around 260 nm and the ratio of absorbance at 260/280 nm was 1.8–2.0, suggesting that the quality of the extracted DNAs was satisfactory.

Genomic DNAs could be recovered from 15–90 g of fruit juice. Although the obtained DNAs less than 500 bp in size showed a low molecular weight (Fig. 2), estimation using a spectrophotometer suggested that the quality was rather high. The amount of extracted DNAs was 1.6 µg per 1 g of fruit juice.

**Evaluation of extracted DNAs**

PCR amplification was tested by using 3 sets of primers to amplify high copy sequences, i.e., the NS5-NS6 rDNA region and the *atpB-rbcL* and *trnL-F* cpDNA regions in order to evaluate the extracted DNAs from samples of fresh, dried, canned fruits and fruit juice. One discrete fragment of ca. 300 bp in size was obtained for amplification in the NS5-NS6 region using genomic DNAs from all the samples (Fig. 3b). Amplified fragments from samples of fresh and processed fruits showed the same size as that from leaf samples of ‘Housui’ and ‘Bartlett’ (Fig. 3b). However, the amplified bands from the samples of canned fruits and fruit juice were faint compared with those of the other samples, suggesting that the quantity and/or quality of the extracted DNAs were low. These results indicated that the size and quality of the genomic DNAs from samples of fresh, dried, canned fruits and fruit juice were sufficient for amplification of the NS5-NS6 sequences.

Amplified fragments ca. 500 bp in size were detected using genomic DNAs obtained from samples of fresh and dried fruits for *trnL-F* amplification, whereas no bands were observed for DNAs from samples of canned fruits and fruit juice (Fig. 3a). Since the nucleotide sequences in the *trnL-F* cpDNA regions were determined in ‘Housui’ and ‘Bartlett’ and their lengths were 477 and 485 bp, respectively (Kimura et al., 2003b), the target sequences were amplified from samples of fresh and dried pear fruits. In contrast, no products could be obtained from samples of canned fruits and fruit juice, suggesting that the size and quality of their DNAs were not satisfactory for amplification.

In the amplification of the *atpB-rbcL* sequences, the target PCR products were detected with DNAs obtained from samples of fresh and dried fruits, whose size of ca. 800 bp was the same as that of DNAs from leaf samples of ‘Housui’ and ‘Bartlett’. However, PCR products could not be obtained from samples of canned fruits and fruit juice.
SSR analysis

Fifteen SSR markers were used for the profiling of genomic DNAs from samples of fresh and processed fruits. All the SSR markers could successfully produce 1 or 2 discrete amplified bands for fresh fruits of the 5 pear cultivars (Fig. 4). SSR genotypes obtained from fresh fruits of ‘Housui’ by using the 4 extraction methods were identical with those from the leaves of ‘Housui’. Similarly, SSR analysis revealed that the genotypes obtained from fresh fruits were identical with those from the leaves for the other 4 cultivars ‘Chojuro’, ‘Okusankichi’, ‘Yali’ and ‘Bartlett’ (data not shown). Since the SSR markers used in the present study could be utilized for cultivar differentiation and parentage analysis in pear (Kimura et al. 2002, 2003a), it was concluded that cultivar identification could be successfully conducted by SSR analysis with genomic DNAs extracted from samples of fresh fruits.

Amplified SSR fragments were obtained from genomic DNAs from samples of dried fruits at the tested 15 SSR loci (Fig. 4, lane 7). All the SSR genotypes from dried fruits were identical with those from the leaves of ‘Bartlett’ at all the SSR loci among dozens of European pear cultivars previously identified (Kimura et al. 2002, Yamamoto et al. 2002a, Yamamoto et al. 2004). Although the cultivar name was not indicated, it was suggested that fruits of ‘Bartlett’ were used for the production of the examined dried fruits. It was revealed that the quality of the genomic DNAs extracted from the samples of dried fruits was sufficiently high to enable the analysis by SSR markers and the identification of the names of the cultivars used as production materials.

In the samples of canned fruits and fruit juice, 9 SSR markers (KA16, BGA35, NB103a, NH001c, NH004a, NH007b, NH009b, NH014a and NH015a) could successfully produce 1 or 2 reproducible amplified bands (Table 2). The other 6 SSR markers (NH011b, NH002b, KA14, BGT23b, KU10 and NH005b) did not produce amplified bands (Table 2). The size of the amplified bands by the 9 SSRs was less than 150–160 bp, ranging from 70 bp for NH014a to 158 bp for NH001c. In contrast, amplification by the 6 SSR markers was not observed for the samples of canned fruits and fruit juice, and the markers produced 160 to 350 bp fragments when DNAs extracted from samples of young leaves were used as templates.

By comparing the SSR genotypes at 9 SSR loci obtained from samples of canned fruits with those of major European pears (Kimura et al. 2002, Yamamoto et al. 2002a, Yamamoto et al. 2004), the ‘Bartlett’ genotype was identified (Table 2). Therefore, it was considered that ‘Bartlett’ pears were actually used for the production of the canned fruits, as indicated in the “Bartlett Pear” label. Similarly, SSR genotypes from fruit juice samples were identical with those of ‘La France’ pears, suggesting that the fruit juice was produced from ‘La France’ pears, as indicated in the “100% juice of La France” label. These results confirmed that SSR markers with rather short target sequences could be utilized for cultivar identification in the case of genomic DNAs extracted from samples of canned fruits and fruit juice.

Discussion

DNA extraction is sometimes difficult in samples of woody plants such as pear, apple, grape, conifer, etc. due to

<table>
<thead>
<tr>
<th>SSR marker</th>
<th>Canned pear fruits</th>
<th>Bartlett (leaves)</th>
<th>Pressed fruit juice</th>
<th>La France (leaves)</th>
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<tr>
<td>NH014a</td>
<td>70/72</td>
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<td>72/84</td>
<td>72/84</td>
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<td>90/96</td>
<td>90/96</td>
<td>90/111</td>
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<td>103/123</td>
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<td>107/119</td>
<td>106/119</td>
<td>106/119</td>
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<tr>
<td>NH001c</td>
<td>122/158</td>
<td>122/158</td>
<td>100/104</td>
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<td>122/124</td>
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<tr>
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nd indicates no detection.
the degradation of DNAs mediated by phenolic terpenoids, polysaccharides and other secondary metabolites (Kim et al. 1997). It is rather difficult to obtain high quality DNAs from juicy fruits and old leaves in fruit tree species, whereas a large amount of genomic DNAs with a high quality can be isolated from young leaves. In the present study, 4 different DNA extraction methods were evaluated for the isolation of genomic DNAs from samples of fresh pear fruits, including the highly popular CTAB-based extraction method and 3 methods based on DNA extraction kits. Among them, the Genomic-tip method using G2 buffer and Genomic-tip20/G yielded a larger amount of genomic DNAs than the other methods. In our study, the Genomic-tip method also showed good results for processed fruits. Goda et al. (2001) reported that the use of an ion-exchange type column such as that in the Genomic-tip method led to a sufficient and satisfactory quality of DNAs in papaya fruits, especially for processed fruits. It was, therefore, indicated that the Genomic-tip method was a suitable extraction method for genomic DNAs from samples of fresh and processed fruits in pear.

Quality of extracted genomic DNAs from samples of fresh and processed fruits was evaluated by electrophoresis, spectrophotometry and PCR analysis of high copy sequences. In the case of genomic DNAs from fresh and dried fruits, high molecular weight DNAs more than 5 kbp in length were observed by electrophoresis, which could be successfully used for cultivar identification by SSR markers. On the other hand, PCR products were not obtained for the cpDNA regions *atpB-rbcL* and *rrnL-F*, but were generated for the rDNA region NS5-NS6, using genomic DNAs from samples of canned fruits and fruit juice. Since the number of chloroplasts (plastids) is very small in mature fruit tissues and genomic DNAs of canned fruits and fruit juice were severely degraded, evaluation by electrophoresis and PCR of a rDNA region was suitable for these processed fruits.

DNA profiling of samples of canned seafood was sometimes difficult because DNAs were severely degraded during the canning process of fish muscle (Ram et al. 1996, Quinteiro et al. 1998). Ram et al. (1996) reported that DNAs of samples of canned tuna and bonito were degraded during the canning process and that the PCR primers designed to amplify DNAs less than 123 bp in size could generate target fragments in mitochondrial sequences. Quinteiro et al. (1998) observed that severely degraded DNAs ranging from 100 to 200 bp in size were extracted from samples of canned tuna muscle, whose species could be identified based on the 126 bp nucleotide sequences of the mitochondrial cytochrome b gene. The transgenes could be amplified from samples of canned papaya fruits by using a combination of DNA extraction by ion-exchange column and PCR primers for short fragments of 151 to 211 bp (Goda et al. 2001). The results we obtained for the samples of canned pear fruits were in good agreement with these descriptions. Our results indicated that DNA extraction methods leading to high quality DNAs and PCR amplification for short target sequences are indispensable to conduct DNA profiling, when using samples of canned fruits and fruit juice.

Heat treatments exceeding 90–95°C are commonly applied at several stages, e.g., processing and sterilization, to produce canned fruits and fruit juice (Sato et al. 1991). In the case of canned fruits, fruit tissues are stored in sugar solutions with a high concentration for more than several months. It is considered that heat treatments and storage in aqueous solutions result in the breaking of DNAs into small fragments. Although detailed manufacturing process of processed fruits is a company secret in most cases, it is necessary to analyze DNAs in fruit tissues at several processing and storage stages.

**Acknowledgements**

Contribution No. 1403 of the National Institute of Fruit Tree Science. The authors are grateful to Drs. H. Iketani, N. Matsuta, J. Soejima, K. Kotobuki and C. Nishitani for their valuable suggestions. They also thank M. Osono, T. Imai, I. Nakajima and T. Iida for their useful discussions and technical assistance.

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