Species Identification of Poultry Egg Products

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ABSTRACT Species-specific primers for duck were deduced from the mitochondrial ATPase8 gene sequence. Species-specific PCR for turkeys and ducks showed no cross reaction with mixtures from chicken and guinea eggs and detection was possible to a concentration of 0.1% of homogenized duck egg and 5% of homogenized turkey egg when a PCR of 35 cycles was applied. A PCR of 30 cycles detected 10% of homogenized duck egg. The same sensitivities were obtained in dilutions of homogenized egg yolk; however, no PCR signals were obtained in egg white. Analysis of 13 industrial egg product samples showed the practical relevance of the species-specific PCR tests described herein.

(Key words: egg products, PCR, species identification)

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INTRODUCTION

Checking food products for accurate labeling is an integral part of food regulatory control with respect to economic fraudulence (e.g., selling cheaper products at higher prices). Egg products are mainly produced from chicken, turkey, and duck eggs. Mixtures of chicken eggs with eggs from turkeys and ducks are sometimes sold as pure chicken egg products. However, because of a lack of adequate analytical methods, these fraudulent operations cannot be easily proved.

Analytical methods for identification of some poultry species in meat products are available and based on protein analysis by electrophoresis (Kim and Shelef, 1986), liquid chromatography (Ashoor et al., 1998), DNA analysis by dot blot hybridization (Ebbehoj and Thomsen, 1991), randomly amplified polymorphic DNA PCR (Calvo et al., 2001), RFLP analysis of mitochondrial DNA (Bellagamba et al., 2001), and species-specific PCR (Herman, 2001). The advantage of protein-based methods is the possibility of obtaining (semi) quantitative results. Randomly amplified polymorphic DNA PCR generates fingerprint patterns on the total DNA derived from the different ingredients mixed in the products leading to complex fingerprints when complex mixtures are analyzed. Species-specific PCR on the other hand has the potential to reach higher detection sensitivity and specificity compared with protein-based methods and randomly amplified polymorphic DNA fingerprinting.

For species identification, a specific PCR can be developed by targeting a DNA segment with sufficient species-to-species variation. Mitochondrially encoded genes such as the ATPase subunit 8 and subunit 6 (Tartaglia et al., 1998) and the cytochrome 6 gene are studied for this purpose (Kocher et al., 1989, Herman, 2001). Herman (2001) published species-specific primers for chickens and turkeys based on cytochrome b sequence variations.

In this report, the ATPase subunit 8 gene is used to develop species-specific primers for identification of ducks. Species-specific PCR for chickens, turkeys (Herman, 2001), and ducks are subsequently applied for the origin identification of egg products.

MATERIALS AND METHODS

Samples and DNA Extraction

Whole chicken eggs and chicken and duck meat were purchased from a retail shop. Industrial egg product samples and eggs from ducks, turkeys, and guineas were obtained from food inspectors. DNA was extracted by the DNeasy protocol provided with the DNeasy tissue kit. Extraction was performed on 100 µL of mixed whole eggs, egg white, and yolk; for meat, an internally located 25-mg portion of pure meat without fat and connective tissue was used; for egg powder, a 30-mg sample was hydrated with 70 µL of H2O before extraction. Mixing of eggs was performed by whirlmixing for 1 min followed by pipetting 20 times.
DNA Sequencing and Analysis

Sequences were performed using the ABI PRISM Dye Terminator Cycle Sequencing Kit on amplified DNA after purification with the High Pure PCR Product Purification kit. The forward PCR primers were used as sequencing primers. Results were analyzed on an ABI 310 Genetic Analyzer. Sequence alignment was made with the Kodon software package.

PCR Amplification

Polymerase chain reaction was performed in a final volume of 50 µL containing 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM MgCl2, 200 µM of each dNTP, 1.5 U of AmpliTaq DNA polymerase, 50 pmol of each primer, and 1 µL of DNA. The mixture was subjected to 30 or 35 cycles of amplification in a thermal cycler (Cetus 9600). The first cycle was preceded by denaturation for 1 min at 95°C. Each cycle consisted of denaturation for 15 s at 95°C, annealing for 30 s at 56, 67, or 63°C for duck-, chicken-, or turkey-specific primers, respectively, and elongation for 30 s at 72°C. A final elongation for 8 min at 72°C followed the last cycle. The PCR products were analyzed on a 1.5% (wt/vol) Seakem ME agarose gel.

Primers for the identification of chicken DNA (forward 5’ CGGTGGGCTATGAGTGTGAGG 3’ and reverse 5’ AGCACCTGCTCATG 3’) and turkey DNA (forward 5’ TAGAGGTTGAGAATTA 3’ and reverse 5’ TAGCATTGCTCTACACT 3’) were derived from Herman (2001). Primers for the discrimination of duck DNA are described in this work.

Determination of PCR Sensitivity in Mixed Eggs

An egg from each species (chicken, turkey, duck, and guinea) was broken and shaken. From a second egg of each species, egg white and yolk were separated and mixed as previously described. Dilutions of chicken egg, egg white, and yolk with 10, 5, 1, 0.1, and 0.01% of turkey, duck, and guinea egg, egg white, and yolk were made. DNA was extracted from 100 µL of each of these mixtures.

RESULTS AND DISCUSSION

Development of a Species-Specific PCR for Duck

The mitochondrial ATPase8 gene sequence of chicken and duck (GenBank Accession numbers X52392 and L22476, respectively) were aligned and primers were deduced which allow discrimination of duck from chicken (Figure 1). The primer pair Duck F1-Duck R1 was tested for specific identification of duck DNA. No cross-reaction with DNA isolated from meat samples of chicken, turkey, guinea, cow, pig, sheep, and goat were obtained. Duck DNA was diluted in a mixture of 50 ng of chicken, turkey, and guinea DNA and a specific reaction was obtained to a concentration of 5 ng of duck DNA in the PCR. For the chicken and turkey specific PCR, sensitivity to a concentration of 0.05 ng was obtained (Herman, 2001).

Origin Identification of Eggs and Egg Products by Species-Specific PCR

Homogenized eggs from ducks, turkeys, and guineas were diluted in a homogenized chicken egg. The experiment was performed in triplicate. The extracted DNA could not be measured spectrophotometrically nor could it be visualized by agarose gel electrophoresis. Species-specific PCR for turkey and duck showed no cross-reaction with mixtures from chicken and guinea eggs and detection was possible to a concentration of 0.1% of homogenized duck egg and 5% of homogenized turkey egg when a PCR of 35 cycles was applied. A PCR signal was obtained after 30 cycles in samples containing 10% of homogenized duck egg. The same PCR sensitivities were obtained in dilutions of homogenized egg yolk. In egg white, no PCR signals were obtained although PCR was positive when 250 ng of pure DNA was added. This indicates that the negative PCR reaction is not due to PCR inhibition but due to the absence of a low concentration of DNA in egg white.

It is clear that duck DNA could be more sensitively detected in the same amount of mixed egg compared with turkey DNA. Because of the even lower PCR sensitivity of the duck PCR on pure DNA mixtures (see above), this difference in sensitivity could not directly be correlated with differences in efficiency of primer binding. The different dimensions of turkey eggs compared with duck eggs could not explain the established difference. Although turkey eggs are larger than duck eggs (average weight of analyzed turkey eggs was 90.5 g compared with 65.6 g for duck eggs), this difference in total weight was entirely due to a difference in egg white content (55.4 and 26.4 g for turkey and duck eggs, respectively). Because the same difference in detection sensitivity was seen between total egg and egg yolk, the lower sensitivity of the detection of turkey

TABLE 1. Detection of duck and turkey DNA in commercial egg products

<table>
<thead>
<tr>
<th>Sample</th>
<th>Chicken 30 cycles</th>
<th>Duck 30 cycles 10%</th>
<th>Duck 35 cycles 0.1%</th>
<th>Turkey 35 cycles 5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 egg powders</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>1 egg powder</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1 egg powder</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>1 egg powder</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>7 egg powders</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

1The minimum percentage of egg product present in the mixture if the PCR reaction is positive (based on PCR detection sensitivity).
DNA is most probably explained by a difference in efficiency of DNA extraction. This difference in extraction sensitivity could be due to differences in egg composition. There is a higher protein content in turkey eggs (13.7 g/100 g) compared with duck eggs (12.8 g/100 g) and a higher content of cations as calcium, iron, and sodium in turkey eggs (respectively 99, 4.1, and 151 mg/100 g) compared with duck eggs (respectively 64, 3.8, and 146 mg/100 g) (Anonymous, 2004).

**Determination of Duck and Turkey in Commercial Egg Products**

Thirteen pure whole egg powders were analyzed for the presence of chicken, duck, and turkey DNA (Table 1). In 3 samples only chicken DNA was detected. Two samples of egg powder reacted positively with the turkey-specific PCR of 35 cycles, indicating the presence of at least 5% of turkey egg product as compared with the results obtained with the mixtures of whole eggs. Ten samples, including the turkey PCR positives, reacted positively with the duck-specific PCR of 35 cycles; 8 reacted negatively with the PCR of 30 cycles and were estimated to contain at least 0.1% duck egg powder, 2 reacted positively with the PCR of 30 cycles and were estimated to contain at least 10% of duck egg powder.

From one sample of egg powder, which was positive for chicken, duck, and turkey egg, the PCR fragments amplified with each species-specific PCR were sequenced. Alignment with the sequences from the database indicated only 2, 1, and 1 mismatches for the chicken, duck, and turkey sequences, respectively, whereas the DNA homology for the fragments between duck and chicken and between turkey and chicken are 76.3 and 82.2%, respectively. These results demonstrate the possibility of identifying the presence of different egg products in a mixture by species-specific PCR.

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**REFERENCES**