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Development and optimization of an efficient method to detect the authenticity of edible oils

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ABSTRACT

The authenticity of edible oils has recently attracted increased attention, particularly due to the emergence of swill-cooked dirty oil. There has not been an effective method to detect the authenticity of edible oils due to the diversity of edible oils, the trace DNA in oils and serious DNA degradation. In this study, an efficient method was developed and optimized to detect the authenticity of edible oils. The stability and efficiency of detecting the authenticity of edible oils were dramatically increased by the DNA extraction method, amplification fragment selection and nano-real time PCR. The DNA extraction method was optimized with regard to the type and the amount of organic reagent, the types and the amounts of extraction buffer, and the DNA carriers. The types of organic reagents and DNA carriers determined whether DNA could be successfully extracted from different edible oils. The amount of DNA extracted from edible oils and adulterated edible oils with the optimized extraction method was evaluated by real-time PCR with different amplification fragments and nano-real time PCR. The results showed that 10 mL soybean oil can be detected against a background of 40 mL sesame oil. Additionally, the new nano real-time PCR technology was applied to amplify the DNA in edible oils at the first time. The efficiency and the precision of the PCR were dramatically increased by gold colloid. The results from this research are beneficial for detecting the authenticity of edible oils.

1. Introduction

Edible oils include the following: soybean oil, maize oil, sunflower oil, peanut oil, sesame oil, rapeseed oil, olive oil and a variety of blended oils, and they can provide essential fatty acids, such as linoleic acid and α-linolenic acid, and the fat-soluble vitamins A, D, E and K (http://en.wikipedia.org/wiki/Vegetable_fats_and_oils). As technology advances and people's health awareness increases, people are more concerned about food quality and safety. Thus, food authenticity and traceability have become very important to allow consumers to make informed choices about the foods they buy and eat (Giménez, Pistón, Martín, & Atienza, 2010). Edible oils can be misdescribed with the substitution of one ingredient by a similar but cheaper one or by over-declaring a quantitative ingredient (Woolfe & Primrose, 2004). Thus, the identification of the raw materials in edible oils is important for authentication.

The authenticity of edible oils has been addressed using different techniques such as gas chromatography, infrared spectroscopy, high-performance liquid chromatography, the isotope ratio method (Aluyor, Ozigagu, Oboh, & Aluyor, 2009; Verleyen et al., 2001), proton transfer reaction mass spectrometry (PTR-MS) and nuclear magnetic resonance spectroscopy (NMR) (Giménez et al., 2010). However, these methods are based on the physical and chemical properties of edible oils, and the detection limits of these methods are not sufficient to ensure edible oil authenticity. Thus, there is a growing interest in the application of DNA-based detection methods, such as PCR and real-time PCR, to evaluate edible oil authenticity. Specific protocols for the detection of DNA isolated from soybean oil (Bogani et al., 2009; Costa, Mafra, Amaral, & Oliveira, 2009; Costa, Mafra, Amaral, & Oliveira, 2010; Gryson, Ronsse, & Sewettinck, 2004), maize oil (Pauli, Liniger, Zimmermann, & Schrott, 2000), rapeseed oil (Hellebrand, Nagy, & MoÈrsel, 1998), olive oil (Busconia et al., 2003; Consolandi et al., 2008; Giménez et al., 2010; Spaniolas et al., 2008; Wu et al., 2008) and palm oil (Zhang et al., 2009) have been developed.

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Other DNA-based detection techniques used for edible oils include multiple PCR (Giménez et al., 2010), capillary electrophoresis (Ayed, Kamoun, Moreau, & Rebaï, 2009; Giménez et al., 2010) and biosensors (Bogani et al., 2009). A variety of molecular markers used to physically map genomes have now been successfully adapted for the identification of the geographical origin of olive oil, but there are no studies about the authenticity of other edible oils.

The efficiency of DNA extraction plays an important role in the detection of the DNA from edible oils. Because of the diversity of edible oils, trace DNA in oils and serious DNA degradation, the DNA extraction method needs to be carefully chosen and optimized to obtain enough good quality DNA. Consequently, the selection and optimization of DNA extraction methods are crucial for the successful application of DNA-based techniques to different edible oils.

The amplification fragments should be an appropriate fragment size in food products in which a high degradation of DNA may occur (Murray, Butler, Hardacre, & Timmerman-Vaughan, 2007). Costa et al. (2009) chose primers to result in an amplification fragment of 103 bp, and there was no positive amplification with other primers producing fragments of 118 bp and 120 bp in the detection of DNA in refined oils.

NanoPCR has a higher efficiency compared with PCR without nanomaterials, such as gold nanoparticles, carbon nanopowder and carbon nanotubes. There are currently three hypotheses regarding the mechanisms of nanoPCR. (1) These materials exhibit high thermal efficiency and thermal conductivity, which increase the efficiency of the PCR thermal reaction by dramatically shortening the time required or making the temperature of the PCR system more uniform (Huang et al., 2008; Zhang, Shen, Wang, Han, & Cao, 2008). (2) It was suggested that the selective interaction of the gold particles and single-stranded DNA could minimize mispairing between primers and templates, enhancing the specificity and increasing the amount of PCR products (Huang et al., 2008; Zhang et al., 2008). (3) The interaction between nanoparticles and DNA polymerase is very important (Shen et al., 2009; Vu, Litvinov, & Willson, 2008; Yang et al., 2008). Among nanomaterials, gold nanoparticles have attracted the most research interest, especially in real-time PCR.

The aim of this work was to optimize DNA extraction methods for five different edible oils. The DNA extracted from edible oils and adulterated edible oil was evaluated by optimized real-time PCR. The new technology, nano-real time PCR, was also employed for the first time to amplify the DNA in adulterated edible oil.

2. Materials and methods

2.1. Materials

Five different refined edible oils, soybean, maize, sunflower, peanut and sesame oil, were bought from local markers in Beijing.

2.2. DNA extraction

To identify critical aspects of the DNA extraction from the edible oils, DNA isolation from five different edible oils was carried out with two extraction replicates in five sets of parallel experiments to examine the type and the amount of organic reagents and the types and the amounts of extraction buffers and DNA carriers.

2.2.1. The type of organic reagent

We used hexane, heptane and chloroform to determine the most suitable organic reagent for the extraction of DNA from five different edible oils.

DNA from 30 mL of oil was extracted by magnetic stirring for 3 h with 30 mL of organic reagent and then mixing with the CTAB buffer (2% CTAB, 0.8 M NaCl, 50 mM Tris–HCl, 1 mM EDTA) for another 3 h. The sample was centrifuged at 11,000 rpm for 10 min. The aqueous phase was transferred to a 50-mL tube and was then centrifuged at 11,000 rpm for 10 min again, and the aqueous phase was transferred to a new 50-mL tube. The DNA was precipitated by mixing samples with 1 V of absolute isopropanol, 1/10 V of 3 M NaAc (pH 5.2) and 1/1000 V of acryl carrier, followed by incubating at −20 °C overnight. The samples were then pipetted into a 2-mL tube and centrifuged at 14,000 rpm for 20 min, and the liquid phase was discarded. The residues in all of the tubes were dissolved in 1 mL of TE. Phenol/chloroform/isoamylol (25:24:1) was added, and the mixture was centrifuged at 12,000 rpm for 10 min. Next, 800 μl of the upper part of the solution was transferred to a new tube, and 1 V of absolute isopropanol and 1/10 V of 3 M NaAc (pH 5.2) were added. The samples were incubated at −20 °C for 3 h and centrifuged at 14,000 rpm for 20 min at 4 °C. The supernatant was discarded, and the residue was washed with 700 μl of 70% ethanol, centrifuged at 14,000 rpm for 10 min and dried at room temperature. Dried DNA pellets were resuspended in 50 μl of TE.

2.2.2. The amount of organic reagent

The volumes of the organic reagents were 20 mL, 25 mL, 30 mL and 35 mL. The other procedures were the same as the procedures described in Section 2.2.1.

2.2.3. The type of extraction buffer

The extraction buffer used in the extraction method was substituted by the buffers as follows: TE (10 mM Tris–HCl pH 8.0, 1 mM EDTA pH 8.0), 2% CTAB (2% CTAB, 0.8 M NaCl, 50 mM Tris–HCl, 1 mM EDTA), 5% CTAB (5% CTAB, 0.8 M NaCl, 50 mM Tris–HCl, 1 mM EDTA), 1.4 M NaCl (2% CTAB, 1.4 M NaCl, 50 mM Tris–HCl, 1 mM EDTA). The other procedures were the same as the procedures described in Section 2.2.1.

2.2.4. The amount of the extraction buffer

The volumes of the extraction buffer used were 10 mL, 17.5 mL, 25 mL and 32.5 mL. The other procedures were the same as the procedures described in Section 2.2.1.

2.2.5. DNA carriers

In this study, four different DNA carriers, including three commercially available DNA carriers (acryl carrier, glycogen, yeast tRNA) and one DNA carrier that was the DNA extracted from another species in our lab, were used.

We performed 15 parallel experiments examining the DNA carriers described in Table 1, with sunflower oil as the representative edible oil to determine the best DNA carriers for DNA extraction from edible oils.

2.3. Primers

To assess the quality and quantity of the extracted DNA, we utilized primers for amplification of species-specific endogenous genes (Table 2). The primers for real-time PCR were designed using Primer Express software 3.0. All of the primers were synthesized by Sangon Biotech Co., Ltd. (Beijing).

Table 1
Combinations of DNA carriers.

Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
A	S	N	N	N	S	S	S	N	N	N	S	S	S	N	S
G	N	S	N	N	S	N	N	S	S	N	S	S	N	S	S
Y	N	N	S	N	N	S	N	S	N	S	S	N	S	S	S
D	N	N	N	S	N	N	S	N	S	S	N	S	S	S	S

A: Acryl carrier; G: Glycogen; Y: Yeast tRNA; D: DNA extracted from other species.
S: DNA carriers included in the combination.
N: DNA carriers not included in the combination.