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Application News

MultiNA

Qualitative Determination of Edible Corn Oil Using the MultiNA and the PCR Method

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■ Abstract:

Currently, edible oil adulteration is a common practice. This not only affects the physical health of consumers, but also lowers their confidence and has a serious impact on their interests. This paper proposes the design of a PCR primer for the specific gene of the species to be identified by DNA-based molecular biological means because different species have different DNA sequences, and determines the existence and chain length of the PCR amplified product using the MultiNA, thereby establishing a MultiNA-based method for edible oil identification. The specific gene PCR extracted from corn oil was amplified, and the size of its amplified product as determined by the MultiNA was 196 bp, largely consistent with the size of the PCR target product of the corn gene (190 bp). The experimental results indicate that this method can realize the qualitative determination of edible oils.

■ Introduction

Together with rice and flour, edible oils are foods essential to people's daily lives. In order to reduce costs and earn extra profits, some illegal traders sell lower-cost edible oils in place of higher-cost types, or substantially blend lower-cost edible oils with higher-cost types. This not only affects the physical health of consumers, but also lowers their confidence and has a serious impact on their interests.Edible oil identification and adulteration detection using molecular biological techniques feature high sensitivity and reliability. A PCR primer is designed to identify the specific gene of the species because different species have different DNA sequences, and the existence and chain engths of the PCR amplified product are determined to realize substance identification.This paper establishes a MultiNA-based method for edible oil identification. A modified reagent kit method was used to extract the corn genome from corn oil. A primer was designed for the corn endogenous gliadin gene, and subject to PCR amplification using PCR instrument. The size of its amplified product based on the MultiNA was 196 bp, largely consistent with 190 bp of the expected product amplified from the primer. The experimental results indicate that through edible oil gene extraction using this method, PCR amplification, and MultiNA determination, edible oil identification can be realized.

■ 1. Experimental Materials and Methods

1.1 Instruments

MCE-202 MultiNA, PCR instrument

1.2 Reagents

Vegetable oil gene extraction reagent kit
(Beijing Kwinbon Biotechnology Co., Ltd.) FZ-002
SYBR® Premix Ex TaqTM II (Takara Bio Inc.) RR820A
Primers: 5'-TGAACCCATGCATGCAGT-3'
5'-GGCAAGACCATTGGTGA-3
' (primer synthesized by Sangon Biotech)
DNA-500 Reagent Kit for MultiNA
(Shimadzu Corporation) 292-27910-91
SYBR® Gold Nucleic Acid Gel Stain (Invitrogen) S-11494
1xTE Buffer
25 bp DNA Ladder (Invitrogen) 10597-011
DNA-500 Reagent Kit for MultiNA
(Shimadzu Corporation) 292-27910-91
Sample: commercially available edible pure corn oil

1.3 Analysis Conditions

DNA-500 on chip mode

1.4 Analysis Procedure

1.4.1 DNA Extraction and Purification in Sample

1.4.1.1 Mix the sample oil with the 1x extract in the extraction reagent kit at a ratio of 2:1, and strongly stir the mixture with a magnetic stirrer for 30 minutes. Add the 1x extract obtained after centrifugation to the new sample oil again, and repeat this step. The total amount of sample oil used is 4,000 mL.

1.4.1.2 Centrifuge the thoroughly mixed mixture at high speeds (at 12,000 rpm for 10 min), then remove the upper oil phase completely, and put the aqueous phase extract in a rotary evaporator for dry concentration at 65 ℃.

1.4.1.3 Dissolve the freeze-dried matter with 1 mL of 1x extract, extract 0.375 mL of the dissolved matter and place in a 1.5 mL centrifuge tube, add 0.375 mL of extract A, mix, and put in a 65 ℃ water bath for 1 hour.

1.4.1.4 After bathing, add 0.75 mL of extract B: extract C=1:1 mixture to the tube, mix for 30 seconds, and then centrifuge at 12,000 rpm for 5 minutes.

1.4.1.5 Pipette the upper aqueous phase into a new 1.5 mL centrifuge tube, add double volume of the pre-cooled anhydrous alcohol (4 ℃), 10 % volume of settling agent 1 and 1.5 mL of settling agent 2, mix, and allow to settle at -20 ℃ for 1 hour.

1.4.1.6 After settlement, centrifuge at 12,000 rpm for 15 minutes, and pour away the supernatant carefully. At this point, white sediment can be seen at the bottom of the EP tube. This sediment is the extracted DNA.

1.4.1.7 Add 1 mL of pre-cooled washing solution (4 ℃), flip the EP tube to mix, centrifuge at 12,000 rpm for 5 minutes, then discard the supernatant, and invert the EP tube onto a filter paper for drying.

1.4.1.8 Add 30 mL of dissolving solution to the dried EP tube for sediment dissolution, and maintain the resultant solution at -20 ℃. Preheat the resultant solution at 65 ℃ for improved dissolution. The resultant solution can be used directly for subsequent PCR determination.

1.4.2 PCR reaction system

See Tables 1 and 2 for the PCR reaction reagents and conditions.

Table 1 PCR Reaction Reagents

	Amount	Final concentration
SYBR® Premix Ex Taq II (Tli RNaseH Plus) (2×)	10.0 μl	1×
PCR Forward Primer (10 mM)	0.8 μl	0.4 μM
PCR Reverse Primer (10 mM)	0.8 μl	0.4 μM
DNA template	2.0 μl	<100 ng
dH2O (sterile purified water)	6.4 μl	
Total volume	20.0 μl	

1.4.3 MultiNA Determination

After PCR amplification, MultiNA determination begins. When the MultiNA is used for high-accuracy DNA fragment length analysis, a ladder analysis should be performed first to prepare a standard curve. When an unknown sample is analyzed, fragment length can be determined from the standard curve as long as its migration time is measured.

Table 2 PCR Reaction Parameters

Impact	Time/s	Temperature/℃
Active DNA enzyme and initial denaturation	30	95
PCR (45 cycles)		
Denaturation	30	95
Annealing	30	55
Extending	60	72
Holding after cycles	180	72

■ Discussion of Results

Fig. 1 and Fig. 2 are the gel diagram and electropherogram obtained by analyzing the ladder, DNA amplified product extracted from corn oil, positive control (used the corn gene template during PCR) and negative control (did not use the corn gene template during PCR), using the MultiNA, respectively. A remarkable 197 bp band was obtained from the positive control. Since the chain length of the PCR target product was 190 bp, and no corresponding band near this area was obtained from the negative control, the PCR procedure has been executed successfully. The DNA amplified product extracted from corn oil was subject to a MultiNA analysis, and the results indicate that a 196 bp band was detected, showing that this corn oil contains a corn endogenous gene. The fragment length determined using the MultiNA differs slightly from that of the PCR target product. This result is deemed rational in consideration of the instrument error of 5 %.

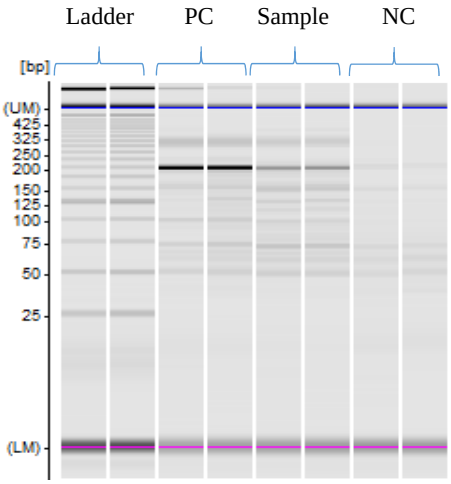

Fig. 1 Gel Analysis Results of Edible Corn Oil
(PC: positive control; NC: negative control)

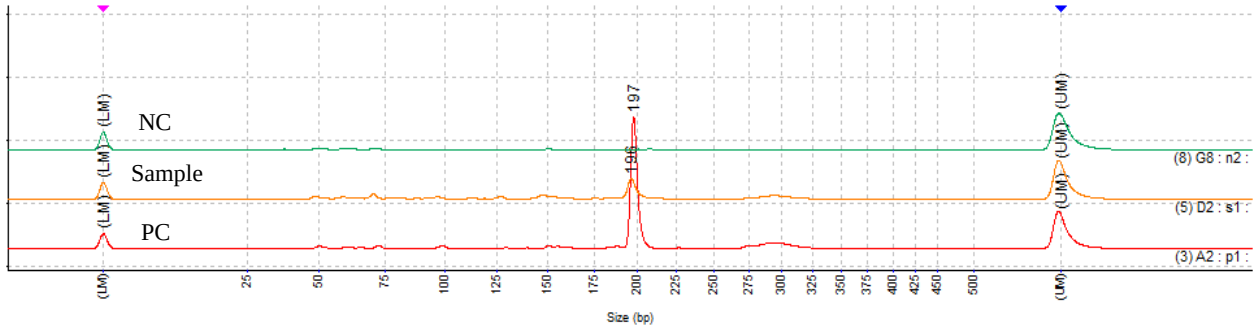

Fig. 2 Electrophoresis Results of Edible Corn Oil (PC: positive control; NC: negative control)

■ Conclusion

This paper establishes a method for the qualitative determination of edible vegetable oils using the Shimadzu MCE-202 MultiNA based on molecular biological technology. For the species identification of edible oils, this method is reliable and easy to operate, and can be applied for the qualitative and quantitative measurement of edible oils in complex systems, thus allowing full exposure of edible oil adulteration.