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Analysis of DNA isolated from different oil sources: problems and solution

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Abstract:

BACKGROUND: One of the major aspects of traceability in food authenticity assessment is to explore practical methods to find the origin of food. **OBJECTIVES:** The aim of the present study was to find a DNA based method for authentication and traceability of food, which are of great importance in health management. **METHODS:** Four different DNA extraction methods were applied to obtain high pure DNA in some oil samples including olive oil, sunflower, canola and soybean oil to improve the traceability. The isolated DNA was analyzed by PCR using common primer pair, derived from the region harboring 18S rRNA/5.8S rRNA genes. Extraction methods were developed based on specific binding of DNA molecules to the silica membrane (column) or resin. **RESULTS:** Our results showed that amplifiable DNA could only be extracted from olive oil in method 1, whereas the isolated DNA from other samples needed to be purified. In method 2, by pre-treating oil with PBS and subsequent precipitation with Isopropanol, the amplification of isolated DNA was observed in sunflower, crude canola and olive oil. To remove the contaminants more effectively, method 2 was combined with chloroform and resin/Isoporopanol precipitation as method 3. Interestingly, the extracted DNA from all examined oil samples could be amplified with the mentioned primers. To eliminate the disadvantages of chloroform, method 4 was set up by direct usage of lysis and binding buffer. The extracted DNA from all refined oil samples could be amplified successfully. **CONCLUSIONS:** Based on our findings, the major problem in DNA extraction from oils is the PCR inhibitors in extracted DNA, which can be resolved by the presented methods 3 and 4.

Introduction

Vegetable oils play significant roles in human consumption, chemical, pharmaceutical and cosmetic industries. The presence of various vegetable oils with a wide variety

of nutritional values and difference in prices provides a potential tendency for adulteration in oil composition. Therefore the authentication and traceability of food are of great importance in health management. European Commission defines traceability as the ability to trace and follow food, feed, and ingredients through all stages of production, processing and distribution (http://ec.europa.eu/food/food/foodlaw/traceability/index_en.htm). For this purpose valid methods and gold standards must be developed.

The conventional methods for identifying the traceability of the oils are proton transfer reaction mass spectrometry (PTR-MS) (Van Ruth et. al., 2010), nuclear magnetic resonance spectroscopy (NMR) (Vigli et al., 2003), high performance liquid chromatography (HPLC) (Fasciotti et al., 2010) and gas chromatography (GC) (Burian et al., 2011). Recently, (Mossoba et al., 2017) reported a new spectroscopic method (FT-NIR spectroscopic method) for identifying the adulteration in olive oil. Temiz et al. (2017) decribed the synchronous fluorescence spectroscopy for detection of adultration in tahini. Since the chemical composition of vegetable oils may differ among seasons and growing area, the use of chemical markers for authenticity assessment of the oils can be be associated with some problems (Gimenez et al., 2010). In recent years, there has been an increasing consideration towards the application of methods based on the analysis of DNA regarding food authentication (Mafra et al., 2008), to support or complement the methods based on the chemical markers (Gimenez et al., 2010, (Uncu et al., 2017, Vietina et al. 2013, Kumar et al. 2011).

For the DNA analysis different methods were developed. The first method used for DNA extraction from oil samples was based on cetyltrimethylammonium bromide (CTAB). Although this method was used in many studies (Busconi et al., 2003, Consolandi et al., 2008, Gimenez et al., 2010, Martin-lopes et al., 2008, Muzzalipo et al., 2002, Testolin et al., 2005) the purity of the extracted DNA was not very high (Nikolic et al., 2014). Therefore, some investigators have modified the CTAB method with Hexane and chloroform in order to obtain high pure DNA (Consolandi et al., 2008, Gimenez et al., 2010). Although the modified CTAB method had a better effect on the purity of extracted DNA, recently many researchers have used the DNA extraction method based on the specific binding of DNA to the silica membrane such as Nucleospin food kit (Consolandi et al., 2008), Nucleospin plant kit (Martin-Lopes et al., 2008), QIAamp DNA Stool kit (Ayed et al., 2009, Costa et al., 2010, Testolin et al., 2005) and DNeasy Plant mini kit (Testolin et al., 2005) were also used. Some studies were performed with kits based on magnetic separation method such as Wizard Magnetic Purification System for food (Breton et al., 2004, Consolandi et al., 2008, Testolin et al., 2005).

Common problems in DNA extraction from oil which nearly all previous studies showed, were the low amount and purity of DNA in oil samples. It is a routine practice to refine crude oil prior to market for human consumption. Refinement process includes physical and chemical steps. Chemical steps including degumming, neutralization, washing, bleaching and deodorization, are applied on crude oil to remove unpleasant odor and color. The oil extraction and refinement processing cause defragmentation

of genomic DNA (Gryson et al., 2004). On the other hand, food samples contain some components such as polysaccharides and phenolic components which can act as inhibitors for polymerase chain reaction(Pinto et al. 2007). Taken together, the extracted DNA for different oils can be accompanied with some PCR inhibitors. In the present study, the residual genomic DNA in different oil samples were extracted with 4 DNA extraction methods in order to obtain high pure DNA to improve traceability of the oil samples.

Materials and Methods

Samples and reagents: This study included a total of seven different refined and crude vegetable oils (crude/refined sunflower oil, crude/refined canola oil, crude/refined soybean oil and refined olive oil). Refined olive oils were supplied from Etka factory (Iran-Gilan). Crude and refined (canola, soybean and sunflower) oils were supplied from Margarine factory (Iran-Tehran). The origin of the oil samples was confirmed by GC analysis at the corresponding factory. Olive leaf and soybean seeds were used as positive control for PCR analysis. All used kits (lysis buffer, Binding buffer, Wash buffer, Resine and column) were provided by research group Molecular Biological System transfer (MBST, Iran/Germany). The abovementioned buffers could be used from other commercial kits such as Quiagen as well.

DNA extraction from olive leaf and seed of soybean

For extraction of DNA from plant materials (olive leaf and soybean seed) as positive control for primer analysis, Rapid DNA isolation kit from plant material was used. Briefly, each sample (1cm2 olive leaves and 2 embryo of soybean seed that was grounded to fine powder), was added to 1.5 ml test tube and mixed thoroughly with 300 µl lysis buffer. Sample was supplemented with 20µl proteinase K and incubated at 56 ºC for 2 hours. The mixture was centrifuged for 5 minutes at 8000 x g (Eppendorf, 5810R, Germany) and the supernatant transferred into a new sterile test tube. In the next step, 540µl binding buffer was added to the solution, mixed well and incubated for 10 min at 70 ºC. The mixture was centrifuged for 5 min at 8000 x g and the supernatant transferred spin column A. The column was centrifuged for 1 min at 8,000 x g. The spin column A was removed. 410µl absolute ethanol (Merck, Germany) was added to the mixture and transferred into a spin column B. The column B was centrifuged for 1 min at 8,000 x g. Subsequently, the column was washed twice by using 500 µl wash buffer. Finally, the column was centrifuged for a further 2 min at 8000 x g to remove the ethanol completely. The genomic DNA was eluted with 40µl prewarmed sterile water (70 ºC).

DNA extraction method 1: This method was based on the specific binding of DNA to the silica based membrane placed in the column. For extraction of DNA from oil, one milliliter of each sample (crude/refined sunflower oil, crude/refined canola oil, crude/refined soybean oil and refined olive oil) was added to 1.5 ml test tube and mixed thoroughly with 300 µl lysis buffer. The mixture was incubated at 70 ºC for 1 hour, vortexed for 1 minute and centrifuged for 5 minutes at 8000 x g (Eppendorf, 5810R, Germany). The lower phase plus interphase was transferred into a new sterile test tube. The sample was supplemented with 20 µl proteinase K and incubated at 56 ºC for 20

minutes. In the next step, 540 µl binding buffer was added to the solution, mixed well and incubated for 10 min at 70 ºC. After addition of 410 µl absolute ethanol (Merck, Germany) to the mixture, the mixture was transferred into a spin column. The column was centrifuged for 1 minute at 8,000 x g. Subsequently, the column was washed twice using 500 µl wash buffer. Finally, the column was centrifuged for a further 2 min at 8000 x g to remove the ethanol completely. The genomic DNA was eluted with 40µl prewarmed sterile water (70 ºC).

The extracted DNA was analyzed on 1% agarose gel, visualized using ethidium bromide or SYBR green dye using UV-transilluminator. The quantity of the extracted DNA was additionally analyzed by spectrophotometer under OD260.

DNA extraction method 2: Method 1 was improved by dilution of oil using PBS and subsequently DNA precipitation using Isopropanol. For extraction of DNA from oil, 5 mL of each oil sample (crude/refined sunflower oil, crude/refined canola oil, crude/refined soybean oil and refined olive oil) was used. Briefly, five ml oil sample was diluted with 5 ml PBS (8 g of NaCl, 0.2 g of KCl, 1.44 g of Na2HPO4, 0.24 g of KH2PO4, pH 8.0 in 1000 ml aqua bidest, Merk, Germany) and 1 ml tween 80 (Merck, Germany) and incubated at 70 ºC for 3 h with occasional shaking. After that, the emulsified solution was centrifuged at 4000 x g (Eppendorf, 5810-Germany) for 20 minutes. After centrifugation, 3 separated phases were observed. The top supernatant layer, which consisted of oil, was discarded carefully. The remaining two layers (middle and bottom) were transferred into a sterile 15 ml tube. The precipitation of DNA was achieved by adding of 0.1 volume of Sodium Acetate (3M, pH= 5.5) and 1 volume Isopropanol (Merck, Germany), incubation at -20 ºC for 20 min and subsequently centrifugation. The precipitated DNA was washed twice with 70% ethanol (Merck, Germany), re-suspended in 180 µl lysis buffer and incubated at 70 ºC for 10 min. After that, 20 µl proteinase K was added to the solution and the solution was incubated for 1 h at 56 ºC. Subsequently, 360µl binding buffer was added to the solution, mixed well and incubated for 10 min at 70 ºC. After adding 270 µl absolute ethanol (Merck, Germany) to the solution, the mixture was transferred into a spin column. The column was washed twice with wash buffer and the DNA was eluted in 40 µl sterile double distilled water.

The extracted DNA was analyzed on 1% agarose gel, visualized using ethidium bromide or SYBR green dye using UV-transilluminator. The quantity of the extracted DNA was additionally analyzed by spectrophotometer under OD260.

DNA extraction method 3: Method 2 was improved by washing the oil suspension with chloroform and replacingthe column through the silica base resin. For extraction of DNA from oil, 5 mL of each oil sample (crude/refined sunflower oil, crude/refined canola oil, crude/refined soybean oil and refined olive oil) was used. Briefly, five ml oil sample was diluted with 5 ml PBS and 1 ml tween 80 (Merck, Germany) and incubated at 70 ºC for 3 h with occasional shaking. After that, the emulsified solution was centrifuged at 4000 x g (Eppendorf, 5810-Germany) for 20 minutes. After centrifugation, 3 separated phases were observed. The top supernatant layer, which consisted of oil, was discarded carefully. The remaining two layers (middle and bottom) were washed twice with 5 ml chloroform for 5 min at

4000 x g. The top supernatant layer, which consisted of PBS solution containing DNA, was transferred into a new tube. The precipitation of DNA was achieved by adding 0.1 volume of Sodium Acetate (3M, pH= 5.5), 1 volume Isopropanol (Merck, Germany) and 60 µl resin, incubation at -20 °C for 20 min and subsequent centrifugation. The DNA precipitant was then re-suspended in 300 µl lysis buffer and 540 µl binding buffer and transferred into a sterile 1.5 ml tube and incubated at 70 °C for 10 min. After the incubation time, 410 µl absolute ethanol (Merck, Germany) and 30 µl resins was added to the same mixture and incubated in room temperature for 1 h. The former solution was centrifuged for 5 min at 8,000 x g, and the supernatant was discarded. Resins were washed twice with wash buffer and the genomic DNA was eluted with 40 µl sterile water.

The extracted DNA was analyzed on 1% agarose gel, visualized using ethidium bromide or SYBR green dye using UV-transilluminator. The quantity of the extracted DNA was additionally analyzed by spectrophotometer under OD260.

DNA extraction method 4: To avoid chloroform, the fourth method was developed. For extraction of DNA from oil, 3 ml of each oil sample (crude/refined sunflower oil, crude/refined canola oil, crude/refined soybean oil and refined olive oil) was used. Briefly, three milliliters of oil sample was diluted with 1500 µl lysis buffer and 2700 µl binding buffer and incubated at 70 ºC for 3 h with occasional shaking. The solution was centrifuged for 20 min at 4000 x g (Eppendorf, 5810R, Germany). After centrifugation, 3 separated phases could be observed. The top supernatant layer, which consisted of oil, was discarded carefully. The remaining two layers (middle and bottom) were transferred into a sterile 15 ml tube. 2050 µl absolute ethanol (Merck, Germany) and 60 µl resin were added to the same mixture and incubated at room temperature for 1 h. After centrifugation, the supernatant was discarded completely. Collected resins were washed twice with wash buffer. DNA was eluted with 40 µl sterile water.

The extracted DNA was analyzed on 1% agarose gel, visualized using ethidium bromide or SYBR green dye using UV-transilluminator. The quantity of the extracted DNA was additionally analyzed by spectrophotometer under OD260.

DNA purification: In some samples, the extracted DNA was further purified. For purification of extracted DNA, 100 µl of DNA was used. A hundred micro liters of DNA sample was diluted with 200 µl of binding buffer (purification Kit). After the addtion of 150 µl absolute ethanol (Merck, Germany) to the solution, the mixture was transferred into a purification spin column. The column was washed twice with wash buffer and the DNA was eluted in 40 µl double distilled sterile water.

Polymerase chain reaction: Amplifications by PCR were carried out by 1, 2 or 5 µl of DNA solution respectively. The PCR was performed on 100 µl total volume including 1 x PCR buffer, 2.5 U Taq Polymerase (Cinagene, Iran), 2 µl of each sense and antisense primer (20 mM, MWG, Germany), 200 µM of each dATP, dTTP, dCTP and dGTP (Fermenta) and 1.5 mM MgCl2 in automated thermocycler (MWG, Germany) with the following program: 5 min incubation at 95 °C to denature double strand DNA, 35-38 cycles of 45 s at 94 °C (denaturing step), 45 s at 56-60 °C (annealing step) and 45 s at 72 °C (extension step). Finally, PCR was com-

pleted with an additional extension step for 10 min. The common primers were derived from the corresponding region harboring 18S rRNA/5.8S rRNA genes registered under accession numbers of KF767534 (from nucleotide 3466 to 3803) for sunflower, KF704394 (from nucleotide 7 to 338) for canola, FJ609734 (from nucleotide 27 to 320) for soy bean and AJ585193 (from 26 to 341) for olive. The nucleotide sequence for forward primer was 5`TGCGGAAGGATCATTGTCG3`and for reverse primer was 5`ATTTCGCTACGTTCTTCATCGATGC 3. The nucleotide sequences of the used primers were identical to the corresponding sequence of the mentioned genes in the genomic DNA occurring in different used oil species. The PCR products were analyzed on 1.8% agarose gel in 0.5 x TBE buffer (5.4 g Tris base, 2.75 g boric acid and 2 ml of 0.5 M EDTA, pH 8.0 in 1000 ml aqua bidest) visualized using ethidium bromide or cyber green dye using UV-transilluminator.

PCR product purification and sequence analysis: PCR products were purified from the salts and proteins using PCR purification kit. Briefly, 200 µl binding buffer was added to 100 µl PCR product solution. After adding 150 µl absolute ethanol (Merck, Germany) to the sample, the mixture was applied into the column. The column was washed twice with 500 µl washing buffer and PCR product was eluted from the column using 100 µl elution buffer. The purified PCR product was then send to Takapousit Company (Iran-Tehran) for sequence determination.

Results

In the present study, we extracted DNA from different vegetable oil samples using four methods. To examine the quality of primer, DNA was extracted from olive leaf and soybean and subsequently amplified successfully by common primer pair derived from the region harboring 18S rRNA/5.8S rRNA gene. First, the DNA was extracted from different mentioned oil sources using DNA extraction kit, based on the specific binding of DNA to the silica based membrane placed in the column. In this method, DNA was extracted from 1ml of each oil sample. Our experiments showed that using this method, the amplifiable DNA can be extracted from refined olive oil. The amplification was performed using common primer pair derived from 18S rRNA and 5.8S rRNA genes resulting in PCR product of 316 bp in length (Fig. 1, A and A´). The amplifiable DNA could not be extracted from sunflower oil (crude, refined), canola oil (crude, refined) and soybean oil (crude, refined) (table 1). It seems that one of the main problem in DNA extraction from oil is the purity of the extracted DNA, therefore the purity of the extracted DNA was measured by spectrophotometer and found that the amplifiable DNA extracted from olive oils had no detectable DNA amount and the amount of unamplifiable DNA extracted from above-mentioned oils were between 11.5± 0.2 and 17.8± 0.4 ng µl-1 (Table 2). Interestingly, the analysis of measured DNA on agarose gel showed no detectable DNA bands. Therefore the extracted DNA with high OD260 was first purified using DNA purification kit and subsequently amplified by PCR. Fig. 1 (part B) showed that after purification of DNA, the Sunflower DNA could be amplified by PCR. Interestingly, in such cases, the purification process could not bring the OD260 to undetectable, the DNA could also not be amplified (data not

Table 1. DNA from different oils was extracted with 4 different methods and amplified with common primer pair. - was negative in PCR, + was positive in PCR. NT: not tested sample, C: crude oil, R: refined oil.

Oil / Extraction methods	Refined Olive oil	Sunflower oil		Canola oil		Soybean oil	
		C	R	C	R	C	R
Method 1 DNA	+	-	-	-	-	-	-
Method 2 DNA	+	+	+	+	-	-	-
Method 3 DNA	+	+	+	+	+	+	+
Method 4 DNA	+	+	+	+	+	+	+

Table 2. The DNA extracted from different oils was analyzed by spectrophotometry. ND: not detected.

	Refined Olive oil	crude Sunflower oil	Refined Sunflower oil	Crude Canola oil	Refined Canola oil	Crude Soybean oil	Refined Soybean oil
Method 1 DNA (ng/µL)	ND	11.5± 0.2	14.2± 0.4	13.4± 0.3	17.8± 0.4	17.1± 0.5	15.3± 0.3
Method 2 DNA (ng/µL)	ND	ND	ND	ND	17.2± 0.3	16.3± 0.2	14.8± 0.4
Method 3 DNA (ng/µL)	ND	ND	ND	ND	ND	ND	ND
Method 4 DNA (ng/µL)	ND	ND	ND	ND	ND	ND	ND

shown). To reduce the inhibitory factors for DNA polymerase, the oil was first emulsified with PBS and the DNA was subsequently precipitated using Isopropanol (method 2). After this procedure, the extracted DNA from refined olive, refined/crude sunflower and crude canola oil could be successfully amplified using the mentioned primer pair (Table 1). Figure 1 (part C) showed the PCR products of 337 bp, 316 bp and 332 bp in length for refined sunflower, refined olive and crude canola oils, respectively. The extracted DNA from refined or crude soybean oil and refined canola oil could not be amplified using the mentioned primer pair (Table1). To eliminate the PCR-inhibitors from DNA extracted from soybean oil and refined canola oil, the third DNA extraction method was developed. For this aim, the oil was first emulsified with PBS and the mixture was centrifuged and after separating the top supernatant layer (oil), the mixture was washed with chloroform (method 3). Subsequently, the DNA was precipitated with Isopropanol in presence of resin. The precipitated DNA was then purified using resin Kit. Interestingly, DNA extracted from all examined vegetable oils using third method, could be amplified by PCR (Table1). Figure 1(part D) showed the PCR products of 293 bp, 337 bp, 332 bp and 316 bp in length for refined/ crude soybean oil, refined/ crude sunflower oil, refined/ crude canola oil and refined olive oil respectively. To avoid the use of chloroform, in the next experiment, we extracted DNA from all used oil samples by method 4. The amplifiable DNA could be extracted from all examined oil samples (Table1, Fig. 1, E and E´). Our results showed that the most important

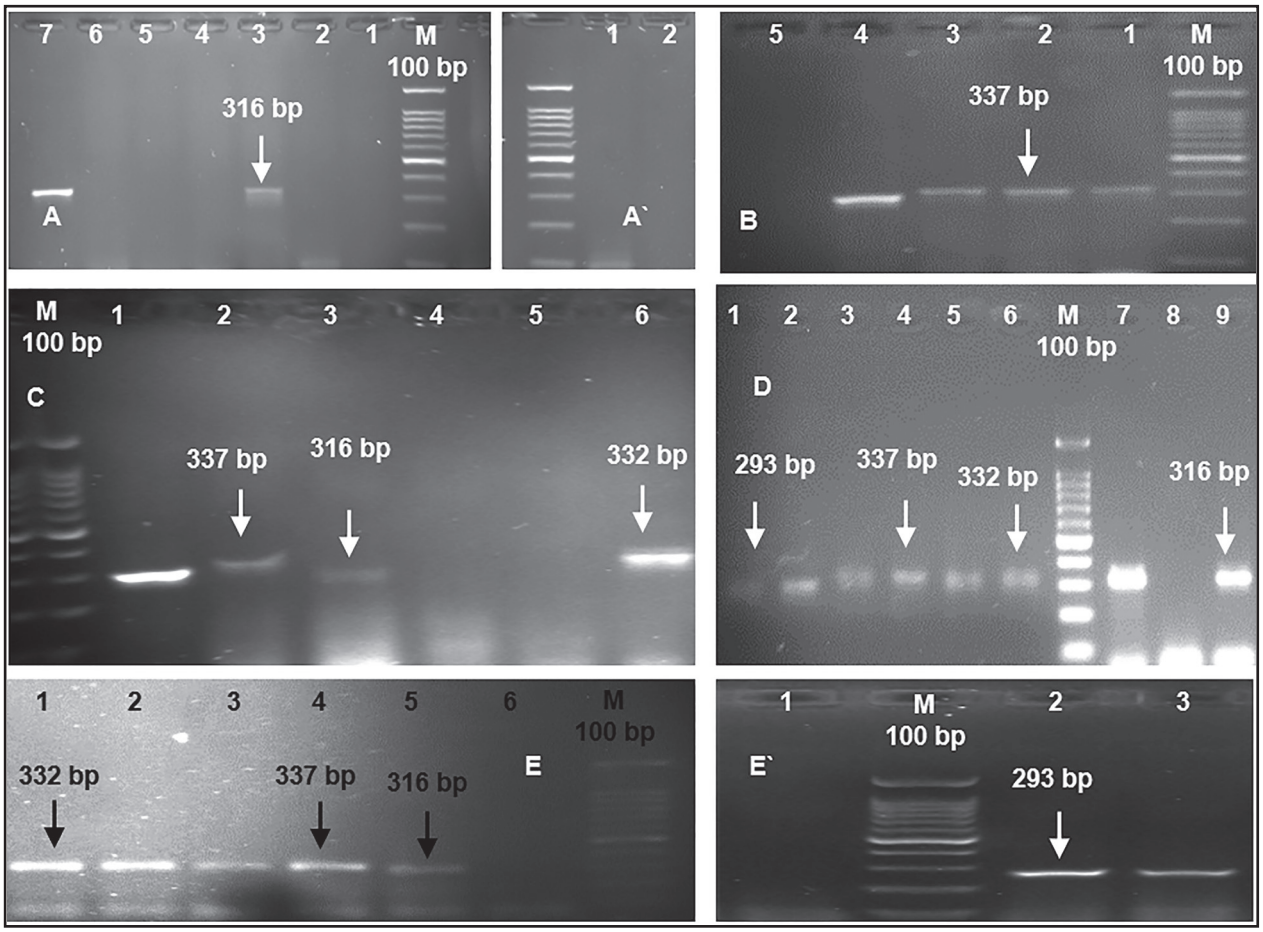

Figure 1. Agarose gel electrophoresis of PCR products achieved by amplification of DNA extracted from refined olive oil, refined/ crude canola oil, refined/ crude sunflower oil and refined/ crude soybean oil with 4 different methods. A: PCR of 0.5 µl DNA extracted with method 1 from refined (lane 2) and crude sunflower oil (lane 4), refined olive oil (lane 3), refined a (lane 5) and crude canola oil (lane 6), lane 7 was positive control (olive leaf) and lane 1 was negative control. A´ (continue method 1): from refined (lane 1) and crude soybean oil (lane 2). B: PCR of 0.5,2,5 µl DNA extracted with purification MBST kit from refined sunflower oil (lane 1, 2, 3 respectively),PCR of 0.5 µl of DNA extracted from refined olive oil(lane 4), negative control (lane 5). C: PCR of 0.5 µl DNA extracted with method 2 from crude sunflower oil (lane 2), refined olive oil (lane 3), refined (lane 4) and crude soybean oil (lane 5), crude canola oil (lane 6) and lane 1 was positive control (olive leaf). D: PCR of 0.5 µl DNA extracted with method 3 from refined (lane 1) and crude soybean oil (lane 2), refined (lane 3) and crude sunflower oil (lane4), refined (lane 5) and crude canola oil (lane 6), refined olive oil (lane 9), lane 7 was positive control (olive leaf) and lane 8 was negative control. E: PCR of 0.5 µl DNA extracted with method 4 from refined (lane 1) and crude canola oil (lane 2), refined (lane 3) and crude sunflower oil (lane 4), refined olive oil (lane 5) and lane 6 was negative control. E´ (continue method 4): from refined (lane 2) and crude soybean oil (lane 3) and lane 1 was negative control.

problem with DNA extraction from vegetable oils is the purity of extracted DNA. Sequence analysis showed 100% homology between the sequenced PCR products of canola oil and sunflower oil with corresponding sequences registered in GenBank under accession numbers KF704394 and KF767534 respectively. Additionally, we amplified successfully the extracted DNA from refined/ crude soybean oil with the primer pair derived from lectin gene (Nikolic et al. 2014) to confirm the specificity of the extracted DNA (data not shown).

Discussion

One of the major aspects of traceability in food authenticity assessment is to explore

practical methods to find the origin of food through its whole production procedure. Therefore, some chemical methods such as proton transfer reaction mass spectrometry (PTR-MS) (Van Ruth et al. 2010), nuclear magnetic resonance spectroscopy (NMR) (Vigli et al. 2003), high performance liquid chromatography (HPLC) (Fasciotti et al., 2010) and gas chromatography (GC) (Burian et al., 2011) were developed. One of the most important problems of such methods is seasonal and growing area variations which can lead to the change in the chemical components of the vegetable oils. This change can affect the validity assessment of these methods. Such problems can be solved by genetic traceability analysis. It is important to emphasize that the genetic analysis alone can not be used as a gold standard method, because in some cases such as determination of the growing areas with cultivars it cannot be performed by genetic analysis. Therefore, the application of the chemical and genetic methods can complete each other and be used as gold standard methods for traceability.

Some oils such as olive oil have essentially been a topic of authenticity and traceability studies due to their high price value. Some investigators used PCR method based on microsatellite markers for identifying the single cultivar virgin olive oils (Busconi et al., 2003, Testolin et al., 2005). In recent years, many different methods have been applied to determine the suitable DNA extraction techniques (Costa et al., 2010., Gimenez et al., 2010, Nikolic et al., 2014, Pauli et al., 1998). Nicolic et al.(2014) reported that the isolated DNA from crude soybean oil by using CTAB method was not pure enough to be amplifiable by PCR (Nikolic et al., 2014). The most recommended method for the DNA extraction from oil was described as the method based on the specific binding of the DNA to silica membrane (Costa et al., 2012, Nikolic et al., 2014) which was also confirmed by the present study. The superiority of this extraction method is due to less loss of DNA in the DNA extraction compared with the CTAB method. Gimenez et al. (2010) showed the purity of DNA extraction was increased by use of CTAB method combined with hexane and chloroform extraction.

In the current study, we extracted successfully amplifiable DNA from various vegetable oil samples by methods 3 and 4. We believe that the purity of DNA extracted from vegetable oils is responsible for the successful PCR amplification. The spectrophotometric examination showed that the undetectable DNA by OD^{260} could be amplified by PCR, whereas the concentration of unamplifiable DNA was between 11.1± 0.2 and 17.8± 0.4 ng μl^{-1}. Therefore, we are of the opinion that the high measured absorbance by OD^{260} in DNA samples extracted from some oils was associated with the contaminants and not with the DNA, since the analysis of the extracted DNA with high amount of the DNA showed no detectable DNA bands by agarose gel electrophoresis.

To reduce the contaminant, the DNA was purified using PCR purification kit. Interestingly, the purification of some DNA samples lead to amplification of DNA by PCR. This means that the purity of DNA is the most critical aspect by DNA extraction methods. To obtain pure DNA from oil samples, we used method 2. With this method only the extracted DNA from olive oil, sunflower and crude canola oil could be amplified by PCR. It seems that more contaminant could be removed from the DNA samples by this

method. In order to remove PCR inhibitors from different oil samples, method 3 was used. In this method chloroform as solvent of organic molecules (non DNA) was used. Interestingly, DNA extracted from all examined vegetable oils could be amplified by PCR. The absorbance of OD^{260} nm by all DNA samples extracted from different oils was not detectable. This means that the purity of DNA in the sample is decisive for amplification of DNA by PCR and not necessarily the low amount of DNA in samples. To avoid the use of chloroform in DNA extraction method, method 4 was developed. This method could also be performed successfully by all oil samples. Our results were in agreement with the results of Costa et al. (2012) regarding the low amount of DNA in vegetable oils, but we believe that the low amount of DNA is not responsible for the lack of PCR amplification. Our results support the reported results of Nicolic et al. (2014) and Costa et al.(2010) according to the importance of the purity of DNA by PCR amplification.

Our results showed that in all DNA samples extracted from different oils, DNA could be amplified with primer pairs resulting in PCR product of 293 to 337 bp. It is assumed that refining processes (chemical and mechanical steps, the deodorization phase (240 ^{o}C), acidified with phosphoric acid and neutralized with NaOH) cause DNA fragmentations (Costa et al., 2010). Therefore, some investigators used primer pairs for their study to amplify small PCR products about 150 bp in length (Costa et al., 2010, Nikolic et al., 2014). Costa et al. (2010) reported that they could amplify the DNA from oil with only primer pair giving PCR product of 103 bp in length but not those with 118 bp or 120 bp (Costa et al. 2010). Nikolic et al.(2014) recommended the use of DNA region with approximately 150 bp in length for processed food by PCR analysis (Nikolic et al., 2014). Even though in this study we were able to detect PCR product with 337 bp in length, we also follow the suggestion of Costa et al. (2010) and Nikolic et al. (2014) to amplify the small DNA region for processed food traceability because we used a multicopy DNA region (18S rRNA and 5.8S rRNA) and the others most probably used another gene with less copy number in genome.

Conclusion: Based on our findings, the major problem in DNA extraction from oils is the PCR inhibitors in extracted DNA. According to our study, the best methods for DNA extraction from oil was that method which was able to remove the PCR inhibitors. Methods 3 and 4 could be used as suitable DNA extraction methods for all oil samples.

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آنالیز ماده وراثتی استخراج شده از منابع مختلف روغن: مشکلات و راه حل

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چکیده

زمینه مطالعه: یکی از جنبه های اصلی ردیابی مواد غذایی در جهت آزمایشات اعتبار سنجی آنها شناسایی و تعریف روش‌های عملی جهت شناسایی منشأ ماده غذای می‌باشد. **هدف:** هدف از مطالعه حاضر، پیدا کردن یک روش مبتنی بر DNA برای احراز هویت و قابلیت ردیابی از مواد غذایی است که از اهمیت زیادی در مدیریت بهداشت و درمان برخوردار می‌باشند. **روش کار:** در این مطالعه ۴ روش مختلف جهت استخراج DNA خالص از تعدادی روغن خوراکی شامل روغن زیتون، آفتابگردان، کانولا، و سویا برای بهبود ردیابی این روغن‌ها بررسی گردید. DNA استخراج شده به روش PCR با استفاده از پرایمر عمومی واقع شده در ناحیه ژن ۵/۸S rRNA/۱۸S rRNA انجام شد. روش استخراج بر اساس اتصال DNA به لایه ی سیلیکایی موجود در ستون‌ها و یا اتصال به رزین بنا شده بود. **نتایج:** نتایج به دست آمده از روش اول نشان داد فقط DNA استخراج شده از روغن زیتون توانایی تکثیر را داشت. در روش دوم با استفاده از محلول PBS و بدنبال آن ایجاد رسوب با ایزو پروپانول تکثیر DNA از روغن های آفتابگردان، کانولا خام و روغن زیتون با موفقیت انجام شد. برای حذف موثر تر ناخالصی‌ها روش دو با کلروفرم و رزین و رسوب دهی با ایزوپروپانول بعنوان روش سوم مورد استفاده قرار گرفت. خوشبختانه با این روش DNAهای استخراج شده ازتمامی نمونه‌های روغن با موفقیت تکثیر شدند. برای حذف اثرات سوء کلرفرم روش چهارم با استفاده ی مستقیم از بافر لیز کننده و بافر اتصال طراحی شد. با این روش DNAهای استخراج شده ازتمامی نمونه های روغن با موفقیت تکثیر شدند. **نتیجه گیری نهایی:** بر اساس یافته‌های ما، مشکل عمده DNA استخراج شده از روغن‌ها، باقی ماندن مهار کننده‌های آنزیم‌های مختلف از قبیل DNA پلیمراز می‌باشدکه این مشکل با استفاده از روش‌های ارائه شده ۳ و ۴ قابل حل می باشد. است.

واژه های کلیدی: تقلبات مواد غذایی، ماده وراثتی ژنومی، واکنش زنجیره ای پلیمراز، سویا، روغن های گیاهی

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