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Potential biological markers by DNA-based tools for determination of Greek PDO geographical origin and authenticity: "Avgotaracho Mesolonghiou" and "Vostizza currant"

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Abstract

Background: Food traceability and authentication had become mandatory for food industry and global food trade. Numerous DNA-based methods could contribute against food frauds, because of their advantages such as simplicity, accuracy, and robustness. The aim of this study was to explore whether unique biological markers for two high valuable and popular Greek protected designation of origin (PDO) products could be indicated. For this purpose, "Avgotaracho Mesolonghiou" known as Greek caviar and "Vostizza" currant were subjected to DNA-based analysis. PCR-RAPD, PCR-RFLP, and PCR-DGGE were performed for Greek PDO products and potential biological markers were explored, based on either genomic DNA or bacteria communities.

Results: Band profiles resulted in molecular techniques, could be used as a "barcode" to certify the origin and authenticity of PDO products.

Conclusion: These methods are proposed, as alternative traceability tools, in order to provide unique markers and could be the key to "farm to table" challenge. Therefore, biological markers could be used throughout the entire commercial supply chain and protect food products, which hold a quality scheme, from adulterations.

KEYWORDS
biological markers, DNA-based techniques, food authentication, geographical origin, PDO

INTRODUCTION

Protected designation of origin (PDO) and protected geographical indication (PGI) are EU quality schemes, which introduced by the EEC Reg. 2081/92 (recently substituted by EC Reg. 510/2006).[1] Products carrying PDO or PGI Origin EU label (PDO) protect their origin, are certified by a full compliance with EU standards and thus, command a higher price in global food trade.[2] As a result, consumers expect these valuable products to have some particular characteristics. One of the most important characteristics refers to product's traceability and authenticity. Therefore, by the European Union law EC No. 2065/2001, food traceability and accurate labeling of products were established.[3]

Moreover, food products which hold a PDO or PGI quality scheme because of their uniqueness and economic impact become prone to adulteration.[4] Several independent agencies and quality authorities are looking for reliable tools for validating food products' geographical origin and authenticity. Due to this demand and the impact befallen on public opinion when a food fraud incident appears, researchers try to address this issue with innovative and analytical approaches.[5,6] Nuclear magnetic resonance, infrared spectroscopy, isotopic methods, DNA-based techniques, mass spectrometry, chromatographic techniques, and combinations of these methods as well, proved to be very promising in fulfilling this role.[3]

The highest percentage of developed DNA-based methods aim to species authentication among a variety of foodstuff and consist on the highly specific amplification of DNA fragments by means of polymerase chain reaction (PCR).[7,8] DNA analysis present several advantages regarding food authentication and food traceability, especially when "biological" markers of food products are identified.[9] New traceability strategies focus on biological markers using either DNA genome such as plastid, chloroplast, mitochondrial, or even microorganisms' population.[6] PCR-Random Amplification of Polymorphic DNA, PCR-Restriction Fragment Length Polymorphism, PCR-Denaturing Gradient Gel Electrophoresis are widely known and used for food authentication purposes. PCR-RAPD method was utilized for potential fraud of Mediterranean oregano, of seafood such as tilapia and of beef meat.[10–12] PCR-RFLP has subsequently found widespread use for meat and fish authentication, for example, beef, pork, deer, cattle, canned tuna, prawn, shrimp, and salmon.[13–17] In addition, PCR-DGGE has applied broadly for determination of food geographical origin and food authentication. DGGE analysis of bacteria or fungi communities isolated from marine salts, Pangasius fish from Vietnam, traditional Minas cheeses, traditional Wielkopolska fried ripened curd cheese are some cases examined.[18–21] Interestingly, the majority of the publications regarding food traceability by application of DNA markers, target to major crops', such as olive and grape due to their great diffusion.[22,23] Therefore, biological markers could be developed, based on either microflora communities or specific regions of genome of a food product.

Avgotaracho Mesolonghiou (eggs from *Mugil cephalus L.*) and currant Vostizza (*Vitis vinifera* L., var. Apyrena) are traditional and high valuable Greek PDO products and thus, considered to be vulnerable for counterfeit. More specific, avgotaracho Mesolonghiou, known as Greek caviar, is semi-dried, salted ovaries of fish *M. cephalus* L., which caught in lagoon system of Mesolonghi-Etolikon. Avgotaracho Mesolonghiou has a well-known reputation due to its unique flavor, aroma, and its commercial price.[24] This food product holds quality scheme PDO since 1996 by EU (07/02/1996) with the code EL/PDO/0017/0446 (EC1263/96) and therefore it is the oldest PDO of the category of "Fresh fish, molluscs, crustaceans and products derived there from".[24,25] As far as Vostizza currants concern, their cultivation and production take place in a semi-mountainous and mountainous area of Aeghioin, in North Peloponesse. Vostizza currants are sun dried vine products and according to nutrition scientists, they are an excellent source of antioxidants, fibers, and polyphenol compounds.[26,27] Moreover, currants considered to provide beneficial health effects on digestive system, on bones (preventing osteoporosis and arthritis), on insulin levels and on cardiovascular and neurodegenerative diseases.[28–30] In our knowledge, there is a study regarding quality control of Avgotaracho Mesolonghiou and Vostizza currant, by means of Next Generation Sequencing.[31] Although, this is the first time, that these Greek PDO products analyzed by DNA-based methods in terms of authenticity and traceability.

The main aim of this study was to investigate potential biological markers by DNA-based tools, able to discriminate Greek PDO products among other samples. A DNA-based approach for studying bacteria microflora of food products and a DNA-based analysis of genomic DNA were conducted and evaluated regarding food traceability.

MATERIALS AND METHODS

Samples collection

An aquaculture Greek PDO product (avgotracho Mesolonghiou) and an agricultural Greek PDO product (Vostizza currant) were examined in this study. Vostizza currants PDO were collected from producers from Aighio (Panegialios Agricultural Union), while Avgotaracho Mesolonghiou PDO was collected from aquaculture company in Mesolonghi lagoon (Stefos). In order to analyze Greek PDO products and examine whether unique biological barcodes by DNA-based techniques could characterize them, we purchased same food products from other geographical origins from producers and local market. Fish eggs of *M. cephalus* from Australia and Mauritania were included in this study in order to compared to fish eggs from Mesolonghi. Corinthian currants from four other districts of Greece, Kalamata, Amaliada, Zante and Nemea, were selected for this experiment as well. Nine samples from each geographical region were subjected to analysis by DNA-based methods and three technical replicates were performed (Table 1). Figures shown technical replicates.

DNA-based tools for fish eggs

PCR-RAPD for *Enterobacteriaceae* analysis

Ten grams of fish eggs samples were homogenized with 90 ml Buffer Peptone Water in a BagMixer 400 W stomacher (Interscience). A series of 10-fold dilutions of each sample was prepared and plated in Violet Red Bile Glucose (VRBG) Agar (Oxoid) according to ISO 17025:2017. Once VRBG had solidified, an extra layer of medium was poured onto the surface of the plate to prevent spreading growth. *Enterobacteriaceae* were enumerated after 1 day of incubation at 37°C. Five purple/pink colonies were selected and plated on a non-selective medium (Nutrient agar). Plates with Nutrient Agar were incubated for 24 h at 37°C. Well isolated colonies were confirmed by oxidase and fermentation tests. Genomic DNA from colonies of *Enterobacteriaceae* was isolated by Ultraclean Microbial Kit (Qiagen), according to manufacturers' instructions and checked as described above. Extracted DNA from *Enterobacteriaceae* of each sample was amplified by PCR-RAPD. PCR-RAPD amplification was perfomed using following primers: M13: 5′-GAGGGTGGCGGTTCT-3′, 1247: 5′-AAGAGCCCGT-3′, 1290: 5′-GTGGATGCGA-3′, OPA10: 5′-GTGA TCGCAG-3′, OPA15: 5′-TTCCGAACCC-3′.[32–34] PCR was performed in a total volume of 50 μl and contained 1X PCR buffer, 5 mmol L^{-1} $MgCl_2$, 200 μmol L^{-1} dNTPs, 2 μmol primer, 1,25 Taq polymerase (Life Technology) and 2μl of bacterial DNA. PCR conditions were subjected to initial denaturation at 94°C for 2 min, followed by 35 cycles

TABLE 1 Number of samples of each geographical area that were included in the study

Samples *currants*	Geographical origin	Samples *fish eggs*	Geographical origin
9	Aighio (PDO Vostizza)	9	Mesolonghi (PDO)
9	Kalamata	9	Australia
9	Nemea	9	Mauritania
9	Zante		
9	Amaliada		

of denaturation at 94°C for 1 min, annealing at 45°C for 40 s, elongation at 72°C for 2 min, and final extension at 72°C for 10 min. PCR products were separated by electrophoresis 2% agarose gel and 100 bp DNA ladder (Lonza) was used as DNA molecular weight marker.[31]

PCR-RFLP for *COI* gene

For genomic DNA extraction from fish eggs, Food Merikon Kit (Qiagen), was used according to manufacturers' instructions and checked by 0.8% agarose gel electrophoresis. Genomic DNA extracted from fish eggs was subjected to PCR amplification. Approximately 655 bp were amplified from the region of the *coi* gene from mitochondrial DNA of fish eggs using primers: FishF1 5′-TCAACCAACCACAAAGACATTGGCAC-3′and FishR1 5′-TAGACTTCTGGGTGGCCAAAGAATCA-3′.[35] Amplification reactions were carried out in 25 μl volumes containing 2.5 μl of 10X PCR buffer with Mg, 0.2 μM dntps, 0.1 μM of primers, 0.5 μl $MgCl_2$, and 0.3 μl Taq polymerase. Using a thermocycler (Bio-Rad), these reactions were subjected to a cycle of 2 min at 95°C followed by 30 cycles; each of which consisted of 94°C for 30 s, 53°C for 30 s, and 72°C for 1 min and then, a final extension cycle at 72°C for 10 min. Generated PCR products were electrophoresed on 2% Gel red (Biotium) stained agarose gels. A 100 bp ladder (Lonza) was used to estimate the approximate molecular weight of amplicons. Electrophoresis was performed at 100 V for 2 h, and amplification profiles were photographed under UV light using Gel Documentation System (GDS8000; UVP).

However, in order to select restriction endonucleases that provide a discrimination RFLP pattern among samples, we proceed to *coi* gene sequencing analysis. We analyzed sequences of *coi* gene by Gene Runner software. Finally, we selected restriction enzymes, *Hin*fI, *Alu*I, *Pae*I, and *Aqu*III (Nippon Genetics Europe). Each digestion was performed in 50 μl of mixture, containing 9 μl PCR product, 3 μl of restriction enzyme, 15 μl buffer and 23 μl sterile nuclease-free water. Reactions were performed separately in Bio-Rad thermal cycler, set at 37°C for enzyme digestion and finally at 65°C for 20 min or 80°C for 20 min to inactivation. RFLP results were analyzed on 2% agarose gel electrophoresis with GelRed staining.

DNA-based tools for currants

Genomic DNA extraction from currants

For genomic DNA extraction from currants, commercial kit, Food Merikon Kit (Qiagen), was used. Procedure of DNA extraction of currants from all geographical regions, were according to manufactures instructions. Furthermore, three replicates from each sample were performed. Then, the DNA each sample was quantified spectrophotometry with Nanodrop™ 1000 by measuring the absorbance at 260 and 280 nm and analyzed by 0.8% (wt/vol) agarose gel electrophoresis, before PCR amplification.

PCR-RAPD analysis of currants

DNA extracted from currants by Food Merikon Kit (Qiagen), were amplified by PCR-RAPD. Same primers (M13, 1247, 1290, OPA10, OPA15) were used for PCR-RAPD amplification. PCR-RAPD protocol for DNA from currant samples, was as described above as well.

PCR-DGGE for 16s rRNA gene

Bacterial DNA from each currant from different geographical origin was extracted by Power Food Kit (Qiagen) and was used as template for PCR amplification. To amplify 240bs from V3 region of 16s rRNA gene, the following universal primers: 338F (5′-ACTCCTACGGGGGCAGCAG, Sigma, France), 518R (5′-ATTACCGCGGCTGCTGG; Sigma) were used.[19,36] PCR reaction was performed in a final volume of 50 μl, containing 100 mg DNA template, 2.5 μl buffer A with Mg, 0.5 μl dNTPs (10 mM), 5 μl primers (0.2 μM), and 0.1 μl Taq polymerase (Kapa TAq PCR kit) (Sigma). PCR amplification was performed using Bio-Rad thermo cycler and an amplification program as follows: initial denaturation 95°C for 3 min, 30 cycles of denaturing at 95°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 1 min with a final extension at 72°C for 10 min. The resulting amplicons of PCR were checked spectrophotomatically and by agarose gel. Then, PCR products from conventional PCR were subjected to DGGE analysis on 8% polyacrylamide gel (acrylamide: bisacrylamide, 37.5:1) with 30%–60% denaturing gradients (100% denaturant contained 7 mol/L urea and 40% [vol/vol] formamide).[37] Therefore, electrophoresis run was carried out in the VS20-DGGE system (Cleaver Scientific) in 0.5X TAE buffer at 60°C, 65 V for 16 h. After electrophoresis, gel was stained with GelStar nucleic acid (Lonza) for 30 min.

RESULTS

PCR-RAPD analysis of *Enterobacteriaceae* isolated from fish eggs

PCR-RAPD technique was carried out in replicates using DNA isolated from colonies *of Enterobacteriaceae*. The selected primers

amplified DNA fragments across the three samples of different geographical locations, with the number of amplified fragments varied from 4 to 9. Moreover, the amplicons size varied from 200 to 900 bs. All fish eggs samples were discriminated by the presence or absence of unique DNA fragments, resulted by RAPD analysis. Figure 1. depicts RAPD profiles of *Enterobacteriaceae* extracted from each sample and carried out with OPA10 primer. At 48% observed a cluster that includes samples of Mesolonghi and samples from the other two regions. At 75%, samples from Australia and Mauritania were discriminated by a second cluster.

PCR-RFLP for *COI* gene

DNA was extracted from fish eggs from all regions and amplified for *coi* gene. Figure 2 shows gel electrophoresis of PCR products amplified. Figure 3 presents multiple alignment of PCR products based on the region of *coi* gene of samples. High similarity in sequence analysis of *coi* gene, indicates that such region had been highly conserved during evolution. However, within the region, there were nucleotide, that could be used in order to discriminate fish eggs according to their provenance. The multiple alignment of PCR products in the region of *coi* obtained from fish eggs samples revealed *Hin*fI, *Alu*I, *Pae*I, and *Aqu*III restriction sites which could be used to generate distinguishable PCR-RFLP pattern (Table 2). *Hin*fI and *Alu*I generated PCR-RFLP patterns are presented in Figure 4. More in detail, by *Aqu*III enzyme digestion, "avgotaracho Mesolonghiou" can be distinguished from the other two samples from Mauritania and Australia (data not shown). *Aqu*III restriction enzyme (GAGGAG), revealed different fragments of *coi* gene among samples. *Coi* gene of Greek PDO was not digested by *Aqu*III restriction enzyme, while the other two resulted 200–300 bp fragments.

PCR-RAPD analysis of DNA extracted from currants

Figure 5 shows PCR-RAPD fingerprinting of DNA isolated from currants from five different geographical origins, while Figure 6 shows cluster analysis of samples each location. Interestingly, samples from each provenance resulted a unique PCR-RAPD fingerprint. Data shown PCR-RAPD analysis with M13 primer.

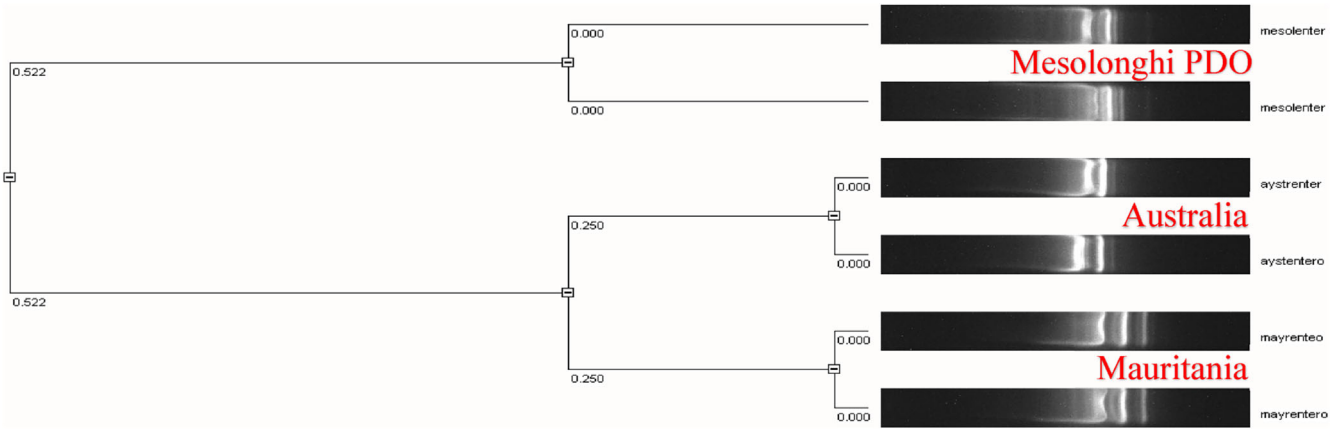

FIGURE 1 Cluster analysis of 16r DNA banding profiles for fish eggs bacterial communities from three geographical origins by PCR-RAPD analysis

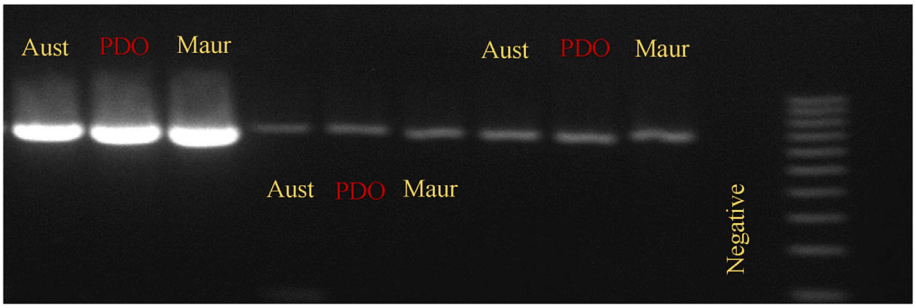

FIGURE 2 PCR products of COI gene of fish eggs from Australia, Mesolonghi PDO, Mauritania amplicon size 655 bp

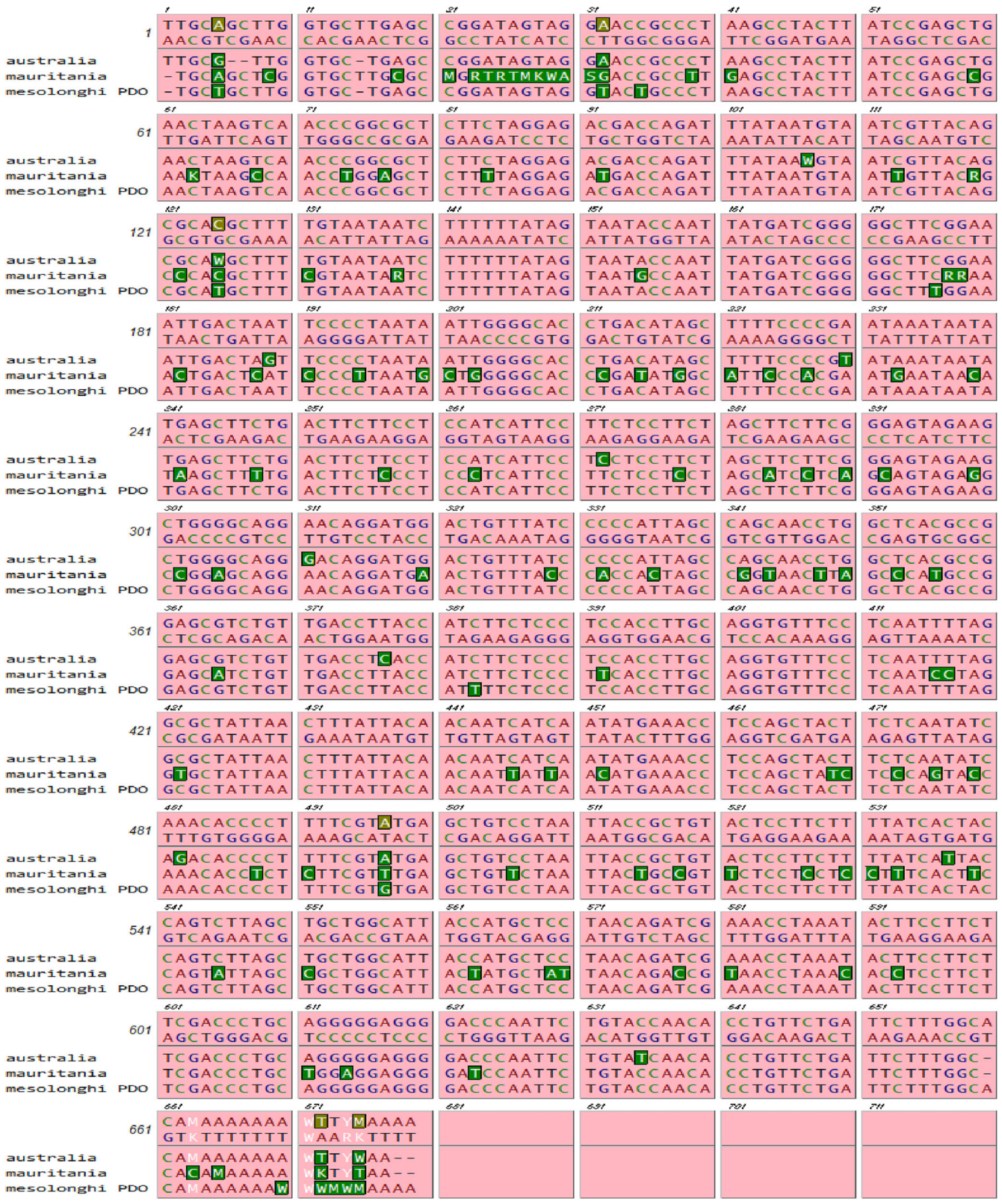

FIGURE 3 Multiple alignment of the sequences of COI gene obtained from fish eggs of *Mugil cephalus* from three different geographical origins

PCR-DGGE analysis of 16s rRNA from currants

The genetic diversity of bacteria in currants from five geographical origins (Nemea, Kalamata, PDO, Zante, Amaliada) was determined and compared by means of PCR-DGGE. PCR-DGGE patterns obtained, revealed individual bands reflecting microorganisms present in samples from five locations. Figure 7 depicts PCR-DGGE profiles of bacterial communities isolated from currants.

TABLE 2 The predicted restriction sites of *Hin*fI, *Alu*I, *Pae*l, and *Aqu*III generated PCR-RFLP of *coi* gene in fish eggs of *Mugil cephalus* from three different geographical origins

	Fragments (bp) restriction sites of enzymes			
Provenance of fish eggs (*M. cephalus*)	***Hin*fI (GANTC)**	***Alu*I (AGCT)**	***Pae*l (GCATG/C)**	***Aqu*III (GAGGAG)**
Australia	646	53,215,240,278,296,461,497,545	N.D	272
Mesolonghi PDO (Greece)	647	54,216,241,279,297,462,498,546	120	N.D
Mauritania	183,648	4,76,242,461,497,545	N.D	277,526,529,612

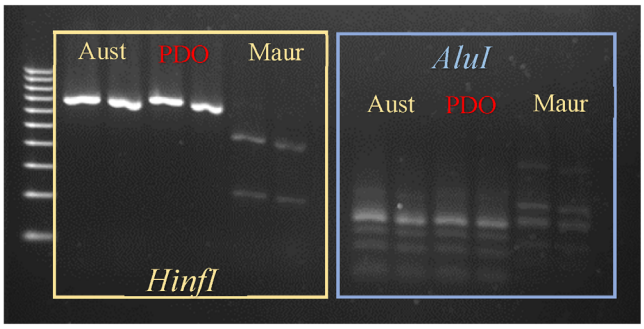

FIGURE 4 PCR-RFLP results of coi gene of two replicates of Australia, Mesolonghi, Mauritania fish eggs. (a) *Hin*f1. (b) *Alu*I

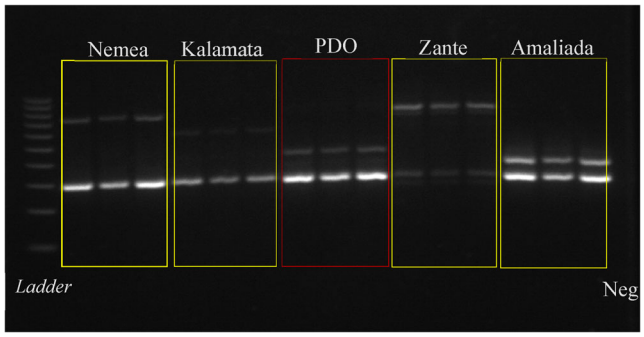

FIGURE 5 PCR-RAPD profiles analysis for genomic DNA of currants from five different geographical origins

DISCUSSION

Several scientists have already utilized DNA-based methods in order to analyze either bacteria or fungi communities of foodstuff or genomic DNA among samples. DNA-based techniques considered to be the most appropriate and accurate regarding food adulteration.[38,39]

In our study, we selected two popular Greek PDO products (avgotaracho Mesolonghiou and Vostizza currant) and we examined if biological barcodes could be settled in order to protect them from counterfeits. The effort of our study was to optimize an appropriate, rapid and accurate method for these Greek PDO products' traceability and authenticity. Therefore, we examine and analyze both bacteria populations and genomic DNA of food products by DNA-based approaches. PCR-RAPD was conducted for analyzing differences in Enterobacteria species, present in fish eggs, while the same method was performed for pointing out variances in genomic DNA of currants. Furthermore, PCR-RFLP was used for analysis of mitochondrial *coi* gene fragment of fish eggs. Finally, PCR-DGGE technique was applied in bacterial microflora of currants, in order to detect representative strains from each provenance.

At first, in our work, we proved that populations of *Enterobacteriaceae* isolated from fish eggs and genomic DNA from currants from different geographical locations can be differentiated by PCR-RAPD analysis. RAPD profiles of *Enterobacteriaceae* isolated from fish eggs revealed genetic diversity among samples. Discrimination ability of PCR-RAPD method is unlimited because of the use of a variety of random primers. In this study, we tested five primers (OPA10, OPA15, M13, 1290, 1217) and finally we selected OPA10 for fish eggs samples analysis, as amplification with OPA10 primer resulted better band profiles, while M13 primer was selected for currants DNA amplification. PCR-RAPD band profiles of currants samples proved to be unique for each sample and can clearly be discriminated regarding their provenance. There are several studies that have already utilized PCR-RAPD analysis for this purpose, such as geographical origin of sea cucumber, authentication of tissues of animal origin, authentication of plant *Senna angustifolia* or herb *Lonicera japonica*.[40–43]

Furthermore, analysis of PCR-RFLP results after digestion with selected restriction enzymes provided a rapid discrimination technique among samples. This method can be applied as a tool for improving mislabeling issues, traceability, and authentication. Determination of beef-jerky species variation, authentication of camel meat and shrimp species are some recent applications of PCR-RFLP method.[44–46] In the present study, sequencing analysis of *coi* gene of fish eggs, and digestion with selected restriction enzymes was performed and proved to be efficient for authentication purposes. From the above results, it is clear that Greek PDO "avgotaracho Mesolonghiou" can be fully discriminated from other samples by restriction enzymes and PCR-RFLP protocol. More specific, the amplicons were digested with four restriction endonucleases (*Hin*fI, *Alu*I, *Pae*l, *Aqu*III) that were selected based on sequencing analysis of *coi* gene. Different levels of polymorphism were detected among samples from different geographical locations. The level of *coi* variation revealed using *Aqu*III was sufficient to generate specific restriction profiles that could distinguish Greek PDO, while *Hin*fI, *Alu*I, and *Pae*l generated different restriction profiles among samples from Mesolonghi and Mauritania.

As far as currants traceability concerns, both PCR-RAPD and PCR-DGGE analysis proved to be very promising for investigating

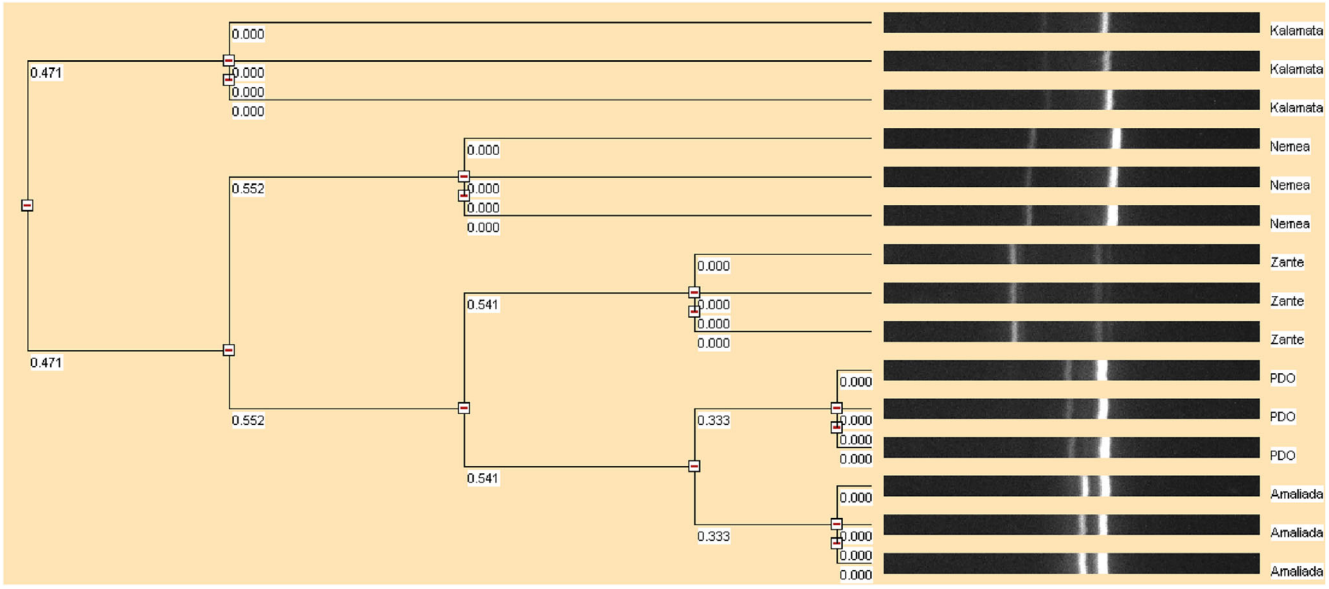

FIGURE 6 Cluster analysis of genomic DNA banding profiles for currants bacterial communities from five geographical origins. Three replicates from each location

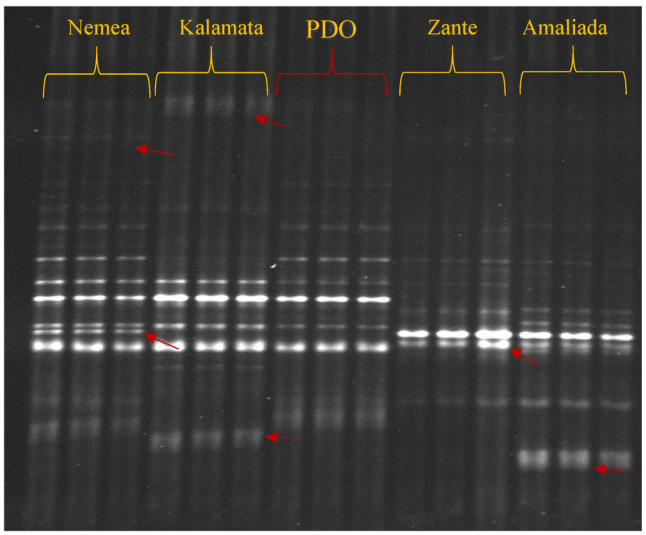

FIGURE 7 PCR-DGGE 16s rRNA band profiles of currants for five different geographical origins

potential biological barcodes. DGGE fingerprinting was used in this study to determine the geographical origin of currants from five geographical origins and to indicate potential biological markers for currants' traceability, as well. PCR-DGGE technique has widely demonstrated for foodstuff authentication and determination of geographical origin. Traditional Wielkopolska fried ripened curd cheese, or marine salt, *Oreochromis niloticus* from lakes of Cameroon, cultured seabass *Dicentrarchus labrax* are food matrixes that have analyzed by PCR-DGGE in terms of food traceability.[18,21,47,48] In this study, results highlighted the dominant species of bacteria populations present in currants. PCR-DGGE profiles of bacteria communities of currants could be demonstrated as possible biological markers of PDO products. However, more studies with a greater number of samples are necessary, in order to identify and analyze sequences of dominant microorganisms in samples according to their geographical origin.

Considering the importance of global food trade and the economic impact to food industry, it is essential need to address challenges regarding food authenticity and traceability. Therefore, obtained results indicate that DNA-based tools can be applied in Greek PDO food products and could reveal information about their provenance by identifying unique biological markers. The present study, reported for the first time, the application of DNA-based techniques for certifying origin and identity of Greek PDO products by means of biological markers. Thus, adoption of unique biological markers of PDO and PGI products could be the key for "farm to table" mission.

CONCLUSION

In summary, this was the first study to successfully applied DNA-based methods for Greek PDO (Avgotaracho Mesolonghiou and Vostizza currant) traceability and authentication. Considering the results of this study, the adoption of DNA-based tools seems to be very promising for determination of food geographical origin or authentication among different samples. In terms of global food trade, the implementation of these techniques in food industry or in quality authority's laboratories could provide a great impact regarding quality schemes, food labeling, food safety, and food fraud incidents. Further DNA analysis and application to a variety of foodstuff in a large scale, may be the proper solution to manage adulterations and to strengthen consumers' confidence, as well.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

1. Belletti G., Burgassi T, Manco E, Marescotti A, Pacciani A, Scaramuzzi S. The roles of geographical indications (PDO and PGI) on the internationalisation process of agro-food products. 105th EAAE Seminar International Marketing and International Trade of Quality Food Products, Bologna, Italy. 2007:517–40.
2. Bonetti E, Mattiacci A, Simoni M. Communication patterns to address the consumption of PDO products. Br Food J. 2020;122:390–403.
3. Dimitrakopoulou M-E, Vantarakis A. Does traceability lead to food authentication? A systematic review from a European perspective. Food Rev Int. 2021;1–23.
4. Di Pinto A, Mottola A, Marchetti P, Savarino A, Tantillo G. Fraudulent species substitution in e-commerce of protected denomination origin (pdo) products. J Food Compos Anal. 2019;79:143–7.
5. Ahmed N, Sangale D, Tiknaik A, Prakash B, Hange R, Sanil R, et al. Authentication of origin of meat species processed under various Indian culinary procedures using DNA barcoding. Food Control. 2018;90:259–65.
6. Galimberti A, De Mattia F, Losa A, Bruni I, Federici S, Casiraghi M, et al. DNA barcoding as a new tool for food traceability. Food Res Int. 2013;50:55–63.
7. Lockley AK, Bardsley RG. DNA-based methods for food authentication. Trends Food Sci Technol. 2000;11:67–77.
8. Mafra I, Ferreira IMPLVO, Oliveira MBPP. Food authentication by PCR-based methods. Eur Food Res Technol. 2008;227:649–65.
9. Costa J, Mafra I, Oliveira MBPP. Advances in vegetable oil authentication by DNA-based markers. Trends Food Sci Technol. 2012;26:43–55.
10. Marieschi M, Torelli A, Poli F, Sacchetti G, Bruni R. RAPD-based method for the quality control of mediterranean oregano and its contribution to pharmacognostic techniques. J Agric Food Chem. 2009;57:1835–40.
11. Mojekwu, T. O. & Megbowon, I. Genetic status of tilapia at Badore site using RAPD markers. 28th Annual Conference of the Fisheries Society of Nigeria (FISON). 2013:214–6.
12. Lin CC, Tang PC, Chiang HI. Development of RAPD-PCR assay for identifying Holstein, Angus, and Taiwan yellow cattle for meat adulteration detection. Food Sci Biotechnol. 2019;28:1769–77.
13. Murugaiah C, Noor ZM, Mastakim M, Bilung LM, Selamat J, Radu S. Meat species identification and halal authentication analysis using mitochondrial DNA. Meat Sci. 2009;83:57–61.
14. Fajardo V, González I, López-Calleja I, Martín I, Hernández PE, García T, et al. PCR-RFLP authentication of meats from red deer (*Cervus elaphus*), fallow deer (*Dama dama*), roe deer (*Capreolus capreolus*), cattle (*Bos taurus*), sheep (*Ovis aries*), and goat (*Capra hircus*). J Agric Food Chem. 2006;54:1144–50.
15. Ram JL, Ram ML, Baidoun FF. Authentication of canned tuna and Bonito by sequence and restriction site analysis of polymerase chain reaction products of mitochondrial DNA. J Agric Food Chem. 1996;44:2460–7.
16. Pascoal A, Barros-Velázquez J, Cepeda A, Gallardo JM, Calo-Mata P. Survey of the authenticity of prawn and shrimp species in commercial food products by PCR-RFLP analysis of a 16S rRNA/tRNAVal mitochondrial region. Food Chem. 2008;109:638–46.
17. Mueller S, Handy SM, Deeds JR, George GO, Broadhead WJ, Pugh SE, et al. Development of a COX1 based PCR-RFLP method for fish species identification. Food Control. 2015;55:39–42.
18. Dufossé L, Donadio C, Valla A, Meile JC, Montet D. Determination of speciality food salt origin by using 16S rDNA fingerprinting of bacterial communities by PCR-DGGE: an application on marine salts produced in solar salterns from the French Atlantic Ocean. Food Control. 2013;32:644–9.
19. Le Nguyen DD, Ngoc HH, Dijoux D, Loiseau G, Montet D. Determination of fish origin by using 16S rDNA fingerprinting of bacterial communities by PCR-DGGE: an application on Pangasius fish from Viet Nam. Food Control. 2008;19:454–60.
20. Arcuri EF, El Sheikha AF, Rychlik T, Piro-Métayer I, Montet D. Determination of cheese origin by using 16S rDNA fingerprinting of bacteria communities by PCR-DGGE: preliminary application to traditional Minas cheese. Food Control. 2013;30:1–6.
21. Rychlik T, Szwengiel A, Bednareka M, Arcuri E, Montet D, Mayo B, et al. Application of the PCR-DGGE technique to the fungal community of traditional Wielkopolska fried ripened curd cheese to determine its PGI authenticity. Food Control. 2017;73:1074–81.
22. di Rienzo V, Miazzi MM, Fanelli V, Savino V, Pollastro S, Colucci F, et al. An enhanced analytical procedure to discover table grape DNA adulteration in industrial musts. Food Control. 2016;60:124–30.
23. Pasqualone A, Montemurro C, di Rienzo V, Summo C, Paradiso VM, Caponio F. Evolution and perspectives of cultivar identification and traceability from tree to oil and table olives by means of DNA markers. J Sci Food Agric. 2016;96:3642–57.
24. Dimitriou E, Katselis G, Moutopoulos DK, Milios K, Malamis A, Koutsikopoulos C. Description of the processing stages of a protected designation of origin fish product: the Greek caviar ‘Avgotaracho Messolongiou’. Agric Econ Rev. 2016;17:50–62.
25. (h) Labelling: PDO " MESSOLOGI example, the code ME-00005 / 94 . with, for (i) National requirements: (if any) Implementation as applicable of the general provisions of Presidential EEC NO: Date of receipt of the application: ../. **21**, 1–2.
26. Nikolidaki EK, Chioua A, Christea M, Gkegka AP, Karvelas M, Karathanosa VT. Sun dried Corinthian currant (*Vitis Vinifera* L., var. Apyrena) simple sugar profile and macronutrient characterization. Food Chem. 2017;221:365–72.
27. Chiou A, Panagopoulou EA, Gatzali F, De Marchi S, Karathanos VT. Anthocyanins content and antioxidant capacity of Corinthian currants (*Vitis vinifera* L., var. Apyrena). Food Chem. 2014;146:157–65.
28. Effie V, Antonia T. Greek raisins: a traditional nutritious delicacy. J Berry Res. 2014;4:117–25.
29. Yanni AE, Efthymiou V, Lelovas P, Agrogiannis G, Kostomitsopoulos N, Karathanos VT. Effects of dietary Corinthian currants (*Vitis vinifera* L., var. Apyrena) on atherosclerosis and plasma phenolic compounds during prolonged hypercholesterolemia in New Zealand white rabbits. Food Funct. 2015;6:963–71.
30. Papadaki A, Kachrimanidou V, Lappa IK, Eriotou E, Sidirokastritis N, Kampioti A, et al. Mediterranean raisins/currants as traditional

superfoods: processing, health benefits, food applications and future trends within the bio-economy era. Appl Sci. 2021;11:1605.
31. Dimitrakopoulou M-E, Kotsalou C, Stavrou V, Vantarakis A. Advancing quality control of food samples by next generation sequencing compared to culture-dependent techniques. J Food Sci Nutr Res. 2021;4:118–30.
32. Venieri D, Vantarakis A, Komninou G, Papapetropoulou M. Differentiation of faecal Escherichia coli from human and animal sources by random amplified polymorphic DNA-PCR (RAPD-PCR). Water Sci Technol. 2004;50:193–8.
33. Thomas PC, Divya PR, Chandrika V, Paulton MP. Genetic characterization of Aeromonas hydrophila using protein profiling and RAPD PCR. Asian Fish Sci. 2009;22:763–71.
34. Koche M, Gade R, Kothikar R, Tekade A. Biochemical studies on genotypic characterization of *Pseudomonas fluorescens* isolates by PCR-RAPD analysis. Int J Chem Stud. 2020;8:2915–7.
35. Ward RD, Zemlak TS, Innes BH, Last PR, Hebert PDN. DNA barcoding Australia's fish species. Philos Trans R Soc B Biol Sci. 2005;360:1847–57.
36. Øvreås L, Forney L, Daae FL, Torsvik V. Distribution of bacterioplankton in meromictic lake Saelenvannet, as determined by denaturing gradient gel electrophoresis of PCR-amplified gene fragments coding for 16S rRNA. Appl Environ Microbiol. 1997;63:3367–73.
37. Muyzer G, De Waal EC, Uitterlinden AG. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl Environ Microbiol. 1993;59:695–700.
38. Rasmussen RS, Morrissey MT. DNA-based methods for the identification of commercial fish and seafood species. Compr Rev Food Sci Food Saf. 2008;7:280–95.
39. Böhme K, Calo-Mata P, Barros-Velázquez J, Ortea I. Review of recent DNA-based methods for Main food-authentication topics. J Agric Food Chem. 2019;67:3854–64.
40. Yun Z, Sun Z, Xu H, Sun Z, Zhang Y, Liu Z. Identifying the geographical origin of protected sea cucumbers (*Apostichopus japonicus*) in China using random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR). Food Sci Biotechnol. 2017;26:357–62.
41. Rastogi G, Dharne MS, Walujkar S, Kumar A, Patole MS, Shouche YS. Species identification and authentication of tissues of animal origin using mitochondrial and nuclear markers. Meat Sci. 2007;76:666–74.
42. Khan S, Mirza KJ, Al-Qurainy F, Abdin MZ. Authentication of the medicinal plant Senna angustifolia by RAPD profiling. Saudi J Biol Sci. 2011;18:287–92.
43. Fu J, Yang L, Khan MA, Mei Z. Genetic characterization and authentication of *Lonicera japonica* Thunb. By using improved RAPD analysis. Mol Biol Rep. 2013;40:5993–9.
44. Chen SY, Liu YP, Yao YG. Species authentication of commercial beef jerky based on PCR-RFLP analysis of the mitochondrial 12S rRNA gene. J Genet Genomics. 2010;37:763–9.
45. Vaithiyanathan S, Vishnuraj MR, Narender Reddy G, Srinivas C. Authentication of camel meat using species-specific PCR and PCR-RFLP. J Food Sci Technol. 2020;58:3882–9. https://doi.org/10.1007/s13197-020-04849-w
46. Wilwet L, Jeyasekaran G, Shakila RJ, Sivaraman B, Padmavathy P. A single enzyme PCR-RFLP protocol targeting 16S rRNA/tRNAval region to authenticate four commercially important shrimp species in India. Food Chem. 2018;239:369–76.
47. Tatsadjieu NL, Maïworé J, Hadjia MB, Loiseau G, Montet D, Mbofung CMF. Study of the microbial diversity of *Oreochromis niloticus* of three lakes of Cameroon by PCR-DGGE: application to the determination of the geographical origin. Food Control. 2010;21:673–8.
48. Pimentel T, Marcelino J, Ricardo F, Soares AMVM, Calado R. Bacterial communities 16S rDNA fingerprinting as a potential tracing tool for cultured seabass *Dicentrarchus labrax*. Sci Rep. 2017;7:11862.

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