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Original Article

Comparison of different DNA isolation methods and use of dodecyle trimethyl ammonium bromide (DTAB) for the isolation of DNA from meat products

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ABSTRACT

Objective: The identification of meat species in meat products is important for protection of human health, economic reasons, religious factors and for controlling the compliance with food regulations. For this purpose, DNA must be obtained in good quality and quantity. The aim of this study was to compare different DNA isolation methods from different meat products.
Materials and methods: Comparison among different DNA isolation methods was done. DNA was isolated from different meat products (*e.g.*, sucuk, salami, sausage, braised meet, meatball and pastrami). The methods included phenol/chloroform, DNA isolation kit, Cetyl Trimethyl Ammonium Bromide (CTAB) and Dodecyle Trimethyl Ammonium Bromide (DTAB).
Results: Although DNA was obtained from all of these methods, the phenol/chloroform and DNA isolation kit methods were found to be the most effective methods for obtaining high quantity DNA. RNA contamination was determined to be common in DTAB method. High quantity of DNA and RNA contamination in terms of quality was detected in CTAB method. Ruminant specific 16S rRNA primer was used to amplify genomic DNA by polymerase chain reaction and all samples were amplified except for some samples of DTAB.
Conclusion: DNA isolation kit, another best method, is recommended due to quality and quantity of DNA for researchers who do not want that phenol/chloroform method have toxic substances. This study is also the first study in which DTAB method is used for DNA extraction from meat products.

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KEYWORDS

CTAB, DTAB, Meat products, Phenol-Chloroform

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INTRODUCTION

Meat is rich in proteins, vitamins, minerals such as iron, phosphor, zinc, and copper, which have a high biological value among the foods of animal origin; and also it is an appetizing, delicious and satisfactory food. Since meat includes exogen amino acids that cannot be synthesized by the body at an adequate and balanced amount, it has an important role in human nutrition. For this reason, meat or meat products must definitely be consumed by human beings (Arslan, 2013).

Sucuk, salami, and sausages are open to adulteration because of their production methods and the texture of the raw materials used. As the price of the meat and meat products increases, many adulterations may be observed in these products without considering the human health. Definition of the meat types used in meat products is important in terms of economic reasons, religious factors, the confirmation of the labels and preventing unjust competition (Sincer et al., 2010; İlhak and Güran, 2015). It has been reported that the methods that are based on sensory qualities, anatomical differences, properties of the tissue and fat, histological properties, the amount of the glycogen in the meat are used in separation of meat and meat products. Also, some immunological, electrophoretic, serologic and genetic methods are reported to be used for this purpose (Hitchock and Crimes, 1985; Ekici and Akyüz, 2003; İlhak and Arslan, 2007a,b; Günşen et al., 2009).

In order to define the types of the meats with various molecular methods, which will be applied with genetic material; first of all, genomic DNA with a high molecular weight (of good quality and amount) must be obtained in a pure way. The DNA isolation method basically consists of three main successive stages which are- 1) Revealing DNA with high molecular weight with the lysis of the cell; 2) Separation of the DNA-protein complex, and having the DNA in soluble state using denaturation or proteolyses process; 3) Separation of the DNA from proteins, RNA and other macromolecules using simple enzymatic and/or chemical methods. The definition of the concentration of the DNAs that are obtained by using different methods is measured by using spectrophotometric methods that are based on absorption. The purity of the DNA molecule measured by spectrophotometer is obtained by rating the values obtained at 260 and 280 nm wavelengths (Topal Sarıkaya, 2004).

In recent years, identification of meat species in meat products obtained from various sales points of different cities by using different methods has become an important matter (Dalmasso et al., 2004; Özgen-Arun et al., 2014; Hou et al., 2015; Stamatis et al., 2015; Özşensoy and Şahin, 2016; Yin et al., 2016). For these studies, the first process is the isolation of the DNA, and it has been performed by using different methods such as DNA isolation kit (Özgen-Arun et al., 2014) and phenol/ chloroform (Krieg et al., 1983; Koh et al., 1998; İlhak and Arslan, 2007b; İlhak and Güran, 2015).

It has been reported in recent studies that DNA hybridization and PCR-based methods are used commonly for identification of meat species in meat products and examining the vegetable protein mixtures (Rahmati et al., 2016). For this reason, it is necessary that firstly, the purity of DNA with high concentration must be obtained in order to use the methods. The objective of our study was to compare four different DNA isolation methods in six different meat products.

MATERIALS AND METHODS

The material of the study consists of sucuk, salami, sausage, braised meat, meatball, and pastrami samples (**Table 1**). The DNA isolation was carried out in these six different meat products by using four different isolation methods. The DNAs obtained were amplified by using 104 base pair (bp)-long ruminant specific 16S rRNA primer (F: 5'-GAAAGGACAAGAGAAATAAGG-3', R: 5'-TAGGCCCTTTTCTAGGGCA-3') (Dalmasso et al., 2004) by polymerase chain reaction (PCR) and the usability of the DNAs was investigated.

DNA isolation methods: Small parts were taken from different points of the meat samples, and were mixed to homogenize. Then, one hundred mg of the samples were taken from this mixture, and the methods were performed. In the DTAB method, one hundred mg sample was taken and the analysis was performed. Another twenty mg sample was taken because the sample amount was in an excessive amount and there was no full-separation in the phases in the supernatant taking stage. The lower phase should also be taken when the supernatant is being taken in order to realize full separation of the phases and to take the supernatant with ease.

DNA isolation using DNA extraction kit: One hundred mg (A) from meat samples was taken. The gSYNC™ DNA Extraction Kit (Geneaid Biotech Ltd., Taiwan) was used for extraction of DNA from the samples. DNA isolation was performed according to manufacturer's protocol. 100 mg meat sample was taken and transferred to a 1.5 mL micro centrifuge tube. Two hundred µL of GST Buffer and 20 µL of Proteinase K

(20 mg/mL) were added and vortexed thoroughly, and then the tube was incubated at 60°C overnight. After incubation, the tube was centrifuged for two min at 16000xg and the supernatant was carefully transferred to a new 1.5 mL micro centrifuge tube. Two hundred µL of GSB Buffer was added to tubes and then shaken vigorously for 10 sec. Two hundred µL of absolute ethanol was added to the sample and mixed immediately by shaking vigorously for 10 sec. A GD Column was placed in 2 mL Collection Tube and all of the mixture (including any insoluble precipitate) was transferred to the GD Column. The tube was centrifuged at 16000xg for 1 min. Collection Tube was discarded then the GD Column was transferred to a new 2 mL Collection Tube. Four hundred µL of W1 Buffer was added to the GD Column and centrifuged at 16000xg for 30 sec then discarded the flow-through. The GD Column was placed back to Collection Tube. Six hundred µL of Wash Buffer was added to the GD Column and centrifuged at 16000xg for 30 sec and then discarded. The GD Column was placed back to Collection Tube and centrifuged again for 5 min at 16000xg to dry the column matrix. One hundred µL of pre-heated Elution Buffer was added into the center of the column matrix. The tube was kept at least 3 min to ensure that Elution Buffer is completely absorbed, and it was centrifuged at 16000xg for 30 sec to elute the purified DNA. The tube was stored along one day at +4°C. The DNA sample more than 100 ng/µL was diluted to a concentration of 100 ng/µL.

DNA isolation using DTAB method: One hundred mg (B1) and twenty mg (B2) from meat samples were taken and Dodecyl Trimethyl Ammonium Bromide (DTAB) method was performed by using isolation as previously mentioned (Kurar et al., 2012). Protocol procedure: 100 mg or 20 mg meat sample was taken and transferred to a 1.5 mL micro centrifuge tube. Eight hundred µL of Nuclear Lysis Buffer (12 g DTAB, 45 mL 5 M NaCl, 15 mL 1 M Tris-HCl pH 7.5, 15 mL 0.5 M EDTA pH 8.0, complete to 100 mL with distilled water) was added and vortexed thoroughly, then incubated at 55°C overnight. After vortexing, 800 µL of chloroform was added and vortexed again. The tube was centrifuged at 12000x rpm for 5 min at +4°C and the supernatant was transferred to a new tube. One mL of 95% ethanol was added and mixed by shaking, and centrifuged at 17000 x rpm for 10 min at +4°C. The supernatant was poured and washed to the pellet with 70% ethanol, again. The tube was centrifuged at 17000 x rpm for 3-5 min at +4°C. The step was repeated with ethanol. The supernatant was removed and the pellet was dried. The pellet was re-suspended 400 µL and 100 µL of TE (10 mM Tris, 1 mM EDTA, pH: 8.0) buffer for 100 and 20 mg of samples, respectively. Then, the tube was stored along one day at +4°C.

DNA isolation using CTAB method: One hundred mg (C) from meat samples was taken and Cetyl Trimethyl Ammonium Bromide (CTAB) method was performed for extraction of DNA. Protocol procedure; 100 mg meat sample was taken and transferred to a 1.5 mL micro centrifuge tube. Two hundred and fifty µL of lysis buffer (0.25% SDS, 0.1 M EDTA pH 8.0) and 3 µL Proteinase K (10 mg/mL) were added, then vortexed thoroughly. The tube was incubated at 55°C for 20 min. Seventy-five µL of 3.5 M NaCl was added and mixed. Four-two µL 10% CTAB/0.7 M NaCl heated at 55°C was added, and mixed well by vortexing, and incubated at 65°C for 10 min. Four hundred µL of chloroform was added and vortexed. The tube was centrifuged at the highest setting (20000xg) for 5 min. The supernatant was transferred to a new micro centrifuge tube. Four hundred µL of phenol:chloroform:isoamyl alcohol (25:24:1) was added and mixed thoroughly up and down. The tube was centrifuged at 14000xg for 10 min at +4°C. The supernatant was transferred to a new micro centrifuge tube. Four hundred µL of 100% ethanol was added and mixed by shaking and incubated at room temperature for 5 min. The tube was centrifuged at 10 000 x rpm for 10 min at +4°C. The supernatant was poured and the step was repeated by adding 100% ethanol. The pellet was dried and fifty µL of TE was added. The tube was stored along one day at + 4°C. The DNA sample more than 100 ng/µl was diluted to a concentration of 100 ng/µL.

DNA isolation using phenol / chloroform method: One hundred mg (D) from meat samples was taken and one hundred µL of TE was added and then the sample was extracted by using a standard organic phenol/ chloroform method (Sambrook et al., 1989). The tube was stored along one day at +4°C. The DNA sample more than 100 ng/µL was diluted to a concentration of 100 ng/µL.

Absorbance definition of the DNA bands and the observing in the gel: In order to measure the purity levels of the DNA samples whose extractions were made, the optic densities of the samples at 260 nm and 280 nm wavelengths were measured by using a nanodrop spectrophotometer (mySPEC, VWR). The DNA samples were observed by using 0.6% agarose gel. Five µL DNA samples, 5 µL bidistillated water and 5 µL dye were mixed and loaded to gel. The DNA samples loaded to gel were separated at 100 V for 90 min. After this procedure was completed, the gel was observed with UV light in gel imaging system (Vilber Lourmat Quantum ST4).

Polymerase chain reaction method: Polymerase chain reaction (PCR) was carried out in 15 µL reaction volume including 1xMg^{++} free PCR buffer (Biolabs, NEB), 0.200 mM dNTPs (Biolabs, NEB), 1.5 mM $MgCl^{++}$ (Biolabs, NEB), 0.375 units of *Taq* polymerase (Biolabs, NEB), 5 pMol each primer (Dalmasso et al., 2004) and 60 to 100 ng of genomic DNA.

The prepared PCR product was amplified using a touchdown PCR profile (Don et al., 1991) in thermal cycler (Bio Rad T-100) device. Touchdown PCR profile was used with two steps. The first step was initial denaturation at 95°C for 4 min, followed by 16 cycles of denaturation at 95°C for 30 sec, annealing beginning at 60°C and ending at 52°C for 30 sec and extension at 68°C for 30 sec. The annealing temperature was decreased 0.5°C per cycle until it reached 52°C. At the second step, 25 cycles of 95°C for 30 sec, 52°C for 30 sec and 68°C for 30 sec was applied. The final extension of 68°C for 5 min was applied in all reactions. The amplified PCR products were separated with 100 V for 60 min and loaded onto electrophoresis device (CBS Scientific) with 2% agarose gel and visualized on 365 nm UV.

RESULTS

In this study, the DNA isolation was carried out by using four different isolation methods in six different meat products (sucuk, sausage, salami, braised meat, meatball, and pastrami samples). DNA purity was measured by calculating the ratio of absorbance at 260 to 280 nm wavelengths, and the values were summarized in **Table 1**. When the table is examined it is observed that generally high quality DNA was obtained at a high concentration with the isolation samples. It has been observed that the highest DNA concentration was found in samples, braised meat and pastrami, and in methods that phenol/chloroform and CTAB in spite of RNA contamination. It has also been determined that DNA with good quality and adequate concentration was obtained in the DNA isolation kit. It was determined that even in four different isolations (by using RNAse and at different amounts) in DTAB method, which was applied by using RNAse, there was RNA contamination. While the DNA amount obtained by DTAB method is relatively lower than other methods, obtained amounts are deemed adequate. **Table 1** summarizes mathematical comparisons of obtained average DNA amounts by different methods. When all the methods are considered, it is observed that the best methods are phenol/chloroform and DNA isolation kit in terms of average DNA concentrations as well as their quality.

The DNA samples that were obtained by DNA isolation methods were observed by using 0.6% agarose gel (**Figure 1**). When a gel image was considered, it was observed that there were smears. These smears show that there are fractures in DNAs.

The DNAs that were obtained from the meat products were amplified by using cattle specific 16S rRNA primers with PCR method (**Figure 2**). Some of the samples which were performed by two different amounts of DTAB method were not amplified. The PCR product was amplified in all samples obtained using the Kit, CTAB and phenol/chloroform methods.

DISCUSSION

It has been reported that taking one hundred mg sample will be adequate in molecular techniques worked on meat products (Özatay, 2012). One hundred mg meat sample was used as standard in all methods in this study. The PCR process was carried out with the DNA obtained as a result of the methods used, and adequate results was obtained.

It has been reported that two methods, which are CTAB as an organic method, and the commercial kit, are the most used methods in meat products. In CTAB method, concentration of DNA was high, and the quality was low (Pinto et al., 2007; Özatay, 2012). Similarly, it was determined that although high concentration of DNA was obtained with the CTAB method in this study, there was RNA contamination.

It has also been reported that as a result of the two methods (DTAB and phenol/chloroform) used in rendering products obtained by exposing to high heat, DNA was obtained and amplified successfully using the PCR method (Kurar et al., 2012). Similarly, in this study, it was determined that all the samples that were obtained using the phenol/chloroform method, and many of the samples that were obtained using DTAB were amplified using PCR. Some of the samples which were performed by using two different amounts of DTAB method (one hundred and twenty mg) were not amplified. The reason for this was considered to be the DNAs obtained with DTAB were dissolved in high-amount buffer, and yet there was still unsolved residue in the tube; and for this reason, the PCR was not obtained in some of the samples.

The phenol/chloroform (Krieg et al., 1983; Koh et al., 1998; Matsunaga et al., 1999; Yetim et al., 2006; İlhak and Arslan 2007b; Kesmen et al., 2007; Kesmen et al., 2010;

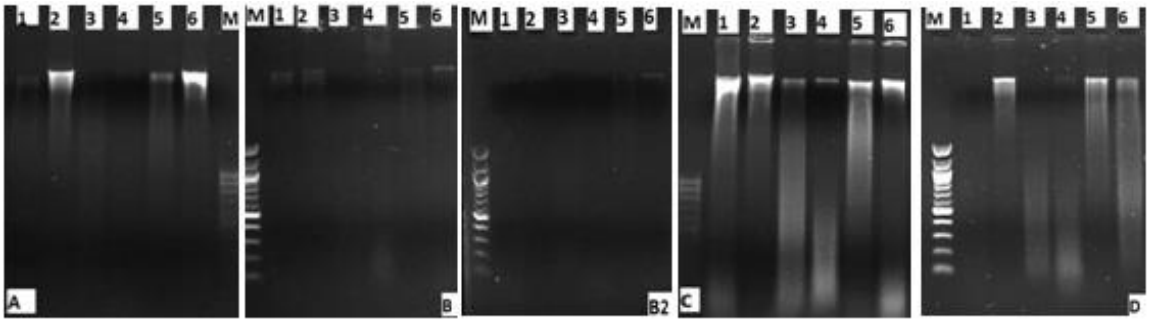

Figure 1. Agarose gel analysis of DNA. A: DNA isolation kit, B: DTAB method (100 mg), B2: DTAB method (20 mg), C: CTAB method, D: Phenol / chloroform method, M: Molecular marker (100 bp), 1) Meatball, 2) Salami, 3) Sucuk, 4) Braised meat, 5) Sausage, 6) Pastrami

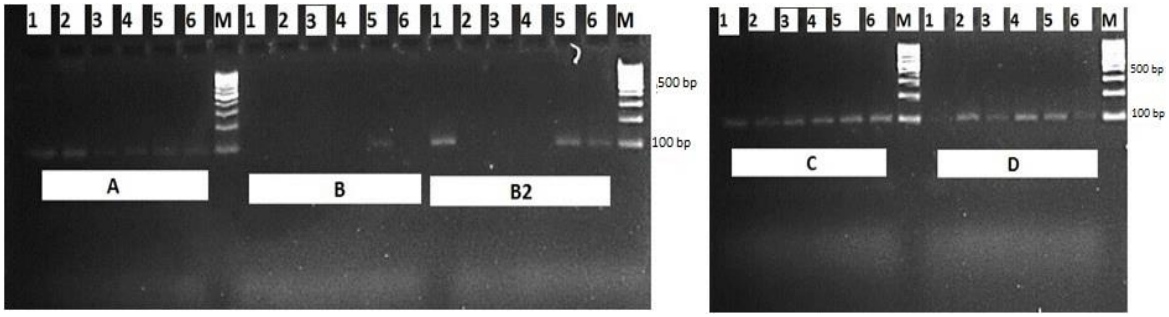

Figure 2. Results of PCR. A: DNA isolation kit, B: DTAB method (100 mg), B2: DTAB method (20 mg), C: CTAB method, D: Phenol / chloroform method, 1) Meatball, 2) Salami, 3) Sucuk, 4) Braised meat, 5) Sausage, 6) Pastrami, M: Molecular marker (100 bp)

Table 1. DNA isolation methods, samples, DNA yield and OD values

DNA isolation Methods	Samples	ng / µL	260/280 OD values
DNA Isolation Kit	Meatball	37.578	1.750
	Salami	71.958	1.799
	Sucuk	51.053	1.787
	Braised meat	154.241	2.077
	Sausage	68.501	1.809
	Pastrami	197.868	1.912
DTAB Method (B)	Meatball	67.266	2.340
	Salami	35.711	2.430
	Sucuk	27.476	2.099
	Braised meat	76.361	1.730
	Sausage	56.102	1.907
	Pastrami	59.209	1.662
DTAB Method (B2)	Meatball	65.093	2.452
	Salami	20.849	3.576
	Sucuk	50.202	2.542
	Braised meat	87.924	2.394
	Sausage	60.612	1.999
	Pastrami	130.274	1.611
CTAB Method	Meatball	423.820	1.940
	Salami	360.284	1.916
	Sucuk	570.127	1.906
	Braised meat	1457.560	2.228
	Sausage	593.343	2.106
	Pastrami	952.918	2.187
Phenol/Chloroform Method	Meatball	86.303	1.672
	Salami	55.124	1.692
	Sucuk	70.897	1.698
	Braised meat	347.503	2.008
	Sausage	92.557	2.000
	Pastrami	338.529	1.418

İlhak and Güran 2015) or DNA isolation kit (Kumar et al., 2011; Kesmen et al., 2012; Mane et al., 2012; Cawthorn et al., 2013; Ulca et al., 2013; Özgen-Arun et al., 2014; Ali et al., 2015; Stamatis et al., 2015; Safdar and Junejo, 2016; Yin et al., 2016) were used in studies, which were conducted for the purpose of isolating DNA and RNA from tissue; and mixtures of other substances were used for the identification of meat species. In this study, four different DNA isolation methods were tested, and DNAs were obtained. It was observed that when DNA yield and quality was considered, the best quality and purity was obtained in the phenol/chloroform method, which was used in previous studies as well, and with the DNA isolation kit. In the DTAB method, although DNA was obtained from meat products, it was not useful in isolation due to the RNA contamination. In addition, DNAs obtained from meat samples using DNA isolation loaded to gel electrophoresis, and obtained traces in the form of a smear in the samples. The reason for this was considered to be the fractures in the DNAs because they were exposed to heat.

CONCLUSION

It has been observed that DNA was obtained in the four different DNA isolation methods, and it may be used successfully in amplified PCR. It has also been observed that the best result was obtained with phenol/chloroform method among the four methods used, and we

recommend that this method may be used successfully in meat products. For the researches who do not want to prefer the phenol/chloroform method because of its toxic effects, the second best method is the DNA isolation kit, which enables qualified and pure DNA isolation.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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