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COMPARISON OF COMMERCIAL DNA KITS AND TRADITIONAL DNA EXTRACTION PROCEDURE IN PCR DETECTION OF PORK IN DRY/FERMENTED SAUSAGES

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SUMMARY

In the present study four commercially available DNA extraction kits (Wizard® Genomic DNA Purification Kit, High Pure PCR Template Kit, DNeasy mericon Food and GeneJET PCR Purification Kit), as well as standard phenol/chloroform isolation technique have been evaluated regarding their concentration, purity and suitability for amplification of porcine DNA in dry/fermented sausages. The isolates were assessed for quantity and quality using spectrophotometer (IMPLEN GmbH, Germany). To verify template usability and quality of isolated DNA, the polymerase chain reaction (PCR) targeting at porcine cytochrome b by species specific primers was used. The comparison of extraction methods revealed satisfactory efficiency and purity of all extraction kits, while with standard phenol/chloroform isolation method high concentrations of DNA with low $A_{260/280}$ were obtained. However, all the investigated techniques proved to be suitable for identification of porcine DNA in dry/fermented sausage. Thus, the standard phenol/chloroform DNA extraction method, as the cost-effective one, can be recommended as a good alternative to more expensive isolation kits when investigating the presence of pork DNA in dry/fermented meat products.

Key-words: dry/fermented sausage, DNA, extraction method, pig

INTRODUCTION

Meat products, especially traditional ones are often being adulated due to the high prices they achieve on the market. However, adding even the smallest amounts of meat into the product other than one stated on declaration is illegal and misleading the consumer. Furthermore, it presents serious health, economical and religious problem. For that reason, identification of meat species in traditional meat products is of great importance. The methods for identification of animal origin are based on electrophoresis, isolectric focusing, chromatography, DNA hybridisation, polymerase chain reaction (PCR), enzyme-linked immunosorbend assay (Ong et al., 2007) and recently used aqueous ionic liquid buffer system (Fujita et al., 2006; Ressmann et al., 2015).

Among these methods PCR based techniques proved to be adequate, as they are fast, reliable and inexpensive. However, as every method, they also have their limitations, especially in cases where much of DNA has been degraded due to changes in pH or temperature (cooking, sterilisation, smoking etc.) foods are often subjected to during the production process (Pascoal et al, 2005; Aslan et al., 2009). This is particularly true for dry/fermented products, such as sausages, where DNA has been subjected to substantial degradation due to salting, smoking and other technological operations involved in making such product. It should be emphasised that DNA quality, purity and quantity has considerable effect in the species identification, and because of that methods for extracting DNA should be carefully selected (Sagi et al., 2009).

There are few methods available for extracting DNA from animal products. First is technique called phenol/chloroform extraction method commonly used for extraction DNA from various samples, but seldom from animal products. It is based on extraction of DNA by adding an equal volume of

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phenol-chloroform to aqueous solution of lysed cells, mixing the phases and allowing them to separate by centrifugation. The extracted DNA is precipitated with alcohol (Ausubel et al., 2000). Although rather laborious, this method is commonly used for DNA extraction because of its rather small price per sample. In recent years, a number of kits for extraction of DNA from different sources have been commercially available. In terms of researching the genetics of the animals, most of them are used for extraction of DNA from mammal blood or tissue. When extracting DNA from those kind of samples high quality DNA with very good yield can be obtained. Price per sample is however higher than in phenol/chloroform extraction method.
In this paper, yield, purity and suitability of two DNA extraction methods (spin column-based and conventional phenol/chloroform extraction procedure), as well as efficiency of four commercially available DNA extraction kits in PCR detection of pork in dry/fermented sausages have been evaluated.

MATERIAL AND METHODS

The investigation was carried out on 10 samples of Croatian traditional dry/fermented sausage called "kulenova seka" produced from pork, salt, garlic, red paprika and pepper, filled in natural casing and exposed to smoking, drying and ripening for approximately 40 days.
DNA was extracted from the sausage using the following commercially available DNA extraction kits: Wizard® Genomic DNA Purification Kit (Promega, USA), High Pure PCR Template Kit (Roche, Germany), DNeasy mericon Food Kit (Qiagen, Germany) and GeneJET PCR Purification Kit (Thermo Fisher Scientific Brand). DNA was isolated according to the manufacturer's protocol. In brief, all kits are based on use of 40 mg – 2 g of the raw material digested with lysis buffer and proteinase K. After the lysis, the lysate was centrifuged through silica membrane and binded to the columns. After series of washing steps, the pure DNA was finally extracted through dilution.
Also, the DNA was isolated using a standard extraction protocol with phenol-chloroform-isoamil (25:24:21) alcohol (Ausubel et al., 2000). In both methods used, the sausages were homogenized with knife mill using liquid nitrogene, mortar and pestle. Concentration and quality of the obtained DNA was determined by measuring the absorbance at 260 nm. DNA quality (purity) was measured by calculating the ratio of absorbance at 260-280 nm. UV/VIS spectrophotometer Nanophotometer® (IMPLEN GmbH, Germany) was used for spectroscopic analyses. The isolated DNA samples were analysed by PCR evaluation of suitability for amplification of porcine DNA. For the detection of pork DNA, a set of species-specific oligonucleotide primers previously reported by Doosti et al. (2014), which yield a 149 bp PCR fragment at porcine cytochrome b were used. The PCR reaction was set up using SapphireAmp® Fast PCR Master Mix (Takara Bio, Inc., Japan) in a 25.0 µL reaction volume containing 12.5 µL of mastermix, 9.5 µL of ultra-pure water, 1.0 µL of each primer and DNA. The PCR was performed in 30 cycles of denaturation at 98°C for 10 sec, annealing at 59°C and 72°C of elongation in a thermal cycler (Eppendorf Mastercycler Gradient). The obtained PCR products were visualised on a 1.5% agarose gel stained with Olerup SSP® GelRedTM Dropper (Olerup SSP AB, Sweden).

RESULTS AND DISCUSSION

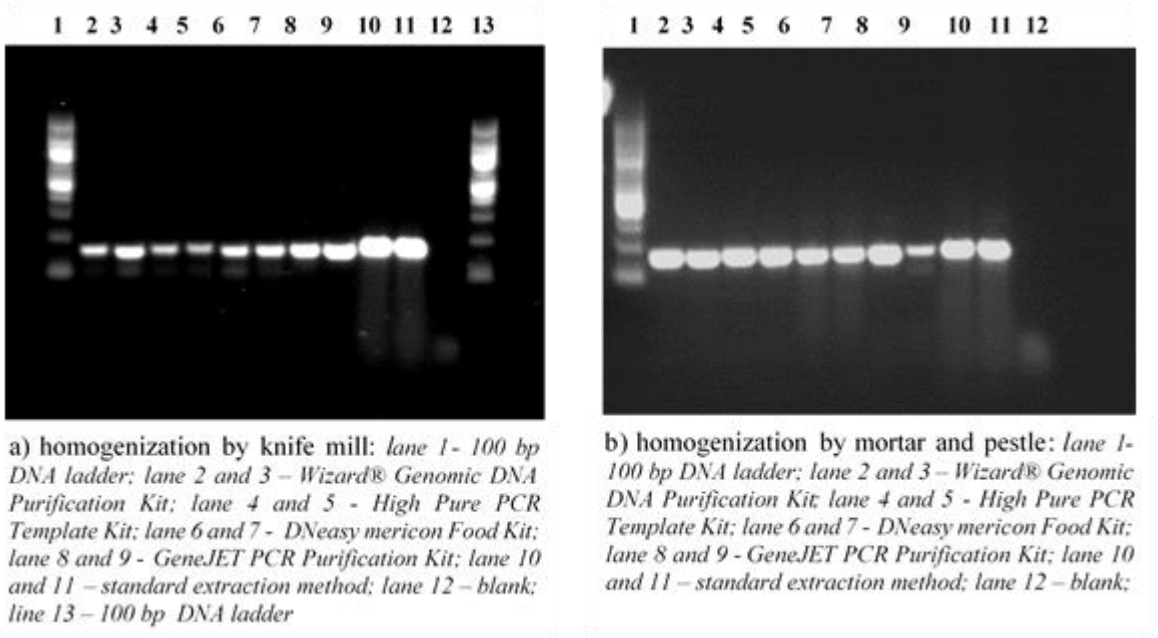
Table 1 presents concentrations and quality of the obtained DNA using of four commercially available DNA extraction kits and standard phenol/chloroform extraction protocol.

Table 1. Concentration and purity of the obtained DNA

Procedure/extraction kit	Homogenisation method	Concentration (ng/µl)	Purity ($A_{260/280}$)
Wizard® Genomic DNA Purification Kit	Knife mill	19.5	1.773
	Mortar and pestle	37.5	1.564
High Pure PCR Template Kit	Knife mill	32.0	1.641
	Mortar and pestle	37.3	1.500
DNeasy mericon Food Kit	Knife mill	126.0	1.780
	Mortar and pestle	164.0	1.777

GeneJET PCR Purification Kit	Knife mill	20.0	1.083
	Mortar and pestle	6.0	0.900
Phenol/chloroform extraction	Knife mill	2123.5	1.149
	Mortar and pestle	1304.0	1.137

As it was expected, the highest concentration of DNA was obtained by conventional phenol extraction method using knife mill for sample homogenisation. However, the purity of DNA was rather unsatisfactory ($A_{260/280}$=1.149 for homogenisation using knife mill and 1.137 for homogenisation using mortar and pestle, respectively) implying a certain amount of phenol in the DNA sample. Among commercially available extraction kits, the highest concentration and DNA purity was obtained using DNeasy mericon Food Kit (both with knife mill and mortar and pestle homogenization method). This was also predictable, as this kit is intended for highly processed foods, where high degradation of DNA can be expected. Amongst the extraction kits originally used for isolation of DNA from muscle tissue or the whole blood, the highest concentration of DNA was obtained with High Pure PCR Template Kit, but the most satisfactory $A_{260/280}$ ratio was obtained with Wizard® Genomic DNA Purification Kit where the sample was homogenized with knife mill. This is opposite to results of Di Pinto et al. (2007), who found a low DNA extraction efficiency of horse meat using Promega Wizard Magnetic DNA Purification for Food kit. In the work of Nesvadbová et al. (2010) aiming at choosing the most effective commercially available DNA extraction kit for chicken identification in different kind of food and feed, the later authors found that the highest DNA yields were obtained by NucleoSpin Food, Wizard Genomic DNA Purification Kit and JetQuick Tissue DNA Spin and the best DNA quality by using NucleoSpin Food, Wizard Genomic DNA Purification Kit and Invisorb Spin Food Kit I (ratio A_{260}/A_{280} close to 1.8). It was concluded that the results are highly depended on different food or feed using and different isolation system.



a) homogenization by knife mill: *lane 1- 100 bp DNA ladder; lane 2 and 3 – Wizard® Genomic DNA Purification Kit; lane 4 and 5 - High Pure PCR Template Kit; lane 6 and 7 - DNeasy mericon Food Kit; lane 8 and 9 - GeneJET PCR Purification Kit; lane 10 and 11 – standard extraction method; lane 12 – blank; line 13 – 100 bp DNA ladder*

b) homogenization by mortar and pestle: *lane 1- 100 bp DNA ladder; lane 2 and 3 – Wizard® Genomic DNA Purification Kit; lane 4 and 5 - High Pure PCR Template Kit; lane 6 and 7 - DNeasy mericon Food Kit; lane 8 and 9 - GeneJET PCR Purification Kit; lane 10 and 11 – standard extraction method; lane 12 – blank;*

Figure 1. The result of primer-specific identification of pig DNA by using commercial kits and standard isolation method (*a*-using knife mill for homogenization; *b*-using mortar and pestle for homogenization)

Figures 1 a) and b) show the obtained PCR fragment using porcine specific primers. It can be noticed that PCR amplification revealed 149 bp PCR product using all commercially available kits and standard phenol/chloroform DNA extraction procedure, regardless the homogenisation method.

CONCLUSION

The results of the present study indicate good efficiency of the all investigated DNA extraction techniques for determination of pig DNA in dry/fermented sausages. Although standard

phenol/chloroform DNA isolation method did not show good $A_{260/280}$ ratio indicating phenol contamination, this contamination can be overcome by diethyl ether extraction or reprecipitation of the gDNA. As this method proved to be reliable and most importantly cost-effective, it can be recommended as a good alternative to more expensive commercial kits when extracting DNA from dry/fermented sausages for detection of pig DNA.

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