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SELECTION OF THE MOST SUITABLE METHOD FOR THE EXTRACTION OF DNA FROM FOODS AND FEEDS FOR SPECIES IDENTIFICATION

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Abstract

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High quality and purity of DNA isolated from food and feed is essential for species identification and has unpredictable influences an effect of analysis. In this study, the efficiency of eight different methods for DNA isolation was investigated. For DNA extraction, the raw chicken meet, ham, sausages, tinned lunch meat, pate, tinned feeds for dogs, complete granulated feeds for dogs and chicken flour were used. Kits of several different producers, i.e.: NucleoSpin Food (Marchery-Nagel), Wizard Genomic DNA Purification Kit (Promega), Invisorb Spin Food Kit I (Invitek), Wizard SV Genomic DNA Purification System (Promega), JetQuick Tissue DNA Spin Kit (Genomed), RNA Blue (Top-Bio), JetQuick Blood & Cell Culture Kit (Genomed), QIAamp DNA Mini Kit and QIAamp DNA Blood Mini Kit (Qiagen) were employed in the study. Gel agarose electrophoresis for primary verification of DNA quality was performed. The isolates were subsequently assessed for quantity and quality using by spectrophotometer Nanodrop 2000 (Thermo Scientific). To verify of template usability and quality of isolated DNA, the polymerase chain reaction (PCR) was used.

Differences between isolated DNA from tinned products and meat, ham, sausage, granulated dog feed and chicken flour were found. In tinned food and feed, the DNA was more degraded, DNA content and DNA purity was lower and also PCR amplification was the most difficult. Overall DNA yield and quality have important influence on PCR products amplification. The best results were obtained with NucleoSpin Food and JetQuick Tissue DNA Spin Kit. DNA extracted by these methods proved highest yields, purity and template quality in all foods and feeds and the results of PCR analysis are excellent reproducible. Analyses showed that results depended on different food or feed using and different isolation system.

The results of this work will be utilized to choose the suitable isolating kit for educational course, which is designed for students and also for following research and analyses.

extraction methods, food, feed, DNA quantity and quality

Species identification of animal tissues in meat products is very important for consumers and pet breeders. Food and feed products must be exactly labelled as to the species they contain. Consumers need high quality products that are labelled correctly in order to assure meat safety (Weibin et al., 2009). Nowadays, various methods for differentiation and identification of meat products are described and discussed. The analyses using nucleic acid have shown to be most appropriate to reveal species in food and feed (Calvo et al., 2001; Girish et al., 2004). The DNA molecule is more thermostable than many proteins and these molecules are present in majority of the cells of organisms (Lockley and Bardsley, 2000). Nevertheless, the basic problem of analysis and detection of animal species in foods and feeds is quantity and quality of the extracted DNA. The DNA molecule suffers degradation due to thermal treatments to which foods and feeds are subjected during the production process such as cooking, sterilisation and extrusion (Saez et al., 2004; Engel et al., 2006; Aslan et al., 2009). Also

the type of liquid added to the product may play a role in the degradation of DNA (Bauer et al., 2003; Chapela et al., 2007). DNA quality, quantity and purity do have substantial effect upon the species identification, therefore the methods for extracting DNA should be carefully selected (Sagi et al., 2009).

The aim of the present study was: 1) to analyse the ability of different commercial methods to extract DNA from several food and pet food samples, 2) to evaluate the quantity and quality of extracted DNA, 3) to select the best extraction protocol for a food and feed samples and 4) to obtain dependable results of isolation for further analyses.

The kits were chosen on the basis of their availability in the market, time demands of the particular procedure, price per individual isolation, toxicity of the kit chemicals and the extraction method (column filter or phenol-chloroform extraction). Methodology of reagents testing should belong to the standard practice of the laboratory. The present results of this work form the part of educational course, which is designed for students of Mendel University in Brno.

MATERIAL AND METHODS

Ten different samples that contained chicken meat were studied: raw meat, ham, sausage, tinned lunch meat, pate, tinned feed for dogs (two samples), complete granulated feed for dogs (two samples) and chicken flour. The samples were homogenized using mixer and DNA was isolated following these extraction techniques according to manufacture's protocols: NucleoSpin Food (Marchery-Nagel), Wizard Genomic DNA Purification Kit (Promega), Invisorb Spin Food Kit I (Invitek), Wizard SV Genomic DNA Purification System (Promega), JetQuick Tissue DNA Spin Kit (Genomed), RNA Blue (Top-Bio), JetQuick Blood & Cell Culture Kit (Genomed) and QIAamp DNA Mini and Blood Mini (Qiagen). Tab. I shows further important characteristic of each kit.

The DNA concentration and purity was determined by measuring absorbance by spectrophotometer NanoDrop 2000 (Thermo Scientific). Methods to evaluate the quality of the extracted DNA were electrophoresis on 1% agarose gel and polymerase chain reaction (PCR). The primers to amplify the chicken-specific PCR product were assumed according to Matsunaga et al. (1999). The size of the obtained PCR product was 227 bp. PCR reactions were performed in total volumes 25 µl containing 200 µM of each dNTP, 0.2 µM of each primer, 1 Units of LA DNA polymerase in standard PCR buffer (Top-Bio, CZ) and 2 µl of DNA template. Amplification was performed in a thermocykler PTC 200 (MJ Research) and the cycling conditions were initial denaturation at 95°C (2min), followed by 35 cycles of 95 °C (30s), 52 °C (30s), 68 °C (30s), with final extension at 68 °C (7min). PCR products were checked by electrophoresis in 3% agarose gel using TBE buffer with ethidium bromide (0.5 µg/ml) staining.

RESULTS AND DISCUSSION

The DNA from ten different samples of foods and feeds, containing chicken meat, was analyzed. DNA was extracted by eight isolation methods. The aim of extraction procedures was to obtain high quality and quantity DNA suitable for for species identification. The methods agarose gel electrophoresis, measuring absorbance by spectrophotometer and PCR were performed. The results of all analysis were in the correlation and corresponded with others authors, as it is demonstrated later. The DNA extraction methods have impact on quantity and quality of the extracted DNA and on the efficiency of the DNA amplification therefore (Di Bernardo et al., 2007). Exposure to heat and physical or chemical treatments are known to cause fragmentation of DNA molecules and random breaks in DNA strands (Quinteiro et al., 1998; Peano et al., 2004). The method of the extraction, the type of liquid employed for canning, cooking and other processing of meet lead to degradation of DNA and can have a great influence on DNA quality, quantity and results in PCR analyses (Chapela et al., 2007; Aslan et al., 2009). DNA quality is a critical factor for most amplification-based analyses, because the amplification of DNA is influenced by the presence of inhibitors from the matrix or the extraction reagents, which can reduce the efficiency of the PCR reaction (Smith et al., 2005). Nevertheless, DNA which underwent thermal denaturation can be still detected

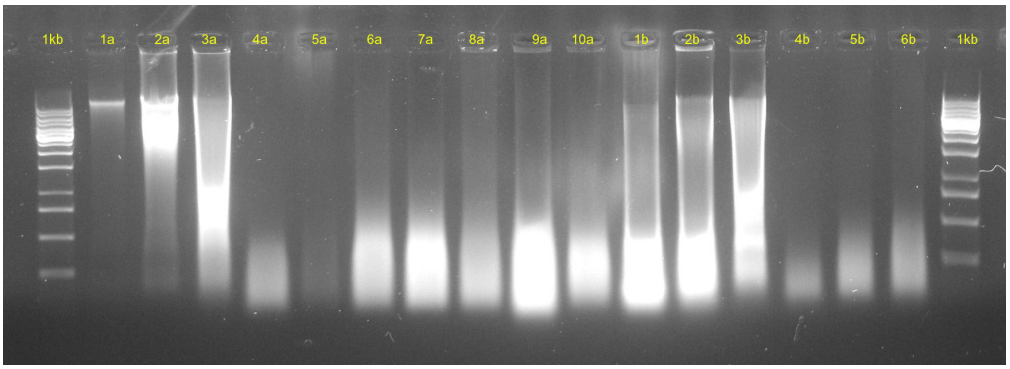
I: *General description of DNA extraction methods*

Method	Base of system	Amount of starting material (mg)	Elution		
			time (min)	volume (µl)	number
NucleoSpin	column	200	5	100	1
Wizard kit	pellet	20	60	100	1
Invisorb	column	40	3	400	2
Wizard SV	column	20	2	250	2
JetQuick tis.	column	20	5	200	2
RNA Blue	pellet	100	-	300	1
JetQuick bl.	column	20	2	200	2
QIAamp	column	20	5	200	2

by short fragment amplification (Krcmar, Rencova, 2001) but the length of PCR amplicon is cardinal aspect for detection of DNA which was denatured by cooking and others thermal processing (Meyer et al., 1994; Hird et al., 2006; Martín et al., 2008). The correct choice of the DNA extraction method and DNA quantification are very important steps in the analytical procedure to ensure optimal results (Barbaro et al., 2004).

Gel electrophoresis showed possibility to detect the band corresponding to the genomic DNA in meat, ham, sausage, granulated dog feed and chicken flour. DNA isolated from tinned lunch meat, pate and tinned feed for dogs, was substantially damaged (Fig. 1). The spectrophotometric measurements (Tab. II and Tab. III) also proved that DNA yield of meat, ham, sausage, granulated dog feed and chicken flour was higher (the average DNA concentration in isolates obtained by all extraction method was 126.4 ng/µl) than of those from tinned samples (the average DNA concentration in isolates obtained by all extraction method was 33.4 ng/µl). Additionally, DNA quality which is determined by the A260/A280 ratio differed between these samples groups (average 1.75 and 1.66, respectively). All eight isolation procedures gave good results in DNA concentration and quality measurement in presence of chicken meat, ham, sausage, granulated dog feed and flour. The highest DNA yields were obtained by NucleoSpin Food, Wizard Genomic DNA Purification Kit and JetQuick Tissue DNA Spin Kit (however, this also depends on different factors such as input amount of samples, elution time, temperature of elution buffer, final elution volume, number of elutions etc.) and the best DNA quality by use NucleoSpin Food, Wizard Genomic DNA Purification Kit and Invisorb Spin Food Kit I (ratio A260/A280 was close to 1.8). With these extraction methods the best concentration values and ratio A260/A208 from tinned lunch meat, pate and also for tinned feed for dogs were attained.

PCR analysis (Fig. 2) confirmed the previous results (Tab. IV). All eight isolation kits gave excellent results of PCR amplification in presence of chicken meat, ham and sausage. Very good results of PCR amplification were also achieved with these extraction methods in granulated dog feed and flour. The weak signal of PCR amplification in granulated feed and chicken flour was detected using the methods Invisorb Spin Food Kit, RNA Blue



1: *Agarose gel electrophoresis of DNA extracted from 1 – Raw chicken meet, 2 – Ham, 3 – Sausage, 4 – Tinned lunch meat, 5, 6, 7 – Tinned feeds for dogs, 8, 9 – Complete granulated feeds for dogs, 10 – Chicken flour; a – Wizard genomic DNA purification kit (Promega), b – NucleoSpin food (Marchery-Nagel)*

II: *Concentrations (ng/µl) of extracted DNA*

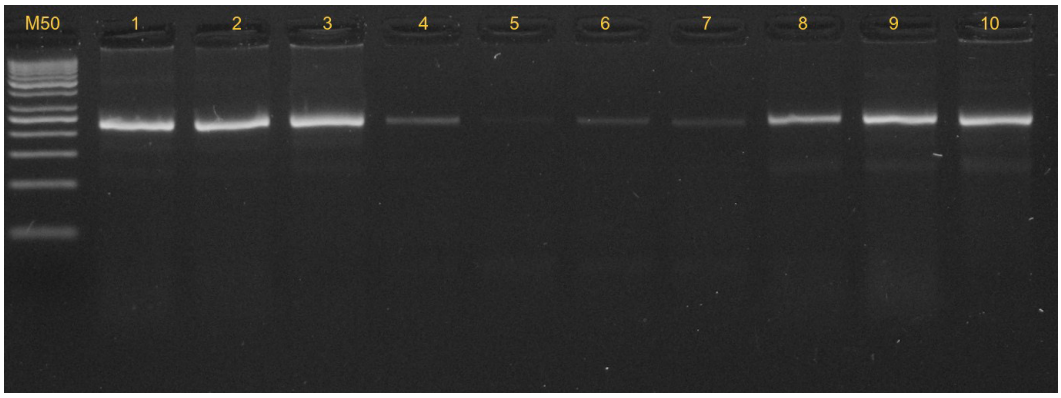
	1	2	3	4	5	6	7	8
Chicken meat	460.5	19.5	10.5	16.2	134.8	23.0	35.2	90.1
Chicken ham	480.4	151.6	9.6	8.4	136.1	11.0	47.9	33.0
Chicken sausage	288.0	67.3	15.4	9.7	751.6	32.8	29.9	30.5
Lunch meet	33.8	105.1	21.9	2.5	24.4	28.1	58.1	5.6
Pate	29.3	20.4	11.2	4.7	34.5	3.5	18.7	2.5
Tinned feed 1	49.9	137.1	17.7	4.9	31.5	2.4	46.0	5.7
Tinned feed 2	75.2	168.1	15.0	4.4	48.5	17.4	30.9	10.4
Granulated feed 1	222.2	171.0	17.8	49.5	105.1	12.8	58.1	47.5
Granulated feed 1	684.1	463.6	77.6	42.6	114.4	23.8	67.9	105.7
Chicken flour	152.2	160.2	40.6	182.6	59.4	7.0	92.3	24.9
Average	266.8	146.4	23.7	32.6	144.0	16.2	48.5	35.6

1 – NucleoSpin food, 2 – Wizard genomic DNA purification kit, 3 – Invisorb spin food kit I, 4 – Wizard SV genomic DNA purification system, 5 – JetQuick tissue DNA spin kit, 6 – RNA blue, 7 – JetQuick blood & cell culture kit, 8 – QIAamp DNA blood mini kit

III: *Relation A260/A280 of the extracted DNA*

	1	2	3	4	5	6	7	8
Chicken meat	2.19	1.66	1.83	1.78	2.09	1.72	1.95	1.99
Chicken ham	1.89	1.76	2.10	1.59	1.56	1.45	1.97	1.96
Chicken sausage	1.87	1.76	1.78	1.54	1.46	1.77	1.60	1.87
Lunch meet	1.84	1.68	1.61	1.64	2.07	0.82	1.73	2.17
Pate	1.39	1.81	1.58	1.54	1.67	0.76	1.49	2.28
Tinned feed 1	1.93	1.79	1.90	1.15	1.84	0.75	1.52	1.86
Tinned feed 2	1.90	1.82	1.79	1.45	1.97	1.52	1.83	1.91
Granulated feed 1	1.90	1.72	1.96	1.43	1.99	1.08	1.61	1.94
Granulated feed 1	1.81	1.81	1.78	1.26	1.83	1.35	1.68	2.01
Chicken flour	1.84	1.79	1.74	1.54	1.87	1.11	1.84	1.80
Average	1.86	1.76	1.81	1.49	1.84	1.23	1.72	1.98

1 – NucleoSpin food, 2 – Wizard genomic DNA purification kit, 3 – Invisorb spin food kit I, 4 – Wizard SV genomic DNA purification system, 5 – JetQuick tissue DNA spin kit, 6 – RNA blue, 7 – JetQuick blood & cell culture kit, 8 – QIAamp DNA blood mini kit



2: *Agarose gel electrophorese of PCR products (227bp) of 1 – Raw chicken meet, 2 – Ham, 3 – Sausage, 4 – Tinned lunch meat, 5 – Pate, 6, 7 – Tinned feeds for dogs, 8, 9 – Complete granulated feeds for dogs, 10 – Chicken flour extracted with QIAamp DNA blood mini kit (Qiagen).*

and QIAamp DNA Blood Mini Kit. It should be noticed that in these samples a low concentration were measured. For PCR amplification from tinned food and feed samples NucleoSpin Food and JetQuick Tissue DNA Spin Kit offered the best results. While using the other extraction methods, weak or no signal was detected in these samples.

IV: *DNA amplification of PCR products*

	1	2	3	4	5	6	7	8
Chicken meat	+	+	+	+	+	+	+	+
Chicken ham	+	+	+	+	+	+	+	+
Chicken sausage	+	+	+	+	+	+	+	+
Lunch meet	/	/	/	/	/	/	/	/
Pate	/	-	/	-	/	-	/	/
Tinned feed 1	/	/	/	-	/	-	/	/
Tinned feed 2	/	/	/	-	/	/	/	/
Granulated feed 1	+	+	/	+	+	/	+	+
Granulated feed 1	+	+	+	+	+	+	+	+
Chicken flour	+	+	/	+	+	/	+	/

+ Signal detected on agarose gel; – no signal detected; / weak signal detected
1 – NucleoSpin food, 2 – Wizard genomic DNA purification kit, 3 – Invisorb spin food kit I, 4 – Wizard SV genomic DNA purification system, 5 – JetQuick tissue DNA spin kit, 6 – RNA blue, 7 – JetQuick blood & cell culture kit, 8 – QIAamp DNA blood mini kit

SUMMARY

In present study, the analysis of DNA isolation by different extraction methods from several food and pet food samples with aim to evaluate the quantity and quality of extracted DNA, the selection of the convenient protocol for a food and feed sample extraction to obtain reliable results of isolation for subsequent analyses were performed. Eight methods for extracting the DNA from ten different samples of food and feed, containing chicken meat were compared. Gel agarose electrophoresis, absorbance measuring by spectrophotometer Nanodrop 2000 (Thermo Scientific) and PCR were performed. The differences between isolated DNA from tinned food or feeds and meat, ham, sausage, granulated dog feed and chicken flour were found. In tinned products, DNA was more degraded, its content and purity was lower and also PCR analyse was the most difficult. As the best extraction method for all analysed foods and feeds NucleoSpin Food and JetQuick Tissue DNA Spin Kit were chosen. These methods show the highest DNA yields, purity and template quality and the results of PCR analyse have an excellent reproducibility.

SOUHRN

Výběr nejvhodnější metody izolace DNA pro identifikaci druhů z potravin a krmiv

V této práci byla izolována DNA z deseti vzorků vybraných potravin a krmiv pomocí osmi různých extrakčních kitů, které jsou běžně dostupné na trhu. Hlavním cílem bylo ověřit kvalitu a kvantitu DNA, vybrat vhodnou metodu pro izolaci DNA z potravin a krmiv a získat spolehlivé výsledky pro další analýzy. Kvalita, koncentrace a čistota izolované DNA byla ověřena pomocí gelové elektroforézy, měření absorbance spektrofotometrem Nanodrop 2000 (Thermo Scientific) a polymerázové řetězové reakce (PCR). DNA extrahovaná z konzervovaných potravin a krmiv byla více degradovaná, její obsah a čistota byla nižší a také PCR analýza byla obtížnější v porovnání s DNA, která byla izolována z masa, šunky, párků, granulovaných krmiv pro psy a kuřecí moučky. NucleoSpin Food a JetQuick Tissue DNA Spin Kit byly vybrány jako nejlepší metody pro extrakci DNA vybraných potravin a krmiv. Extrakce DNA pomocí těchto kitů umožňuje nejvyšší výtěžky DNA, vysokou čistotu a výsledky PCR analýzy mají výbornou reprodukovatelnost.

extrakční metody, potraviny, krmiva, kvalita a kvantita DNA

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