Food Research 5 (4) : 210 - 215 (August 2021)
Journal homepage: http://www.myfoodresearch.com

FOOD RESEARCH

FULL PAPER

Authenticity test of the processed meat products

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Article history:
Received: 20 November 2020
Received in revised form: 23 January 2021
Accepted: 2 April 2021
Available Online: 1 August 2021

Keywords:
Authentication,
DNA,
Food Fraud,
Meat Products,
PCR

DOI:
https://doi.org/10.26656/fr.2017.5(4).679

Abstract

Different and related approaches must be considered to address consumers' concerns regarding the identification of species involved, mainly those of manufactured and derived meat products such as cured and canned meat and canned sausage. Therefore, the development of molecular techniques has been achieved by a mixture containing different shares of meat DNA. This study provided an important indication regarding the validity of the multiplex Polymerase Chain Reaction (PCR) technique in such tests. Based on the fact that the aforementioned method is qualitative; yet, the different percentages of meat DNA contained in the mixtures cannot be determined. Our current study showed that tracing back the origin of the ingredients used in food production is achievable, even if the DNA is degraded as a result of the food transformation processes. A PCR test was conducted at 35 cycles for mixtures at 100%, 75%, 50%, and 25% levels. Our results indicated that the original species of the meat products used were accurately determined in all mixtures tested by PCR technique. Therefore, we concluded that the PCR technique can be useful as a fast, easy to perform, and reliable control for adulterated consumer meat products.

1. Introduction

In contemporary societies, where a mixture of traditions, uses and habits of different people occur, consumers' choices in terms of food based on their religious beliefs (the prohibition of pork meat in Judaism and Islamic faiths), or even their lifestyle (vegetarianism, preference of organic or animal-friendly products) do not simply seem satisfactory to their nutritional needs (Jhon *et al.,* 2004; Michel and Patrick, 2012). Regardless, such choices have always been made according to the information provided in various forms (verbal, written, and/or visual) by the manufacturers. Such information must be true and precise, especially for food products that had been once processed and various ingredients used in their preparation are difficult to be distinguished (Mauro and Maria, 2009). The mismatch between what is declared by the manufacturers and what is actually present in the product ingredients represents fraudulent food (Belkis *et al.,* 2007). Food fraud is perpetrated by the fraudsters against the consumers, in fact, it always exists but is due to regular monitoring. Nowadays, it is difficult to identify a case of adulteration, alteration, sophistication, etc., in food products compared to their previous original patches. The food fraud product in question could have the same taste, format, and price as the original ones and does not necessarily cause damage to the consumer (Mauro and Maria, 2009). For this reason, the determination of food fraud requires accurate and sophisticated testing techniques especially those based on genetic (Partis *et al.,* 2000). The Polymerase Reaction Chain (PCR) is one of the most important DNA investigation techniques that is widely applied in many fields, especially in the identification of the ingredients used in the preparation of foods (Mehrnaz *et al.*, 2018). This bio-molecular technique has been used also in the food production sector for the identification of the species of origin of meat products. Several studies (Miguel *et al.,* 2005; Mehrnaz *et al*., 2018) indicated that the amplification of the mitochondrial mDNA sequences is more abundant than the nuclear one (Galtier *et al.,* 2009; Nicolas *et al.,* 2009; Chandrika *et al.,* 2010). In addition, among the most studied mitochondrial genes is that of cytochrome b (Cyst b) where sequences have been used to design primers able to discriminate between different meats species (Ilhak and Ali, 2007; Khairalla *et al.,* 2007; Zhang *et al*., 2007; Anita *et al*., 2009; Yacoub and Sadek, 2017). This study used PCR techniques to determine the species of origin used in the preparation of meat products. The diagnostic system, in particular, allows the identification of the simultaneous presence of beef and pork genetic materials through the amplification

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eISSN: 2550-2166 / © 2021 The Authors. Published by Rynnye Lyan Resources

of species-specific sequences of mitochondrial DNA. Furthermore, the validity of the method used has been also tested on a processed food product made with beef and pork ingredients.

2. Materials and methods

2.1 Sample preparation

The beef samples were secured from the local markets and the pork samples were purchased from a special market in Jordan and an industrial product (canned sausage), not available in the local Iraqi markets was purchased abroad and also analyzed. As described in the manufacturer's label, it is composed of (sausage pork 80%, water, potato starch, salt, stabilizer, white pepper, paprika powder, nutmeg extract, beef collagen, antioxidant, rosemary extract, smoke-flavored and preservative).

2.2 Primer and DNA extraction

Specific PCR primers for the amplification of beef and pork meat were presented in Table 1. The DNA genome was prepared from beef, pork and canned sausage following the Genomic DNA mini kit for the protocol on animal tissues (Biotech, Taiwan). A total of 30 mg of meat tissue was weighed and transferred into a 1.5 mL microcentrifuge tube, and then the meat tissue was ground to a pulp using the micro pestle provided in the kit. About 200 µL of GT buffer was added to the tube and homogenized by milling, and 20 µL of proteinase K (20 mg/mL) was added to the mixture and incubated the mixture at 60°C for 30 mins afterwards. In addition, 200 µL of GBT buffer was added to the tube and incubated at 60°C for 20 mins to ensure clear the lysate. Then lysate, a 200 µL of absolute ethanol was added immediately and vigorously shaken for 10 s. The mixture was transferred to the GD column, placed in a 2 mL tube, and centrifuged at 14,000 rpm for 2 mins. Subsequently, the 2 mL collection tube was discarded and transferred to the GD column in a new 2 mL tube. Also, 400 µL of W1 buffer was added to the GD column and centrifuged at 14,000 rpm for 30 s, then the flow was discarded into the 2 mL collection tube. The GD column was placed again in the 2 mL tube and 600 µL of wash buffer was added to the GD column for centrifugation at 14,000 rpm for 30 s. Afterwards, the flow was discarded and the GD column was returned to the 2 mL collection tube for final centrifugation at 14,000 rpm for 3 mins to dry the column matrix. The dried GD column was transferred to a new 1.5 mL microcentrifuge tube; a preheated elution buffer of about 100 µL was added to the column matrix until completely absorbed. Finally, the mixture was centrifuged at 14,000 rpm for 30 s to elute the DNA. The DNA quality was verified by an electrophoretic run (in TAE 1X buffer) on agarose gel (1%, w/v) coloured with ethidium bromide and visualized by Gel Document (Bio-Rad).The determination of the extracted DNA concentration in (ng/µL) occurred with the aid of (NanoDrop ND-1000 UV-Vis spectrophotometry) and the DNA was ready to be used for further analysis as exhibited in Table 2.

Table 2. The qualitative parameters of total DNA solutions

Sample	Concentration	Purification	
		A260/280	A260/230
Beef	24.99	1.73	1.90
Pork	28.34	1.65	2.50
Canned sausage	26.5	1.77	2.26

2.3 Preparation of the DNA mixtures

Appropriate dilutions of DNA extracts were performed to standardize samples concentration between (25 ~ 30 ng/µL) to obtain five new samples linking bovine with pork DNA as displayed in Table 3.

Table 3. Preparation of beef and pork DNA mixtures

Mixture No.	Beef DNA (%)	Pork DNA (%)	Total DNA (ng/µL)
1	100	0	25
2	75	25	25
3	50	50	25
4	25	75	25
5	0	100	25

2.4 Polymerase chain reaction-PCR

The amplification of the DNA mixtures (sample of canned sausage) was first verified by PCR simplex. Later on, it was developed by a multiplex PCR according to Henegariu *et al*. (1997). Each reaction, in particular, was performed in a final volume of 50 µL using 0.1 µg total DNA, 10 mM of Tris-HCl (pH 8.8 at 25°C), 1.5 mM of $MgCl_2$, 50 mM of KCL, 0.1% of Triton X-100, 0.2 mM of dNTPs, 0.4 mM per primer as shown in Table 1, and 2.5 U of Taq polymerase. The amplification were made using the MJ Mini personal Thermocycler Bio-Rad with the following protocol: initial denaturation at 95°C for 4 mins, then 35 cycles, 94°C for 30 s, 60°C for 1 min and

Table 1. The primer pairs used in PCR identification of beef and pork meat

Species	Sequence 5'-3'	PCR Product bp	References
Beef	F 5'- CGGAGTAATCCTTCTGCTCACAGT-3' R5'-GGATTGCGATAAGAGGTTGGTG-3'	116	Dooley *et al.* (2004)
Pork	F 5'- CAACAACGCATTCATTGAC-3' R 5'- AAGAATATGGATGCTCCGTTTG-3'	212	Shabani *et al.* (2015)

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72°C for 1 min and a final extension of 72°C for 10 mins. The PCR products were visualized with gel documentation.

3. Results and discussion

The specificity of the primers and the PCR conditions have been set for the identification of beef and pork genetic materials in the meat samples of meat products. The primers amplify particular amplicon for pork and beef as indicated In Table 1. The performed parameters indicated the quality of the DNA solutions that were obtained using suitable dilutions of the starting extracts Table 2. The values of the concentrations of the solutions finals were ranging from ~ 25 (beef DNA, B) – 28.34 ng/µL (pork DNA, P). Furthermore, the authors (Zhang *et al.,* 2007) also reported a value of the same higher index for a pork DNA solution compared to our current study (2.12 vs. 1.65). The DNA solution extracted from canned sausage Unox (Winsted, it is characterized by an intermediate concentration value 26.5 ng/µL) compared to the previous ones, but has indexes of better purity. The first index, A260/A280, has a purity value higher than that of the others (Torsten *et al.,* 2003; Ergün and Mustafa, 2011) which indicate one or less protein contamination. The second index, A260/ A230, also has a purity value higher than the others which indicate the absence of various contaminants, the same results were achieved by analyzing the total DNA profile produced by the following electrophoresis as shown in Figure 1, the beef (B) and pork (P) DNA solutions have greater impurities than those obtained from canned sausage (C). Usually, such impurities, if excessive, interfere with the mobility of nucleic acids which binding to them complicates the amplification processes difficult. The DNA obtained from the canned sausage (C) with higher impurities and lower concentrations than beef and pork DNA. This is due to the various physical processes performed on the meat before it became a sausage. Grinding of meat, mixing with other ingredients, filling the intestines, fumigating (at 80°C for 1 hr 30 mins), cooking (at 85°C for 25 mins), cooling in running water (for 15 mins) and the storage. The difficulties of locating long DNA fragments in meat samples subjected to boiling or cooking at oven (at 180°C) was reported by Hird *et al*. (2006), a result has been confirmed by Aslan *et al*. (2009), who succeeded to amplify only small DNA fragments (<200 bp) (Irene *et al.,* 2007). In autoclaved foods at temperatures ranging from 120–133°C, the size of the fragments of the amplifiable DNA has oscillated between 84 and 122 bp (Irene *et al*., 2007). The DNA decomposition in smaller molecules is a phenomenon observed also in other foods, such as in wheat used for the production of bakery products (Matasaka *et al*., 2004; Tilley, 2004), processed fruit (Toshiya *et al*., 2006) or in canned tuna (Chandrika *et al*., 2010). On contrary, the lanes B and P, it is possible to observe DNA molecules mostly intact and characterized by a very slight degradation.

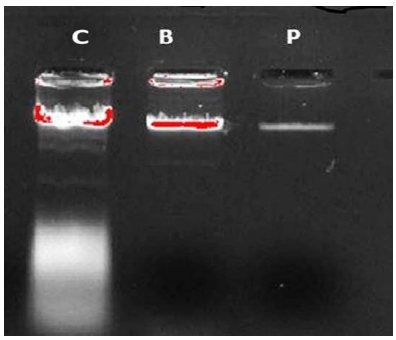

Figure 1. Electrophoresis of extracted DNA. Lane C = Canned sausage DNA; lane B =Beef DNA; lane P = Pork DNA.

In Figure 2 it is possible to observe the amplification results by multiplex PCR. DNA is been amplified successfully in all the mixtures. From the mixtures 2-3,5 and 8, it is possible to observe two amplification.

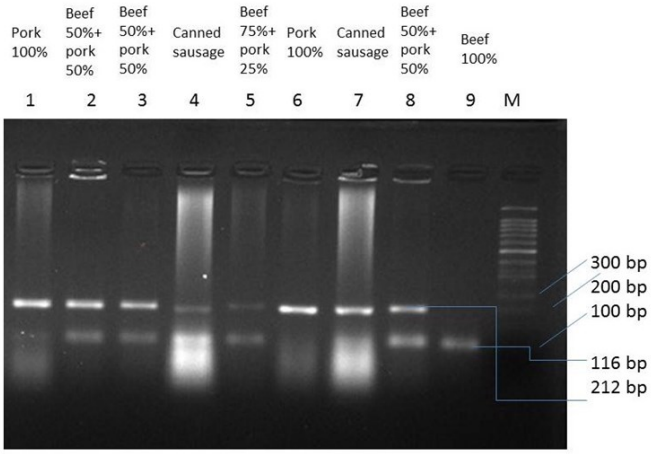

Figure 2. Electrophoretic profile of amplification products obtained by multiplex PCR. Lane DNA Ladder M = 100 bp; lanes 1and 6 = pork (100%); lanes 2-3 and 8 = mixture beef (50%) + pork (05%); lane 4 and 7 = Canned sausage; lane 5 = mixture beef (25%) + pork (75%); lane 9 = beef (100%).

The first, lower, was obtained with the couple of primers that amplify a long section 116 bp inside the gene species-specific *cytb* of beef. The amplicon at 212 bp was the trait species-specific *cytb* of pork, in lanes 2-3, 5 and 8. In lane 9, on the other hand, the sole presence of the product is 116 bp long confirms that the solution contains only beef DNA, as well as the product in lanes 1and 6 confirms the presence of pork DNA. Therefore, these data provide an important point indication about the validity of multiplex PCR. Despite that, multiplex PCR is a qualitative analysis, the different percentages of DNA found in the mixtures can't be confirmed. Looking at the fluorescence of the amplified bands, however, it is possible to draw important indications. The fluorescence

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is due to the bond that ethidium bromide forms with DNA interleaving in the double helix, a bond that, if exposed to ultraviolet rays, is able to emit fluorescence. The intensity of the bands' fluorescence is proportional to the amount of DNA present in that band. That is, it is possible to estimate the amount of amplified DNA present in the sample. In our case, a decrease in this product fluorescence intensity means that the samples contain less beef DNA. Simultaneously, it can be observed a little fluorescence in lane 5 of the band that goes back to pork DNA. This analysis suggests that these mixtures under study hold beef and/or pork DNA in different quantities, one more positive result for this molecular diagnostic system in terms of sensitivity. Similar results were described by Ilhak and Ali (2007), which were obtained by mixing beef and pork DNA in different percentages, a result was subsequently confirmed by Shally (2004). In another study, the same pair of bovine primers were used, and chicken DNA was developed (Zhang *et al*., 2007). In contrast, tracing different species of origin in meat food products was obtained to make preparations of different compositions through one simplex PCR (Ilhak and Ali, 2007). Our results agree with other studies (Meyer *et al*., 1994; Partis *et al*., 2000; Nagappa *et al*., 2013), which reported that the PCR could be used to identify meat mixtures with percentages of 1 and 0.5%. The real results of our PCR multiplex are represented by the possibility of amplifying the DNA contained in a food product.

In fact, our main difficulties were that the DNA could be extracted from the meat product which undergoes irreversible alterations as a result of the various processes to which the ingredients of the product itself are destined. The electrophoretic profile in Figure 1 confirms that the DNA total extracted from canned sausage (C) has suffered a degradation, such as to break down the molecule original DNA in multiple molecules small. Nevertheless, the result of the PCR multiplex was positive, a distinct band can be observed relating to the presence of pork DNA as shown in Figure 2, lane 4 and 7. The result confirms what has been described on the manufacturer's label, or that in the preparation of canned sausage, pork meat has been used. Through a quantitative approach, it would have been also determined the quantities of ingredients used for the realization of this product.

4. Conclusion

This study described the tracing back to the origin of the ingredients used for Food production is possible, even if the DNA is degraded due to the food transformation processes. Whether thermal or physical treatments to which food is exposed during processing, may not affect or cause a significant change on the structure and shape of DNA, which may not affect the result of biological and chemical tests for food. Molecular biology techniques are based on the study of DNA jumped to most through the cases of adulteration reported from the media. The identification of an exoneration of an alleged innocent has often been obtained through the study of biological traces.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

We would like to express our deepest thanks to the chief of Food Contamination Research Center, Environment and Water Directorate, Ministry of Science and Technology, Iraq. For taking part in the useful decision and giving necessary advice and guidance and arranged all facilities to make the work easier.

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