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Detection of species substitution and mislabeling in Albanian meat products by identification of DNA

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Abstract

Meat species adulteration is a worldwide problem, which violates food labeling laws, constitutes economic fraud, and raises ethical, religious and food safety concern. In this study, polymerase chain reaction (PCR) technique is applied for the detection of meat adulterate in processed-meat products samples from Albanian markets / supermarkets. Sixty samples from meat products: 20 raw meatballs, 20 chicken sausages, 20 pork sausages, were subjected to testing. Our study revealed a high incorrect of animal species declaration and substitution of meat products. Out of 60 collected samples, 49 resulted as mislabeling cases as well as with insufficient labeling information. The results of this study emphasize the necessity of interventions by National Food Authority, applying effective control measures to assess compliance with labeling requirements in the market. Considering this study but not only we propose that DNA identification of the animal species used in meat products is one of the efficient analytical methods that can be used in our labs.

Keywords: meat products, DNA, mislabeling, substitution species

1. Introduction

Meat is considered to be an excellent and nutritious source of protein. However, due to the high cost of this animal protein, and unfair competition by producers to gain economic benefits, adulteration frequently occurs in meat products. This includes; use of meat varieties of commercially lower value, use of sub-standard raw materials, presence of unknown species, replacement of animal or plant proteins, incorrect labeling or not declaring ingredients at all [20, 21, 9]. Moreover, consumers now pay closer attention to health issues (e.g., absence of allergenic compounds), diet (e.g., level of nutrients and calories), religion (e.g., absence of pork), and lifestyle (e.g., organic and vegetarian foods), and this increases the need to control fraud and protect consumer rights [21]. Given all of the above, the evaluation and validation of basic ingredients of meat products is one of the major concerns of food industry experts [15]. On the one hand, improper labeling of food products is indicative of another form of adulteration which concerns consumers, and is in fact the replacement of an animal species with a commercially cheaper species. On the other hand, improper labeling may not cite allergens and harm consumers that may be sensitive or allergic to them [20]. According to the European Commission Law 178/2002, validation is a term defined by the ability to identify animal species and products at different stages of the food production chain (from production to distribution stages). Quality and safety assurance of food products for consumers requires rapid confirmation of the validity and reliability of food products. This requirement is gaining importance due to increased global requirements to know the origins of food products, identification of risks associated with the consumption of improper food products on human health, and increased attention on the

effects of genetically modified organisms on the human food chain and the environment [9, 19]. According to Appendix standards, all raw materials used in the formulation of meat and poultry products should be declared, and when a product is made from a mixture of red meat and chicken, the name of the meat that has the highest proportion should be cited on the label of raw materials. In products containing red meat, chicken, and plant protein, if the proportion of plant protein to meat species is less than 1:13 or equal to 1:10, the name of the plant protein should be cited on the ingredients label, as well as by the product's name [13]. Furthermore, in recent years, the addition of soybean protein as a raw material replacing red meat in meat products (over 60 % meat) has increased significantly due to its functional characteristics (including: increased water and fat binding capacity, emulsification ability, and improved organoleptic properties, such as: appearance, (smooth texture, and cutability), nutritional value, as well as its low price. Meanwhile, the Codex Alimentarius Commission, World Health Organization, Food and Agriculture Organization, and European Commission have recently declared a list of 12 groups of allergens according to their prevalence and intensity, and food packages must declare their names on the labels. Soybean is also included in this list [14, 12] as it can cause various allergies, even at low concentrations [12]. As stated in the introductory statement to Directive 2000/13/EC of the European Parliament and of the Council on the approximation of the laws of the Member States relating to the labeling, presentation and advertising of foodstuffs [11], "the prime consideration for any rules on the labeling of foodstuffs should be the need to inform and protect the consumer". Therefore, verification of declared components in food products is essential for the protection of consumer health but also to ensure fair trade and compliance with legislation [2, 16]. In order to verify species origin for meat and meat products, analytical methods mainly rely on protein or DNA analysis. However, protein-based detection technology has its own disadvantage.When the meat is processed with high temperatures and under high pressure, protein in meat has a tendency degenerate. Compared with protein, DNA based methods were more reliable due to their high stability and unique variability that can identify meat tissues from closely related species. Among DNA-based methods, PCR technology is more popular [5, 4]. Considering the widespread distribution of fraudulent misdescription of food contents [7, 10], intensive and continuous monitoring is strongly recommended in order to ensure that consumers can make conscious choices. PCR analysis of species-specific mitochondrial DNA sequences is the most common method currently used for identification of meat species in food [3, 7, 12].

2. Material and Methods

2.1. Collected samples

Sixty samples from meat products: 20 chicken sausages, 20 raw meatballs, 20 pork sausages, were collected from Tirana City retail markets during the year 2015, and analyzed for detection of meat adulteration. The chicken sausage samples, pork sausage and raw meatballs, were labeled respectively as chicken, pork and beef only and containing mechanically separated meat [1].The samples were stored at -20°C until processing.

2.2. DNA extraction

Approximately 25 mg of meat samples was used for DNA extraction. DNA was extracted and purifited using the DNeasy® Tissue Kit (QIAGEN, Hilden, Germany), according to the manufacturer's instructions. The DNA, adsorbed into the QIA amp silica-gel membrane during subsequent centrifugation steps at 6000 g for 1 min, was washed using 500 µl AW1 and 500 µl AW2 washing buffers. Finally, the DNA was eluted with 200 µl of AE Elution Buffer (QIAGEN, Hilden, Germany). DNA concentration and purity was measured by DU-640B Spectrophotometer (Beckman) at 260 nm. Genomic DNA was stored at 4∘C before PCR.

Table 1. Oligonucleotide primer

Primer	Sequence (5'-3')	Length of amplicon
Bovine	5'-CTAGAAAAGTGTAAGACCCGTAATATAAG-3'	274
Chicken	5'-AAGATACAGATGAAGAAGAATGAGGCG-3'	227

Pork	5’-GCTGATAGTAGATTTGTGATGACCGTA-3’	398

2.3. PCR assay

The PCR reactions were performed in a final volume of 25 µl, using 12.5 µl of HotStarTaq Master Mix 2X (QIAGEN, Hilden, Germany), containing 2.5 units of HotStarTaq DNA Polymerase, 1.5 mM of MgCl2 and 200 µl of each dNTP. Then, 0.25 µM of each oligonucleotide primer and 2 µl of DNA were added. The amplification profile involved an initial denaturation step at 95 °C for 15 min, followed by 35 cycles at 94 °C for 30 s, 58 °C for 30 s and 72 °C for 45 s. The positive and negative controls for the extraction and PCR were included. The PCR reactions were processed in a Mastercycler Personal (Eppendorf Milan, Italy). All reactions were performed in duplicate. The sequence analysis was carried out in order to confirm the specificity of the PCR assay. Sequencing reactions were performed by PRIMM Srl (Milan, Italy).

2.4 Detection of amplified products

PCR amplified products were analyzed by electrophoresis on 1.5% (w/v) agarose NA (Pharmacia, Uppsala, Sweden) gel in 1X TBE buffer containing 0.089 M Tris, 0.089 M boric acid, 0.002 M EDTA, pH 8.0 (USB, Cleveland, OH, USA), and stained with ethidium bromide. PCR amplified products were analyzed by electrophoresis on 1.5% (w/v) agarose NA (Pharmacia, Uppsala, Sweden) gel in 1X TBE buffer containing 0.089 M Tris, 0.089 M boric acid, 0.002 M EDTA, pH 8.0 (USB, Cleveland, OH, USA), and stained with ethidium bromide.

3. Results and Discussion

The results of PCR showed that the adulteration rates for bovine raw meatballs, pork raw meatballs, chicken sausages and pork sausages, were 100%, 53.8%, and 65% respectively as presented in Table 2 . DNA extraction for all samples was successful, and all PCR products were clearly visible in agarose gel and in the expected size: respectively 227 bp for chicken, 398 bp pork, 274 bp bovin. Meat species adulteration, substitution or mislabeling of meat products has been reported from different countries such as Italy, Canada, Australia, United Kingdom and Egypt [5,6,8]. Food manufacturers or food processing factories may add different types of meats to species-specific meat product so as to add bulk or make up the volume of the product. Low priced or lower valued meat species may substitute higher valued meat species. These meat products which contain less desirable species may cause health risk and species identification is becoming a common and important practice [6,5]. Fraudulent substitutions of expensive meat with cheaper one or addition of undeclared species in meat products,as our study results, may cause concerns for consumer protection.

Table 2. PCR results of meat products

Samples No.	Product type	Labelled as	Bovine	Chicken	Pork
1.	raw meatballs	Bovine	+	-	+
2.	raw meatballs	Bovine	+	+	+
3.	raw meatballs	Bovine	+	+	+
4.	raw meatballs	Bovine	+	-	+
5.	raw meatballs	Bovine	+	+	-
6.	raw meatballs	Bovine	+	+	+
7.	raw meatballs	Bovine	+	+	-
8.	raw meatballs	Bovine	+	-	+
9.	raw meatballs	Bovine	+	-	+
10.	raw meatballs	Bovine	+	-	+
11.	raw meatballs	Bovine	+	+	-
12.	raw meatballs	Bovine	+	-	+
13.	raw meatballs	Bovine	+	-	+

14.	raw meatballs	Pork	-	+	+
15.	raw meatballs	Pork	-	-	+
16.	raw meatballs	Pork	+	-	+
17.	raw meatballs	Pork	+	+	+
18.	raw meatballs	Pork	-	-	+
19.	raw meatballs	Pork	-	+	+
20.	raw meatballs	Pork	-	-	+
21.	chicken sausages	chicken	-	+	+
22.	chicken sausages	chicken	-	+	+
23.	chicken sausages	chicken	-	+	+
24.	chicken sausages	chicken	-	+	+
25.	chicken sausages	chicken	-	+	+
26.	chicken sausages	chicken	-	+	+
27.	chicken sausages	chicken	-	+	+
28.	chicken sausages	chicken	-	+	+
29.	chicken sausages	chicken	-	+	+
30.	chicken sausages	chicken	-	+	+
31.	chicken sausages	chicken	-	+	+
32.	chicken sausages	chicken	-	+	+
33.	chicken sausages	chicken	-	+	+
34.	chicken sausages	chicken	+	+	+
35.	chicken sausages	chicken	+	+	+
36.	chicken sausages	chicken	+	+	+
37.	chicken sausages	chicken	-	+	+
38.	chicken sausages	chicken	-	+	+
39.	chicken sausages	chicken	+	+	+
40.	chicken sausages	chicken	-	+	+
41.	pork sausages	pork	+	-	+
42.	pork sausages	pork	-	-	+
43.	pork sausages	pork	+	-	+
44.	pork sausages	pork	-	-	+
45.	pork sausages	pork	+	-	+
46.	pork sausages	pork	-	-	+
47.	pork sausages	pork	+	+	+
48.	pork sausages	pork	-	-	+
49.	pork sausages	pork	-	-	+
50.	pork sausages	pork	+	+	+
51.	pork sausages	pork	-	+	+
52.	pork sausages	pork	+	-	+
53.	pork sausages	pork	+	+	+
54.	pork sausages	pork	-	-	+
55.	pork sausages	pork	+	-	+
56.	pork sausages	pork	-	+	+
57.	pork sausages	pork	-	-	+
58.	pork sausages	pork	-	+	+
59.	pork sausages	pork	-	+	+
60.	pork sausages	pork	-	+	+

4. Conclusions

The results of this study emphasize the necessity of interventions by National Food Authority, applying effective control measures to assess compliance with labeling requirements in the market. Considering this study

but not only we propose that DNA identification of the animal species used in meat products is one of the efficient analytical methods that can be used in our labs.

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