Investigation of meat species adulteration in beef-based meat products via real-time PCR in Türkiye

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Abstract

The addition of poultry and other meats to products made from 100% beef is prohibited in Türkiye. In addition, in Türkiye, where the majority of the population is Muslim, the use of pork and single-hoofed meat is a matter of concern due to religious reasons. This study utilized a sensitive and specific real-time polymerase chain reaction (PCR) method to identify the different meat species in meat products marketed as 100% beef and sold in the eastern Türkiye provinces of Erzurum, Erzincan, Kars, Ağrı, and Bingöl. The real-time PCR method was used to investigate the presence of DNA specific to six animal species (chicken, turkey, pork, horse, donkey, and camel). The analysis revealed no traces of horse, donkey, camel, or pork meat in any of the 100 samples of Turkish fermented sausage (sucuk), salami, and sausage. Contrary to the product labels claiming 100% beef content, poultry DNA residues were identified in a salami sample from Erzincan, while turkey DNA residues were found in sucuk samples from both Ağrı and Kars. The study's findings reveal that contrary to label information, various types of meat can be present in meat products sold by both trademarked and local butchers. Consequently, this paper emphasizes the need for routine checks on market-sold products to ensure they comply with legislation and avoid consumer deception. Furthermore, the Real-Time PCR method, with its rapid results and high sensitivity, is deemed beneficial for food safety, consumer rights, and the prevention of unfair competition.

Keywords: adulteration, meat species, mislabeling, real-time PCR

Introduction

The rising awareness of health, social, and environmental implications of food choices has heightened the global demand for safe, high-quality foods with distinct characteristics (Wu *et al*., 2021). Dietary components of animal origin, indispensable to nutrition, play a crucial role in safeguarding health. They contribute significantly to the physical and cognitive development of infants, children, and adolescents, and assist in maintaining physical function as people age (Leroy *et al*., 2022). However, concerns about the quality and content of meat and meat products are prevalent among today's consumers. Therefore, accurate labeling becomes vital in guiding consumer choices (Inguglia *et al*., 2023).

Meat is among the foods most frequently adulterated in commercial markets, often as a result of deliberate fraud. A prevalent form of fraud in meat industries involves substituting more expensive or permitted meat either partially or completely with cheaper meat from different animal species (Afifa Khatun *et al*., 2021; Xu *et al*., 2018). Such adulterated food products can lead to health impacts ranging from mild to severe, including symptoms like diarrhea, nausea, allergic reactions, and chronic conditions such as diabetes and cardiovascular disease

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(Fengou *et al*., 2021; Momtaz *et al*., 2023). Hence, food labeling regulations necessitate that the types of meat in food products be accurately declared to consumers (Roobab *et al*., 2021). Failing to label the animal species present in food not only infringes consumers' rights and trust but also presents a major concern for religious groups due to halal and kosher requirements and preferences of other religious affiliations (Afifa Khatun *et al*., 2021; Hendrickson *et al*., 2021).

Turkish Food Codex regulations specifically prohibit adding poultry and other meats to beef-only products, mandating that such information be included on the label (TFC, 2017; 2019). Food products that have been proven to be counterfeited and adulterated by laboratory results have therefore been routinely disclosed to the public by the relevant ministry since 2012 in order to ensure food safety, to eradicate food counterfeiting and adulteration, to protect health, to prevent misleading consumers, and to avoid unfair competition in the sector. The analysis reports of the food items officially listed on the ministry's website reveal that the most adulterated and counterfeited foods and beverages available for consumption in Türkiye are meat and meat products, milk and dairy products, and vegetable oil. It has been reported that beef-based products often contain additional animal meats, particularly poultry and other animal meats (pork and one-hoofed). Furthermore, in many scientific studies (Kaya *et al*., 2019; Keyvan *et al*., 2017; Ulca *et al*., 2013), poultry, pigs, and single-hoofed animals have been detected in raw, cooked, and processed meat and meat products. Similarly, different animal species have been detected in studies conducted in different regions and countries of the world.

Accurate labeling of animal species in meat products is crucial not only for human health protection but also for fostering fair trade among producers in the meat industry (Kaya *et al*., 2019). Preventing adulteration and fraud in these products relies on analyses to identify the animal species present and regular auditing processes (Doosti *et al*., 2014). The development of rapid and efficient methods to detect meat adulteration is critical for the growth of the food industry and consumer safety (Du *et al*., 2023). The majority of analytical methods developed for verifying meat species labeling claims and detecting adulteration primarily employ protein, metabolite, or nucleic acid-based assays (Kesmen *et al*., 2012).

Though precipitation methods used to identify meat species in meat products are affordable and straightforward, they are time-consuming and demand specific laboratory equipment and temperature conditions (Seddaoui and Amine, 2021). These requirements limit the method's use to only non-heat-treated products, inhibiting its application to the desired extent. In addition, results may be misleading due to the potential for cross-reactions in closely related species (Derinöz *et al*., 2021). Typically, enzyme-linked immunosorbent assay (ELISA) kits, precipitant sera, or qualitative kits used in laboratories for meat species determination do not sufficiently identify the proportions of meat derived from different animal species used in meat mixtures (Ayaz *et al*., 2013). Among DNA-based methods, the polymerase chain reaction (PCR)—a molecular biological technique—is reported to be highly accurate and relatively rapid, and is used in food authentication (Kesmen *et al*., 2012).

Genus-specific PCR can detect very low amounts of genetic material and is accurate in the identification of most food products. However, in addition to species verification, the amount of species in the product is also significant for quality control. Hence, real-time PCR allows real-time monitoring of the amount of DNA during amplification (Nizar *et al*., 2019). Real-time PCR techniques are advantageous when identifying different meat species in meat and meat products. This is due to its quickness, automation, high sensitivity, real-time detection, and quantification of targets, eliminating the need for time-consuming post-PCR analysis, such as electrophoresis (Hossain *et al*., 2023).

There is a growing body of literature describing the use of PCR to identify adulteration in meat to combat the deliberate replacement of a meat species with a cheaper species and also for quality control (Szemethy *et al*., 2021) or to detect the presence of pork DNA in meat products for religious reasons (Maritha *et al*., 2022; Murugaiah *et al*., 2009). The majority of publications report the use of genus-specific primers (Wang *et al*., 2019) and a TaqMan probe (Ali *et al*., 2012; Hossain *et al*., 2017) to assess adulteration in meat products. The majority of publications presenting PCR methods to identify different meat species' DNA in raw, cooked, or processed meat products mainly concentrated on prepared mixtures with known concentrations and left out any information on market screening.

In the eastern provinces of Türkiye, the primary sources of livelihood for most residents are agriculture and animal husbandry. Meat and its products, significant in human nutrition, are a crucial food source in this region and are consumed in large quantities. This study was conducted to investigate potential adulteration in meat products labeled as 100% beef, produced by trademarked and local butchers in the provinces of Erzurum, Kars, Erzincan, Bingöl, and Ağrı. In addition, it evaluates the practical applicability of the Real-Time PCR method for the sensitive, rapid diagnosis and identification of different animal species in meat products.

Materials and Methods

Sample collection

In this study, samples were collected from 50 sucuks, 32 sausages, and 18 salami products that were reported to be made from 100% beef by trademarks and local butchers in the provinces of Erzurum, Erzincan, Kars, Bingöl, and Ağrı in Türkiye. These 100 samples were transported to the laboratory under cold chain conditions and stored at –20°C until analysis.

Genomic DNA isolation

The samples stored at –20°C were taken and pulverized in liquid nitrogen then 10 mg powder was transferred to Eppendorf tubes. The genomic DNA was isolated from the samples using the Genomic DNA Isolation Kit from Hybrid Tissue and Cell Culture (Hibrigen, Ankara, Türkiye). The total nucleic acid content obtained from the samples was measured using the Thermo Scientific Nanodrop 2000 device.

Real-time PCR analysis

Adulterations commonly seen in beef products in Türkiye originated from pork, one-hoofed animals, and poultry meat. In the present study, a kit containing a combination of these could not be found. Therefore, two different kits ("PCR 4plex Pork/Chicken/Turkey + IAAC kit protocol" and "PCR 4plex Camel/Horse/Donkey + IAAC kit protocol") (R-Biopharm, Darmstadt, Germany) were utilized for species identification. Kits of detection limit is 0.1% depending on matrix and DNA preparation. The PCR amplification process was carried out in a total volume of 20.0 µl of a solution containing (19.9 µl Reaction Mix+0.1 µl Taq Polymerase). The amplifications were performed in a thermal cycler (Rotor Gene Q, Qiagen). The cycling conditions were the same as the ones described in Tables 1 and 2.

Results

According to results from the Real-Time PCR analysis, no traces of horse, donkey, camel, or pork meat were found in any of the 100 sucuk, salami, and sausage samples collected. However, poultry DNA residues were detected in a salami sample sourced from a local business in Erzincan, while turkey meat DNA residues were found in a sucuk sample from both Ağrı and Kars (Table 3).

Discussion

Adulteration in meat products has recently become a major concern for consumers all over the globe. For this

Table 1. PCR 4plex pork/chicken/turkey + IAAC kit protocol.

Initial denaturation (HOLD)	1 min, 95°C	
Cycles	35	
Denaturation	10 sec, 95°C	
Annealing/extension (CYCLE)	15 sec, 60°C	
Temperature transition rate/ramp rate	Maximum	
Fluorescence detection setup (exemplary)	Detection	End of Extension Phase
	Detection system pork:	
	Various devices	FAM-channel, Quencher: BHQ
	Rotor gene Q	465 nm–510 nm
	Detection system vertebrates and internal amplification control (IAAC):	
	Various devices	VIC/HEX-channel, Quencher: BHQ
	Rotor gene Q	533 nm–580 nm
	Detection System turkey:	
	Various devices	ROX-channel, Quencher: BHQ
	Rotor gene Q	533 nm–610 nm
	Detection system chicken:	
	Various devices	Cy5-channel, Quencher: BHQ
	Rotor Gene Q	618 nm–660 nm

Table 2. PCR 4plex camel/horse/donkey + IAAC kit protocol.

Initial denaturation (HOLD)	1 min, 95°C	
Cycles	35	
Denaturation	10 sec, 95°C	
Annealing/extension (CYCLE)	15 sec, 60°C	
Temperature transition rate/ramp rate	Maximum	
Fluorescence detection setup (exemplary)	Detection	End of extension phase
	Detection system camel:	
	Various devices	FAM-channel, Quencher: BHQ
	Rotor gene Q	465 nm–510 nm
	Detection system vertebrates and internal amplification control (IAAC):	
	Various devices	VIC/HEX-channel, Quencher: BHQ
	Rotor gene Q	533 nm–580 nm
	Detection system horse:	
	Various devices	ROX-channel, Quencher: BHQ
	Rotor gene Q	533 nm–610 nm
	Detection system donkey:	
	Various devices	Cy5-channel, Quencher: BHQ
	Rotor gene Q	618 nm–660 nm

Table 3. Distribution of adulteration detected in meat products produced from beef by province.

Province	Meat Product	N	Animal species					
			Chicken	Turkey	Pork	Horse	Donkey	Camel
Erzurum	Sucuk	10	ND	ND	ND	ND	ND	ND
	Sausage	6	ND	ND	ND	ND	ND	ND
	Salami	4	ND	ND	ND	ND	ND	ND
Erzincan	Sucuk	10	ND	ND	ND	ND	ND	ND
	Sausage	4	ND	ND	ND	ND	ND	ND
	Salami	6	Detected	ND	ND	ND	ND	ND
Kars	Sucuk	16	ND	Detected	ND	ND	ND	ND
	Sausage	2	ND	ND	ND	ND	ND	ND
	Salami	2	ND	ND	ND	ND	ND	ND
Ağrı	Sucuk	2	ND	Detected	ND	ND	ND	ND
	Sausage	16	ND	ND	ND	ND	ND	ND
	Salami	2	ND	ND	ND	ND	ND	ND
Bingöl	Sucuk	12	ND	ND	ND	ND	ND	ND
	Sausage	4	ND	ND	ND	ND	ND	ND
	Salami	4	ND	ND	ND	ND	ND	ND
Total		100	ND	ND	ND	ND	ND	ND

N: Number of samples; ND: No Detection.

reason, the type of meat from which the meat product is manufactured is a critical issue in terms of both food control and consumer protection. On the other hand, the identification of meat species in various meat products is considered as vital, especially in Islamic countries where people only consume Halal meat (Gholamnezhad *et al.*, 2021). Although the Turkish Food Codex prohibits the addition of poultry and other species' meat to products labeled as 100% beef, chicken genetic material was detected in two samples, and turkey genetic material was found in one sample. In addition, none of the 100 samples contained horse, donkey, or camel meat.

In recent years, the PCR method has emerged as a significant tool for detecting different animal species in meat products and stands as an alternative that could replace existing methods (Kesmen *et al*., 2010). Studies have also demonstrated that the Real-Time PCR technique can be effectively used for the quantitative detection of meat species (Rohman *et al*., 2022). The PCR method enables the accurate, reliable, and timely identification of animal species in meat mixtures, thereby preventing consumer deception. Thus, compared to other methods, it allows for the quicker, easier, and more reliable detection of animal meats that are not typically consumed by society (Hossain *et al*., 2023).

There were meat products which were not in compliance with their labels in various markets in Türkiye, presenting a potential public health risk and economical losses to consumers. In this study, a small number of samples were found to be adulterated, contrary to the information provided on the label. To investigate the compliance of meat products in Türkiye with the current meat products communiqué, Keyvan *et al*. (2017) used commercial kits to find the presence of meat or tissues from different animal species in meat products like salami, sausage, and sucuk. They found that 5 (13.5%) out of 37 sucuk samples contained poultry meat not specified on the label, and 1 (2.7%) contained both poultry and hoofed meat.

The PCR kits offer advantages such as fast, reliable, and a low detection limit (below %0,1 level, w/w) in determining adulteration resulting from the addition of meat from different animal species in both raw and cooked meat products (sucuk, salami, sausage, meatball, cured spiced beef, and doner kebap) (Ulca *et al*., 2013).

Accuracy of serological methods such as ELISA used in the identification of adulteration in meat products from various animal species is considered to have disadvantages in comparison to DNA-based methods. On this basis, in a study conducted by Perestam *et al*. (2017), ELISA and real-time PCR methods were compared in the determination of species in processed meat products. They found that while real-time PCR was less expensive, ELISA was less time-consuming and easier to perform. Both methods successfully identified species in processed meat and meat products, but it was determined that real-time PCR was more appropriate for species identification in processed meat products when a low detection limit was required. Similar results were found in another study comparing ELISA and real-time PCR methods. Both methods were found to be 100% successful in identifying beef in analysis, but while real-time PCR yielded 100% positive results in pork assays, ELISA only achieved 63.7% positive results (Yörük, 2021).

In another study, conducted by Gecaj *et al*. (2021), the levels of pork in commercial beef and poultry products marketed in Kosovo were investigated by ELISA and two confirmatory real-time PCR approaches. The results of this study proved that ELISA was faster than both real-time PCR approaches, but it was also found to be more challenging when handling a high number of specimens.

Due to people's dietary habits and religious beliefs, each nation has particular concerns and requirements regarding authenticity, labeling, and composition regulations. In literature, it can be observed that in studies conducted in numerous countries, cases of adulteration in meat products have been identified by taking into account the sensitivities specific to that country. In Malaysia, buffalo and chicken DNA was detected in sausages, cold cuts, meatballs, and minced meat (Chuah *et al*., 2016), as well as in sausages, hamburger patties, and meatballs produced from beef in Bangladesh (Afifa Khatun *et al*., 2021). While pork species were identified in beef sausage products in Canada (Naaum *et al*., 2018), the most commonly unspecified species in ham sausages produced in China was duck (Song *et al*., 2019). In a study conducted by Premanandh (2013), horse meat residues were detected in ready-to-eat foods labeled as 100% beef in Europe, while it was determined that different meat species were found in meat products in the USA (Kane and Hellberg, 2016).

Conclusions

In this study, a small number of samples were found to be adulterated, contrary to the information provided on the label. Therefore, it is essential to conduct routine checks on these products sold in the market to verify their compliance with legislation and avoid deceiving consumers. Furthermore, it was determined that the Real-Time PCR method is a rapid and effective technique for species identification, providing substantial benefits in terms of food safety, consumer rights, and the prevention of unfair competition. Consequently, it is believed that the potential for producer fraud and violations of meat product criteria set by the food codex can be minimized, thereby preventing consumer deception.

Author Contributions

MA and BÇ were involved in conceptualization and methodology. BÇ, MFS, and BAP did the formal analysis, and BÇ did the validation. HÖ and BÇ prepared the original draft. HÖ was concerned with visualization, and MA was responsible for project administration—review and

editing. All authors have read and agreed to the published version of the manuscript.

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Data Availability

All authors want no new data to be created.

Conflicts of Interest

The authors declare that they have no known financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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