



УНИВЕРЗИТЕТ „КИРИЛ И МЕТОДИЈ“ ВО СКОПЈЕ
ФАКУЛТЕТ ЗА ВЕТЕРИНАРНА МЕДИЦИНА – СКОПЈЕ



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**МЕТОДИ ЗА ИДЕНТИФИКАЦИЈА НА ИЗМАМИ СО ПРОИЗВОДИ ОД
МЕСО ВО КОСОВО**

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**METHODS FOR FRAUD IDENTIFICATION IN MEAT PRODUCTS FROM
KOSOVO**

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Abstract

This doctoral research examines the extent of adulteration in beef sausages produced in Kosovo, with a focus on two key issues: the unauthorized inclusion of non-beef meat species and the incorporation of soy protein. Such practices compromise product authenticity, nutritional integrity, and consumer safety. A total of 131 sausage samples were analyzed, comprising 68 samples (43 industrial and 25 traditional) tested for undeclared meat species, and 63 samples (42 industrial and 21 traditional) evaluated for soy content. Species authentication was conducted using the Liquid Chip Display (LCD) Array System, Meat 5.5.5.0, which revealed that 52.94% of the analyzed samples contained undeclared meat species. Poultry was the predominant adulterant (45.55%), followed by mutton (4.41%) and turkey (2.9%). Notably, 46.42% of industrial sausages and all homemade sausages with mixed meat compositions failed to disclose the presence of additional meat species, raising significant ethical, religious, and regulatory concerns.

Soy DNA detection, performed via real-time PCR, identified soy presence in 85.7% of all samples, with a markedly higher prevalence in industrial sausages (97.6%) compared to traditional products (61.9%). Statistical analysis demonstrated a significant difference in soy content between production methods ($p \leq 0.001$, ANOVA).

Gas chromatography-flame ionization detection (GC-FID) analysis of fatty acid profiles revealed that sausages containing poultry meat exhibited lower levels of saturated fatty acids (SFAs) and elevated levels of polyunsaturated fatty acids (PUFAs), particularly linoleic (C18:2) and alpha-linolenic acid (C18:3). Moderate correlations were observed between soy DNA quantification (Ct values) and alterations in fatty acid composition, specifically a reduction in palmitic (C16:0) and stearic (C18:0) acids and an increase in oleic acid (C18:1), indicative of an improved lipid profile.

These findings indicate that the presence of undeclared meat species and soy protein significantly modifies the nutritional composition of beef sausages, primarily by

shifting fatty acid profiles toward lower SFA and higher PUFA content. Although such modifications may enhance the nutritional quality of the products, the absence of transparent labeling compromises consumer rights, allergen safety, and adherence to dietary or religious standards.

This study underscores the urgent need for enhanced regulatory enforcement, more rigorous labeling practices, and increased public awareness concerning food adulteration. Future research should explore the sensory attributes, economic impacts, and consumer acceptance of such adulterated meat products within Kosovo and comparable markets.

Key words: Beef sausage; Species; Soy detection; Food fraud; Fatty acids; Real-time PCR

МЕТОДИ ЗА ИДЕНТИФИКАЦИЈА НА ИЗМАМИ СО ПРОИЗВОДИ ОД МЕСО ВО КОСОВО

Апстракт

Ова докторско истражување го испитува степенот на фалсификување кај говедските колбаси произведени во Косово, со фокус на две клучни прашања: неовластено вклучување на видови месо кои не се од говедско месо и вклучување на соин протеин. Ваквите практики ја загрозуваат автентичноста на производот, нутритивниот интегритет и безбедноста на потрошувачите. Анализирани се вкупно 131 примерок од колбаси, од кои 68 примероци (43 индустриски и 25 традиционални) тестирани за непријавени видови месо и 63 примероци (42 индустриски и 21 традиционален) оценети за содржина на соја. Автентикацијата на видовите е спроведена со помош на системот за низа со течен чип (LCD), месо 5.5.5.0, кој откри дека 52,94% од анализираниите примероци содржат непријавени видови месо. Живината била доминантна фалсификувачка состојка (45,55%), проследена од овчо месо (4,41%) и мисиркино месо (2,9%). Имено, 46,42% од индустриските колбаси и сите домашни колбаси со мешан состав на месо не откриле присуство на дополнителни видови месо, што покренува значајни етички, религиозни и регулаторни проблеми. Детекцијата на соја ДНК, извршена преку PCR во реално време, идентификуваше присуство на соја во 85,7% од сите примероци, со значително поголема преваленца кај индустриските колбаси (97,6%) во споредба со традиционалните производи (61,9%). Статистичката анализа покажа значајна разлика во содржината на соја помеѓу методите на производство ($p \leq 0,001$, ANOVA).

Анализата на профилите на масни киселини со гасна хроматографија-пламен јонизација (GC-FID) покажа дека колбасите што содржат живинско месо покажаа пониски нивоа на заситени масни киселини (SFAs) и покачени нивоа на полинезаситени масни киселини (PUFAs), особено линолна (C18:2) и алфа-линоленска киселина (C18:3). Беа забележани умерени корелации помеѓу квантификацијата на соја ДНК (Ct вредности) и промените во составот на масните киселини, поточно намалување на палмитинските (C16:0) и стеаринските (C18:0) киселини и зголемување на олеинската киселина (C18:1), што укажува на подобрен липиден профил. Овие наоди укажуваат дека присуството на непријавени видови месо и соини протеини значително го менува нутритивниот состав на говедските колбаси, првенствено со поместување на профилите на масни киселини

кон пониска содржина на SFA и повисока содржина на PUFA. Иако ваквите модификации можат да го подобрат нутритивниот квалитет на производите, отсуството на транспарентно етикетање ги загрозува правата на потрошувачите, безбедноста на алергените и почитувањето на диететските или религиозните стандарди.

Оваа студија ја нагласува итната потреба од засилено спроведување на регулативите, поригорозни практики на етикетање и зголемена јавна свест во врска со фалсификувањето на храната. Идните истражувања треба да ги истражат сензорните атрибути, економските влијанија и прифаќањето од страна на потрошувачите на вакви фалсификувани месни производи во Косово и споредливите пазари.

Кје зборови: Говедска колбас; Видови; Детекција на соја; Измама со храна; Масни киселини; PCR во реално време

"I declare that the doctoral thesis was completed by myself, all sources and literature used have been properly cited, and that the work has not been used in the context of other university studies or for obtaining any other title."

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List of abbreviations

| Abbreviation | Meaning of abbreviation/translation |
|---------------------|---|
| DNA | Deoxyribonucleic acid |
| Real-time PCR | Real-time polymerase chain reaction |
| LCD | Liquid Chip Display |
| GC-FID | Gas chromatography-flame ionization detection |
| SFA | Saturated fatty acids |
| PUFA | Polyunsaturated fatty acids |
| PCR | Polymerase Chain Reaction |
| DFS | Dry Fermented Sausages |
| MSG | Monosodium Glutamate |
| EU | European Union |
| USA | United States of America |
| FDA | Food and Drug Administration |
| TVP | Textured vegetable protein |
| qPCR | Quantitative Polymerase Chain Reaction |
| Cyt b | Cytochrome b |
| ELISA | Enzyme-Linked Immunosorbent Assay |
| UHPLC– MS/MS | Ultra-High-Performance Liquid Chromatography–Tandem Mass Spectrometry |
| HR | Hour |
| EAST | Enzyme Allergosorbent Test |
| RNA | Ribonucleic Acid |
| CTAB | Cetyltrimethylammonium bromide |
| Hz | Hertz |
| FAMES | Fatty acid methyl esters |
| SPSS | Statistical Package for the Social Sciences |
| ANOVA | Analysis of Variance |
| LDL | Low-Density Lipoprotein |

1.INTRODUCTION

Food authenticity and safety represent two of the most critical aspects of public health and consumer protection in the modern food industry. The growing complexity of food production chains, globalization of markets, and the increasing demand for affordable processed foods have simultaneously created opportunities for intentional mislabelling and adulteration. Among meat-based products, sausages are particularly vulnerable to fraudulent substitution practices due to their complex formulations, variable ingredients, and the masking effect of technological processing. The adulteration of meat products through the undeclared addition of non-target animal species or plant-derived proteins poses significant challenges not only to consumers' health but also to ethical, cultural, and economic integrity. This research seeks to address these challenges within the specific context of Kosovo, a country characterized by diverse food production systems that range from industrial-scale processing to traditional, family-based sausage production.

The principal objective of this doctoral research is to comprehensively evaluate the extent and characteristics of adulteration in beef sausages marketed in Kosovo. Specifically, the study focuses on detecting undeclared meat species and soy protein using advanced molecular and chemical analytical techniques. Through the integration of Liquid Chip Display (LCD) Array technology, real-time PCR, and gas chromatography–flame ionization detection (GC-FID), this research aims to establish a clear scientific basis for identifying fraudulent practices and assessing their nutritional and regulatory implications. The investigation provides not only empirical evidence of adulteration but also a broader understanding of how such practices influence the nutritional composition, consumer trust, and compliance with national and international food safety standards.

The selection of this topic is motivated by several interrelated factors. Firstly, adulteration of meat products has become a global concern, with numerous studies reporting undeclared substitution of species and incorporation of inexpensive fillers such as soy. While such practices are often economically motivated, they raise serious ethical and legal concerns, particularly when consumers are misled regarding the true composition of their food. Secondly, Kosovo's rapidly developing food sector presents

unique challenges in quality assurance, as traditional and industrial producers coexist in a regulatory environment that is still consolidating. There remains a paucity of research exploring the authenticity of processed meat products in this region, making this study one of the first to provide systematic molecular and chemical evidence of adulteration within the Kosovar meat industry. Thirdly, the study responds to growing public interest in food integrity, religious dietary compliance, and allergen transparency issues that are central to protecting consumer rights and sustaining trust in local food systems.

From a broader perspective, the importance of this research extends beyond the detection of fraud. Adulteration has multifaceted consequences. It undermines fair competition among producers, distorts market value, and may expose consumers to allergens or substances prohibited by religious dietary laws. In predominantly Muslim regions such as Kosovo, undeclared substitution of beef with other meat species may carry profound religious implications, as the presence of non-halal meat constitutes a violation of faith-based dietary principles. Moreover, the undeclared use of soy protein introduces allergenic risks for sensitive individuals, while simultaneously influencing the nutritional characteristics of the product, particularly the fatty acid composition. Therefore, the present study combines scientific rigor with a commitment to public health, ethical transparency, and cultural respect.

The research also contributes to the global discourse on food fraud detection methodologies. While conventional chemical analyses remain important for assessing compositional quality, molecular biology techniques such as DNA-based assays provide unparalleled precision in species identification and allergen detection. The study employs the Chipron LCD Array System (Meat 5.0) for broad-spectrum species identification, complemented by real-time PCR for the quantitative detection of soy DNA. These molecular results are then correlated with the fatty acid profiles obtained via GC-FID analysis, enabling a novel exploration of the relationship between adulteration and nutritional quality. The integration of these analytical approaches represents a significant methodological advancement in food authenticity research in Kosovo and the Western Balkans.

The structure of this dissertation is designed to systematically address the objectives and to ensure a logical progression from background understanding to practical conclusions. Following this introductory chapter, Chapter 4 (Materials and Methods) provides a detailed account of the experimental design, sampling strategy,

and analytical techniques used to detect and quantify undeclared meat species and soy protein in beef sausages. It outlines the rationale behind the selection of molecular and chemical methods, emphasizing their reliability, sensitivity, and applicability in complex food matrices. The chapter also discusses rigorous quality control measures and the statistical tools applied for data validation.

Chapter 5 (Results and Discussion) presents the core findings of the research, beginning with the molecular detection of DNA from non-beef species in both industrial and traditional sausages. The results reveal a high prevalence of poultry DNA, particularly chicken, indicating widespread substitution practices likely motivated by economic factors. The chapter further analyzes the detection and quantification of soy DNA, demonstrating its substantial presence in industrial products and its statistical significance when compared with traditional sausages. Subsequent sections examine the impact of undeclared ingredients on the fatty acid composition, showing that adulterated sausages exhibit reduced saturated fatty acids (SFAs) and increased polyunsaturated fatty acids (PUFAs). These alterations are discussed in relation to nutritional implications, authenticity concerns, and consumer protection. Comparative analysis with international studies contextualizes the findings within broader patterns of global meat adulteration.

Chapter 6 (Conclusions) synthesizes the results to provide a comprehensive understanding of the authenticity, safety, and nutritional implications of beef sausage production in Kosovo. It highlights the dual nature of adulteration—where certain substitutions may improve nutritional value yet simultaneously violate ethical, legal, and allergenic standards. The conclusions emphasize the urgent need for enhanced regulatory oversight, more transparent labeling practices, and public awareness initiatives. Furthermore, the chapter identifies avenues for future research, including economic analyses to assess the financial motivations behind adulteration, sensory studies to evaluate consumer acceptance of soy- and poultry-containing sausages, and policy-oriented research to strengthen national food authenticity monitoring systems.

In summary, this research contributes to the advancement of food authenticity science in Kosovo by providing the first integrated molecular and chemical analysis of beef sausage adulteration. It brings empirical clarity to the scope of undeclared substitutions and offers practical insights for regulators, producers, and consumers alike. Beyond its scientific contributions, the study serves as a foundation for

developing a national framework for food authenticity assurance, aligning Kosovo with European standards for transparency, traceability, and consumer protection. By combining modern analytical technology with a culturally and ethically informed perspective, this research aspires to reinforce public confidence in the integrity of food products and to promote responsible practices within the meat processing sector. Ultimately, the study affirms that safeguarding food authenticity is not merely a technical endeavor but a moral obligation, ensuring that consumers receive products that are truthful, safe, and respectful of both nutritional and cultural values.

2. REVIEW OF LITERATURE

2.1. Importance of Meat in Human Diets and The Role of Sausages in the Meat Industry

Meat is recognized as a highly nutritious food source that plays a crucial role in human diets worldwide. It provides several essential nutrients that are often challenging to obtain in adequate amounts from other dietary sources. Among these nutrients, bioavailable protein stands out as a key component, supplying the body with all the necessary amino acids required for vital physiological functions such as muscle repair, enzyme production, and immune response. Additionally, meat is an important source of several essential micronutrients that are commonly deficient in many populations' diets. These include iron, which is critical for oxygen transport in the blood; zinc, which supports immune function and cellular metabolism; and vitamin B12, a vitamin exclusively found in animal derived foods that is essential for neurological function and red blood cell formation [1].

When comparing protein sources, animal proteins generally exhibit superior digestibility and nutritional quality compared to plant proteins. This superiority is primarily due to the higher bioavailability of amino acids in animal proteins, meaning these amino acids are more readily absorbed and utilized by the human body. Plant proteins often lack one or more essential amino acids or have them in lower proportions, which can limit their nutritional effectiveness in isolation. Consequently, animal proteins are considered a more complete protein source, fulfilling human dietary requirements more efficiently [2].

2.2. The Role of Sausages in the Meat Industry

Sausage-making is an ancient practice, with historical accounts from the Odyssey and Roman feasts attesting to its long-standing popularity. Creating sausage requires a structured design and a controlled procedure to combine the right ingredients in proper proportions. The quality of the final sausage is always a reflection of the initial raw materials and the process used. Therefore, a carefully designed process depends on standardized raw materials to deliver consistent quality. Every ingredient must be

assessed against the sausage's quality requirements and governed by a specification that details all important physical, chemical, and microbiological criteria, as well as other services impacting the product [3]. Each local variety within each sausage type (raw, scalded, cooked, or raw and fermented/ripened) reflects the availability of raw materials, the climate conditions of each geographical environment, the cultural and religious conditionings, and the ancestral manufacturing knowledge transmitted through generations. It is evident and expected that the different local varieties of sausages and the art of making them were perfected over centuries through experience and the successes/failures in the production processes of the different generations.[4].

Fresh sausages (and eventually the raw-semidry ones) are produced from diverse kinds of meat such as beef, pork, mutton, chicken, turkey, etc., and usually pork fat or fatty tissues. Furthermore, various non-meat ingredients (salt, herbs, spices, juices, vinegar, etc.) and additives (nitrites, phosphates, sorbates, etc.) can be added according to the type of sausage, geographical traditions or manufacturing practices.[5]. Dry fermented sausages (DFS), mainly manufactured with pork and pork fat, are much appreciated in Western countries, and this type of sausages accounts for a significant part of traditional meat products produced in Mediterranean countries. In these countries, there is a wide variety of traditional DFS, produced in small-scale units, characterized by having in their manufacture a spontaneous fermentation [6]. On the other hand, in Islamic countries and in Muslim and Jewish religious communities, there are restrictions on the raw materials that will be in those meat products and on the processing conditions, not allowing the use of pork in the production of sausage.[7].

Otherwise, Beef sausage is typically made by mixing ground beef meat with salt, spices, and other seasonings before being encased in a skin made from animal intestines or synthetic materials. [8]. Of all sausage varieties, dry-fermented types are uniquely complex to produce. Their distinctive sensory properties emerge from a sequence of transformations in the raw materials, driven by the interplay between natural meat enzymes and microbial activity. This biochemical activity is further influenced by ingredients like salt and spices, as well as the specific environmental conditions maintained throughout the ripening stage. [9]. Within the wide variety of meat products available globally, sausages occupy a privileged position. Their popularity stems from several factors: their wide diversity in types and flavors, the notable nutritional value

they provide, their deep-rooted cultural significance in many societies, and their considerable economic importance within the food industry [10].

The production of sausages has experienced remarkable advancements over the years. These improvements encompass nearly every aspect of the process, including the refinement of the product's shape to meet consumer preferences, the diversification of animal species used to create different types of sausages, innovations in casings that affect texture and shelf life, the enhancement of ingredients to optimize flavor and nutrition, and the development of specialized equipment and machinery that increase production efficiency and product consistency [11].



Figure 1. Example of typical beef sausage packaging indicating '100% beef' labeling

Looking forward, it is expected that technological progress will continue to drive further innovations in sausage production. This includes both enhancements in areas already well-explored and breakthroughs in new research areas yet to be fully investigated, thereby continually improving product quality, safety, and consumer satisfaction.

2.3. Sausage production in Kosovo

There are 2 types of sausage produced in Kosovo: industrial sausage and homemade (traditional) sausage.

Industrial sausage in Kosovo

Industrial sausage is produced in large-scale facilities with standardized processes, aiming for consistency, long shelf life, and mass distribution. Made in government-

inspected factories with automated machinery. The production volume is high enough to supply supermarkets across the country and for export. Typically uses a mix of meats, often including chicken, turkey, or lower-cost cuts of beef and pork, to keep the price competitive. Contains preservatives (like sodium nitrite) to prevent spoilage and extend shelf life. It also includes stabilizers, flavor enhancers (like monosodium glutamate - MSG), and sometimes soy protein or other fillers to improve texture and reduce cost. The spice profile is standardized and less complex, usually focusing on salt, black pepper, and paprika. The flavor is consistent from one batch to another. The texture is very uniform and fine, almost emulsified, due to the high-level grinding and mixing. The casing is usually artificial, made of collagen or plastic. The taste is generally saltier, and the smokiness is often imparted by liquid smoke flavoring rather than traditional smoking. The flavor of the meat itself is less pronounced.



Figure 2. Industrial sausage produced in Kosovo

Homemade (Traditional) Sausage (Suxhuku)

This is the heart of Kosovar culinary tradition. It's a seasonal, family-oriented process, deeply rooted in culture and passed down through generations. Made manually at home, on the farm, or in small, artisanal butcher shops. The process is labour-intensive and usually done in late autumn or early winter when temperatures are ideal for air-drying. In the described method, as given by the three butchers, they mainly use beef meat, a combination of neck and rib meat, and fat. The ground meat, salted (NaCl) for 24 h at room temperature, is then mixed with minced onions, red and black pepper, and vegetable seasonings, and some minced bread is added at the end. The ingredients used in the sausage preparation, such as the beef meat, onions, and bread, were not

subjected to any disinfection or pasteurization procedures before processing. The whole mixture is then filled into the gut casings and is kept for 4–5 h at room temperature. The fermentation stage lasts 1–3 days, and the sausages are usually placed in special rooms for fermentation at a moderate temperature, starting at 27 °C on the first day and then rising to reach 34 °C by the end of 3rd day of fermentation. The fermented sausage is then put in storage at 0–3 °C for 3 days, after which it can later be preserved at room temperature. The environmental temperatures involved in the sausage production and storage ranged from 0 °C during refrigeration to 34 °C during fermentation. .[12].



Figure 3. Traditional sausages produced in Kosovo

The difference between industrial sausage and traditional sausage produced in Kosovo lies in the fact that industrial sausage can also contain meat from other species, vegetable proteins such as soy, but it needs to be declared. Traditional sausage contains only beef meat and fat (rarely sheep) and should not contain any meat from other species or vegetable proteins.

2.4. Fraudulent Meat Substitution

While food fraud is an ancient practice, documented as far back as the Roman Empire [13], it has only recently become a major global priority. This heightened attention is evident in the growing volume of scientific research [14]. The majority of available scientific studies have adopted definition of food fraud. They define it as a deliberate act for economic gain involving the substitution, addition, tampering, or misrepresentation of food, food ingredients, or food packaging.[15]. Food fraud types, definitions, and examples are presented in Table 1.

Table 1. Food fraud types, definitions, and examples

| Term | Definition | Example |
|----------------|--|--|
| Adulteration | An ingredient of the finished product is fraudulent | Melamine added to milk |
| Tampering | To render something harmful or dangerous by altering its structure | Changed expiry date, product up-labelling, and religious designation. |
| Simulation | The product is designed to look like but not exactly copy the legitimate product | “Knock-offs” security systems change- |
| Counterfeiting | Made in imitation of something else with the intent to deceive | Copies of a well-known brand of food made with other security systems. |

Source: Spink, Moyer, 2011.

In response, the European Commission (2013) [16], explicitly designated food fraud as a new area for action. Similarly, countries including the United States and China have launched their own programs [17]. Consequently, food fraud is now widely recognized by both regulators and the food industry as a significant food risk [18]. Meat is one of the food items that always make it to the list of most falsified foods in the world. While the true extent of fraud isn't clear to us, the picture is looking grim: in 2015, 483 million USD worth of smuggled meat was seized by Chinese authorities. Some of this meat was repeatedly frozen and thawed meat dating back to the 1970s, and was meant to be sold to consumers at the time it was seized.[19].

Although substitution involving different species, variations in protein or fat content, or the incorporation of plant-based ingredients represents a prevalent form of food adulteration, such tampering with food products carries significant consequences that extend beyond economic fraud. Adulterated food products pose a spectrum of risks ranging from mild to severe threats to public health and can also have detrimental effects on the environment [20].

The consumption of adulterated foods has been linked to a variety of adverse health outcomes. Commonly reported illnesses include gastrointestinal disturbances such as diarrhea and nausea, which result from exposure to harmful contaminants or allergenic substances. More chronic effects include allergic reactions, which can vary in severity from mild discomfort to life-threatening anaphylaxis. Furthermore, there is evidence associating adulterated food consumption with the development or exacerbation of

chronic conditions such as diabetes and cardiovascular diseases, underscoring the far-reaching impact of food adulteration on population health [21]. In light of these significant health risks and ethical considerations, it is imperative that meat production companies transparently declare the presence of any added species or grain flours in sausage products. Such transparency is essential because there exists a disconnect between consumer expectations regarding the accuracy and clarity of meat product labeling and the actual level of transparency achievable under current regulatory frameworks. This discrepancy can lead to consumer confusion and mistrust. Of particular concern is the use of ambiguous terminology such as “and/or” in ingredient lists, which can obscure the true composition of meat products. This ambiguity necessitates intensified efforts in consumer education and the provision of clearer guidance for the food industry to ensure honest and comprehensible labeling practices [22].

Moreover, fostering transparency and accountability within the food industry is critical for enhancing consumer confidence and safeguarding public health. Achieving this goal requires improved communication between regulatory bodies, producers, and consumers, along with more rigorous enforcement of existing food safety standards. Additionally, ongoing education and training initiatives targeting all stakeholders—ranging from food handlers to regulatory inspectors—are vital to raise awareness about food safety regulations and best practices [23]. Failure to declare additions or substitutions in meat products can severely undermine trust not only in food labeling but also in the broader regulatory systems designed to protect consumers. Thus, a clear and accurate declaration of ingredients in sausages is fundamental, as it differentiates between trust in the labeling information itself and the trust consumers place in the overall food supply system mediated through labeling [24].

Fat is a crucial ingredient in salami and other comminuted meat products, significantly influencing the texture, juiciness, and flavor profile. Generally, mammalian fats used in these products tend to have higher levels of saturated fatty acids compared to unsaturated fatty acids, although the exact fatty acid composition varies depending on the animal species from which the fat is sourced. Fat serves as a key reservoir for flavor compounds, which enhances the sensory experience of the meat product, while also contributing to its desirable moistness and tender texture. This explains why the absence of fat in products like beef sausages often results in a dry and

tough texture, which has been noted negatively by consumers. Given the health concerns associated with high saturated fat intake, there is a growing interest in identifying alternative fat sources that are lower in saturated fatty acids but still capable of maintaining or even enhancing the sensory qualities of beef sausages. One promising approach involves the addition of soy to sausages. Soy not only enriches the product with unsaturated fatty acids but also helps balance the fatty acid profile, countering the predominance of saturated fats in mammalian fat. Recent studies have thus focused on partially or fully substituting beef fat in sausages with non-beef fats rich in unsaturated fatty acids, aiming both to improve the nutritional value and to maintain or improve sensory attributes such as flavor, texture, and juiciness [25-29]. This approach aligns with consumer demand for healthier meat products without compromising on quality. Common undeclared ingredients found in beef sausages and their associated risks are presented in Table 2.

Table 2. Common undeclared ingredients found in beef sausages and their associated risks

| Added fraud / undeclared ingredient | Main risks / why it matters | Reference (Author et al., Year) |
|--|---|------------------------------------|
| Poultry (chicken, turkey) added to “beef” sausages | Economic adulteration and consumer deception; violation of halal/kosher or labeling claims; nutritional differences; undermines consumer trust. | Mehmetkaj et al., 2025 [30]. |
| Pork (undeclared pork in beef products) | Religious and ethical violations (halal/kosher); consumer fraud; potential allergenic cross-contact. | Di Pinto et al., 2014 [31]. |
| Soy / textured vegetable protein (TVP) | Major undeclared allergen; risk for soy-allergic individuals; lowers nutritional quality; used as a cheap filler for profit. | Segura-Gil et al., 2022 [32]. |
| Milk/dairy proteins (casein, whey) | One of the most common undeclared allergens can cause severe allergic reactions; frequent cause of food recalls. | Martínez-Pineda et al., 2022 [33]. |
| Egg proteins (ovalbumin, ovomucoid) | Major food allergen; may cause anaphylaxis if undeclared; health risk for allergic consumers. | Costa et al., 2021 [34]. |
| Gluten/wheat starch binders | Harmful for individuals with celiac disease or gluten intolerance; regulatory violation if | Cressey et al., 2019 [35]. |

| | | |
|--|---|-------------------------------|
| | unlabelled. | |
| Starch/plant fillers (potato, tapioca, rice) | Economic adulteration changes texture and composition; it misleads consumers regarding protein content and origin. | Reget et al., 2015 [36]. |
| Horse or other unexpected species (e.g., equine) | Consumer fraud; religious and ethical concerns; major scandal (2013); violates labelling and EU meat authenticity laws. | Premanandh et al., 2013 [37]. |
| Peanut / tree-nut contamination via spice mixes | Severe allergen risk; responsible for many undeclared-allergen recalls; potential life-threatening reactions. | Stone Jr. et al., 2023 [38]. |

2.4.1. Fraudulent Meat Substitution through Addition of Other Animal Species in Beef Sausages

Meat from other species, such as poultry or pork, is sometimes deliberately added to beef sausages primarily for economic motives, often involving fraudulent substitution. [39]. This practice entails replacing more expensive beef with cheaper meats to reduce production costs and increase profits. Such adulteration not only undermines consumer trust but also distorts market dynamics by unfairly disadvantaging producers who comply with labeling regulations and maintain product integrity [40]. The implications of this fraudulent substitution extend beyond economics; they also raise significant ethical concerns. In many countries, strict regulations prohibit the addition of poultry or other non-beef meats to products labeled as 100% beef. This is to prevent misleading consumers and protect cultural and religious dietary restrictions. For instance, the inclusion of pork in beef products is particularly sensitive in regions where pork consumption is forbidden for religious reasons, such as in Muslim or Jewish communities [41].

Beyond ethical and legal issues, the addition of chicken meat to beef sausages can materially alter the product's biochemical composition, particularly its fatty acid profile.



Figure 4. Example of typical beef sausage packaging with declared chicken meat

The fatty acid composition of a sausage is heavily influenced by the types of meats included, as well as other formulation ingredients like vegetable oils and lard. These changes can affect not only the nutritional quality but also the flavor, texture, and shelf life of the final product [42]. For example, in homemade beef sausages, saturated fatty acids constitute approximately 59.10% of the total fatty acid content. This is followed by monounsaturated fatty acids at 38.63%, and a smaller proportion of polyunsaturated fatty acids, about 2.27%. The dominant fatty acids in these sausages are oleic acid (C18:1), making up 34.37%, and palmitic acid (C16:0), accounting for 30.24%. Additionally, short-chain fatty acids are present, contributing to the overall fatty acid profile and influencing sensory properties [43].

In contrast, chicken meat displays a markedly different fatty acid composition. It typically contains a lower proportion of saturated fatty acids, around 36.4%, compared to beef, which contains approximately 53.3%. Conversely, chicken meat has a considerably higher proportion of polyunsaturated fatty acids, about 21.3%, in comparison to just 3.0% in beef. These differences are important because polyunsaturated fatty acids are generally considered more beneficial for cardiovascular health than saturated fatty acids [44]. The fatty acid profile of chicken meat is primarily composed of linoleic acid, oleic acid, and palmitic acid, each contributing distinctly to the nutritional and sensory characteristics of the meat [45].

2.4.2. Fraudulent Meat Substitution through Addition of Soy in Beef Sausage

Food allergy represents a significant health concern worldwide, affecting approximately 3% to 5% of adults and around 8% of children [46, 47]. Beyond allergies, in developed countries, it is estimated that up to 20% of individuals

experience some form of food sensitivity. These sensitivities can arise due to the presence of chemical, microbiological, or pharmacologically active contaminants in food products, which can provoke adverse reactions. Soy is a common allergen frequently incorporated into meat products such as salami. Numerous studies have confirmed the presence of soy content in salami, highlighting its widespread use as a non-meat protein source. The inclusion of soy protein fractions in meat products is driven by both functional and economic motivations. Soy proteins enhance product characteristics such as water and fat binding, texture, and emulsification, which can improve the overall quality and yield of meat products while simultaneously reducing production costs [48, 49].

These functional properties make soy protein fractions highly preferred in processed meat formulations. Nutritionally, soybeans are composed of approximately 35% protein, 31% carbohydrates, 17% oil, 13% water, and 4% ash. Of these components, the protein and lipid fractions hold the greatest commercial value, underpinning the importance of soybeans not only as a nutritional ingredient but also as a functional additive in food production [50].

However, the presence of soy in meat products poses health risks for individuals with soy allergies, emphasizing the need for clear labeling and consumer awareness to prevent allergic reactions.



Figure 5. Example of typical beef sausage packaging with a declaration of soya protein

Adding soy to sausages offers multiple benefits, including increasing the protein content while reducing fat levels. For instance, fermented sausages can be produced with healthier characteristics by reducing fat content from 15% to 10% and incorporating 1% soy fiber, all without compromising product quality [51]. This nutritional enhancement aligns with consumer demand for healthier meat products. The

inclusion of soy in salami and other processed meats is legally permitted and regulated within the European Union. Soy protein and its derivatives are recognized as approved food additives for use in meat products, subject to specific EU regulations and labeling requirements [52]. However, the quantity of soy additives must be carefully controlled.

2.5. Regulatory Framework Governing Meat Labelling in the EU and Kosovo

Regarding the addition of other spices to beef sausage, European Union legislation, notably Regulation (EU) No 1169/2011 on the provision of food information to consumers, establishes a comprehensive legal framework designed to enhance consumer protection by mandating that food business operators provide clear, accurate, and easily understandable information about the contents of their products. Such transparency is essential to allow consumers to make informed and safe choices about the foods they purchase and consume [53]. A critical component of this regulation, as specified in Annex VII, Part B, requires the explicit declaration of the species from which the meat originates. This provision aims to prevent mislabeling and fraud, ensuring consumers are fully informed about the true nature of the meat products they buy [54].

Despite these regulatory safeguards, meat products remain vulnerable targets for species substitution and adulteration. This vulnerability is largely driven by the high market value of certain meats, such as beef, which incentivizes unscrupulous practices aimed at economically benefiting from the substitution with cheaper species. Such acts not only violate regulatory standards but also undermine consumer trust and can pose health risks. Regarding the addition of soy, the European Commission Regulation (EC) No 1333/2008 establishes maximum permissible levels for food additives, including soy-based ones, across various food categories to ensure safety and quality. Furthermore, Regulation (EU) No 1169/2011 mandates clear allergen labeling in prepackaged foods, requiring that allergens such as soy be declared using the name of the source food allergen. This regulation ensures that consumers are properly informed about the presence of soy in food products [55].

In line with this, if soy is part of the formulation, it must be explicitly declared either in the ingredient list or in a separate “contains” statement immediately following the

ingredients, as required by regulatory agencies such as the U.S. FDA and Canadian authorities [56, 57]. Documented international cases of meat adulteration are presented in Table 3.

Table 3. Documented international cases of meat adulteration

| Mislabeling / Fraud Detected | Detection Method | Violation Type / Significance | Reference (Author(s), Year) |
|--|---|--|-----------------------------------|
| Horse meat in beef lasagna/beef burgers | DNA testing / DNA barcoding / species-specific PCR | Economic fraud, labelling fraud, and potential drug residue risk | Walker, M. J. et al. (2013) [59]. |
| Porcine (pork) DNA in meatballs labelled as beef (halal concern) | Real-time qPCR (cytochrome b gene) | Religious dietary violation, fraud | Sajali et al. (2022) [60]. |
| Pork / wild boar in “beef meatballs” | Real-time PCR, species-specific mitochondrial primers | Religious violation (halal), adulteration | Rahmania et al. (2025) [61]. |

For Kosovo, food products placed on the market must comply with Law No. 03/L-016, which requires a declaration of the prescribed list of ingredients along with the quantity and category of certain ingredients, ensuring transparency and regulatory compliance [62]. The responsibility for accurate and transparent labeling of food products lies squarely with every company involved in food production. In Kosovo, this responsibility is governed by national regulations, specifically the Administrative Instruction No. 09/2013, which outlines detailed requirements for the labeling, presentation, and advertising of food products.

2.6. Advances in Detection Methods for Fraudulent Meat Substitution

The development of fast and efficient methods for detecting meat adulteration is crucial for advancing the food industry and ensuring consumer safety. Meat adulteration detection technologies are generally classified based on the target substances they analyze: protein-based, metabolite-based, or nucleic acid-based methods [63]. Traditional limitations of these methods have led to the adoption of polymerase chain

reaction (PCR)-based techniques, which are highly favored due to their superior specificity, sensitivity, rapid processing time, and cost-effectiveness [64].

Among these technologies, DNA sequence examination stands out as one of the most convenient and reliable methods for identifying the species origin in processed meat products. This is especially important because heat processing can fragment DNA, but real-time PCR (qPCR) techniques are capable of amplifying even small DNA fragments, enabling accurate identification [65]. The real-time PCR method has been developed and extensively evaluated for the detection and identification of species in processed meat products, making it a valuable tool for food quality control and fraud prevention. Furthermore, species-specific real-time PCR assays employing TaqMan probes have been successfully developed to verify the labeling of meat from various game bird species, such as quail, pheasant, partridge, guinea fowl, pigeon, Eurasian woodcock, and song thrush. These assays have shown high suitability for detecting the presence of target DNA sequences, thereby confirming the authenticity of meat products [66].

In addition, duplex PCR assays targeting species-specific mitochondrial Cytochrome b (Cyt b) gene primers have been applied for simultaneous identification of cattle and buffalo, as well as chicken and pig species, in meat and milk products. These duplex PCR methods have demonstrated accuracy and reliability in species identification, offering efficient tools for monitoring adulteration in multiple animal products [67].

Beyond PCR methods, enzyme-linked immunosorbent assay (ELISA) combined with PCR has also proven to be effective for detecting mislabeling and adulteration in fish products, such as Grouper.

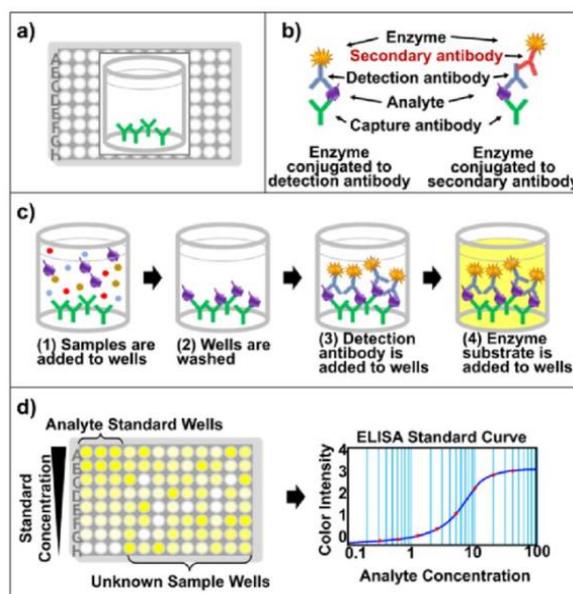


Figure 6. Soy protein detection using a sandwich-ELISA kit in sausage samples [68].

Figure 1. Overview of the sandwich ELISA immunoassay technique. (a) The wells of an ELISA plate are coated with analyte-specific capture antibodies. (b) When analyte is present, interactions with the capture antibody and a separate analyte-specific detection antibody allow formation of a "sandwich" complex, which provides for analyte detection and quantitation. The signaling enzyme (e.g., Horseradish Peroxidase) can be directly conjugated to the detection antibody or to a secondary antibody with specificity for the detection antibody. (c) In the ELISA workflow, (1) samples containing a complex mixture of molecules (e.g., plant tissue extracts) are added to the ELISA plate wells; (2) analyte molecules are specifically bound by the capture antibody and the wells are washed to remove nonspecific molecules; (3) a solution of detection antibody is added to the wells and the antibody binds specifically to the captured analyte; and (4) the enzyme substrate is added to the wells and allowed to develop a visible colored signal. (d) Known amounts of an analyte standard are added to wells on the plate to generate a standard curve. The intensity of the signal in the sample wells is directly proportional to those produced by the standard curve allowing for quantitation of the analyte. [68].

Both ELISA and PCR have been validated as specific and reliable techniques that support accurate traceability and regulatory food control enforcement [69]. Detecting undeclared ingredients in processed meat products, such as sausages, requires highly sensitive and specific analytical methods, especially when these products undergo heat

treatment and complex emulsification. Multiple scientific techniques have been developed and applied to identify both animal species and plant-based additives like soy in meat matrices. Each method varies in cost, sensitivity, and applicability, depending on the target component and food matrix.

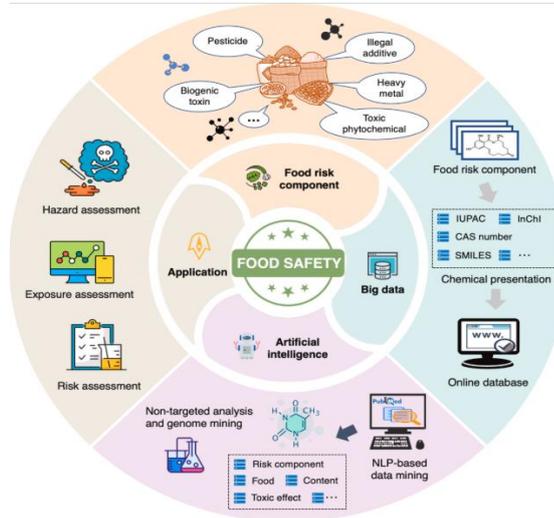


Figure 7. Overview of molecular and chemical techniques used in food authenticity testing [70].

Modern laboratory methods for detecting the presence of soy in sausages typically involve advanced analytical techniques that can identify soy-derived components such as proteins, DNA, or specific compounds unique to soybeans. Polymerase Chain Reaction (PCR): PCR is a sensitive and specific method used to detect DNA sequences unique to soybeans. It can identify the presence of soy DNA in sausage samples. Authors Sónia Soares et al [71] used Quantitative detection of soybean in meat products by a TaqMan real-time PCR assay, and they proposed a normalized real-time quantitative PCR assay to determine the addition of soybean to meat products.

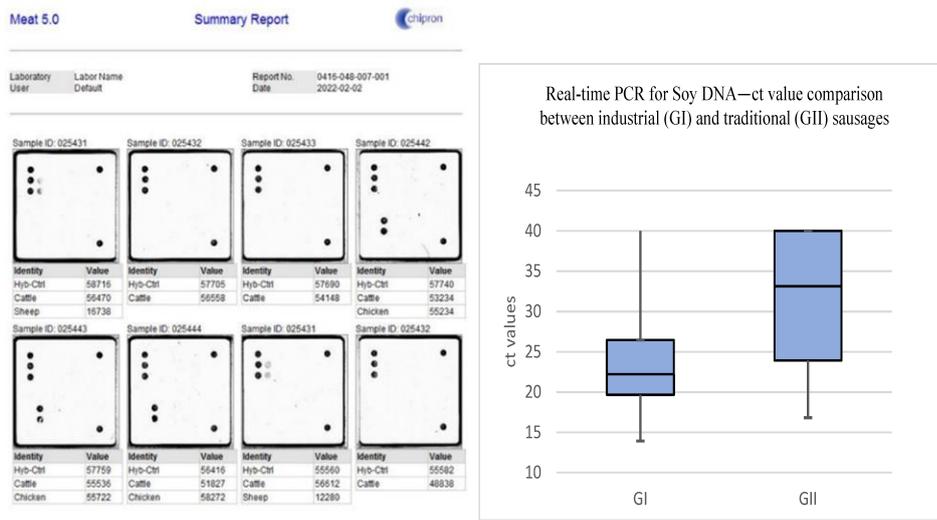


Figure 8. Detection results of undeclared species in sausage samples from Kosovo using qPCR analysis.

The method proved to be a powerful tool for the quantification of soybean protein (dry basis) in the range of 0.01% to 6%, being successfully in-house validated. Its application was effective in the analysis of several meat products, indicating 2% non-compliance with the food allergen labeling legislation, and some inconsistencies when comparing the declared with the estimated amounts of soy-bean. This work highlights the importance of efficient tools to assess labeling statements of meat products, avoiding fraudulent practices. The detection of soy in sausages through this laboratory method is also confirmed by the works of other authors [72, 73, 74, 75, 76]. Comparison of Detection Methods for Meat and Soy Adulteration is presented in Table 4.

Table 4. Comparison of Detection Methods for Meat and Soy Adulteration

| Study (Author et al., Year) | Country/setting | Sample type (sausage context) | Detection method(s) used |
|----------------------------------|--|--|---|
| Mehmetukaj et al., 2024. [77]. | Kosovo (industrial vs traditional producers) | Industrial and homemade sausages (n = 63) | Real-time PCR for soy DNA (targeted assay) |
| Benli et al., 2021. [78]. | Turkey (research lab validation) | Fermented sausages (method study) | Sequential real-time PCR + ELISA (protein + DNA) |
| Montowska et al., 2019. [79]. | Poland / Europe (method development) | Smoked and processed meats (includes sausages) | Targeted UHPLC-MS/MS (mass spectrometry) for soy peptides |
| Kružíková et al., 2022. [80]. | Czech market surveillance | Commercial meat products (salami/sausages) | ELISA and PCR comparison study |
| Pedersen et al., 2008. [81]. | Denmark (method comparison) | Model foods and processed products (relevant to sausages) | Multiple: sandwich/competitive ELISA, PCR, HR, EAST inhibition |
| Abd-Elhafeez et al., 2024. [82]. | Egypt (quantitative methods) | Meat products (histology + microscopy + quantitative analysis) | Histology, SEM, chemical quantification and recovery; also applied molecular approaches |

3. OBJECTIVES OF THE DOCTORAL DISSERTATION

The objectives of this doctoral dissertation are:

- To conduct a comprehensive molecular analysis of beef sausages produced in Kosovo using the Liquid Chip Display (LCD) Array system to detect and identify undeclared meat species and quantify the extent of adulteration in both industrial and traditional products;
- To apply real-time polymerase chain reaction (PCR) techniques for the detection and quantification of soy DNA, to determine the prevalence of soy-based adulteration and assess compliance with food labeling and allergen declaration standards;
- To evaluate the impact of adulteration on the nutritional quality of beef sausages by analyzing the fatty acid profiles using gas chromatography flame ionization detection (GC-FID), and to establish correlations between soy or poultry DNA content and variations in lipid composition
- To perform a comparative assessment between industrial and traditional sausage production systems, examining differences in authenticity, labeling accuracy, and compositional integrity, and identifying key risk factors contributing to food fraud within Kosovo's meat industry.
- To develop scientifically grounded recommendations for national authorities and food producers aimed at enhancing regulatory enforcement, analytical control systems, and public awareness, thereby improving food authenticity and protecting consumer rights in Kosovo.

4. MATERIALS AND METHODS

This section comprehensively describes the experimental design, analytical techniques, and rigorous quality control measures implemented throughout this study. The primary objective was to comprehensively investigate the prevalence and characteristics of adulteration in beef sausages sourced from Kosovo. To achieve this goal, a multifaceted approach was adopted, integrating molecular techniques for precise species and soy identification, sophisticated chemical analyses for detailed fatty acid profiling, and robust statistical methods for accurate data interpretation and meaningful conclusion extraction.

4.1. Sampling Strategy and Sample Preparation: Ensuring Representative Data

A total of 68 beef sausage samples were strategically acquired from a diverse array of markets throughout Kosovo, with careful consideration given to encompassing a balanced representation of both industrial (n=43) and traditional (n=25) production methods. This mixed sampling approach was intentionally designed to capture a representative snapshot of the broader sausage market landscape, acknowledging the inherent differences in production scales, ingredient sourcing practices, and potential variations in processing techniques that may exist between large-scale industrial manufacturers and smaller-scale traditional producers. This representative sampling is essential for ensuring the generalizability of the study's findings to the wider Kosovar beef sausage market.

Acknowledging the inherent potential for undeclared ingredients and fraudulent labeling practices, the sample collection process was deliberately conducted without taking into consideration any pre-existing declarations of meat species or soy content listed on the product labels. This unbiased and non-selective approach ensured a more accurate and realistic reflection of actual adulteration practices prevalent in the market, irrespective of compliance with labeling regulations or the potential for intentional misrepresentation by producers.

To facilitate the execution of a comprehensive and multi-pronged analytical strategy, each sausage sample, meticulously weighed to a precise 250 g, was carefully divided

into two equal portions under strictly controlled sterile conditions to minimize the risk of cross-contamination or degradation. One portion was specifically allocated for downstream molecular analyses, including DNA extraction, targeted species identification utilizing the LCD Array system, and sensitive soy detection via real-time PCR. The other portion was dedicated exclusively to chemical testing, primarily focusing on the generation of detailed fatty acid profiles through the application of advanced gas chromatography-flame ionization detection (GC-FID) techniques.

To ensure the long-term stability and integrity of the collected samples and to mitigate any potential degradation of sensitive analytes such as DNA and fatty acids, all samples were promptly stored at a precisely controlled temperature of 4 °C immediately following collection and maintained under these conditions throughout the entire duration of the study until the initiation of further processing steps. This rigorous sample handling protocol, encompassing careful portioning, sterile techniques, and controlled temperature storage, served as a cornerstone of the study's methodology, ensuring the reliability, reproducibility, and accuracy of all subsequent analytical procedures and data interpretations.

The rationale for employing slightly different sample sizes for the meat species analysis (n=68) and the soy analysis (n=63) stemmed from a combination of factors, including preliminary scoping studies conducted before the main investigation, logistical constraints related to sample processing and analysis, and strategic resource allocation considerations. While acknowledging that a larger and more uniformly sized sample would have offered enhanced statistical power and the potential for more robust and representative results, the selected sample sizes were carefully deemed to be sufficient for achieving the primary objectives of the study. Specifically, these objectives encompassed providing an initial but comprehensive exploration of adulteration practices within the Kosovar beef sausage market and identifying key trends and patterns in the utilization of undeclared ingredients, such as non-bovine meat species and soy-based additives.

4.2. Molecular Analyses for Species and Soy Identification: Unveiling Hidden Ingredients

To achieve a comprehensive understanding of the species composition and potential adulteration practices within the collected beef sausage samples, a dual-pronged

molecular analysis approach was implemented. This approach leveraged the complementary strengths of two distinct DNA-based techniques: the Chipron LCD Array Analysis System for broad-spectrum meat species identification and real-time PCR for highly sensitive and quantitative detection of soy DNA.

4.2.1. Chipron LCD Array Analysis System, Meat 5.0: High-Throughput DNA-Based Meat Species Identification

For the comprehensive and high-throughput DNA-based identification of meat species present within the beef sausage samples, the cutting-edge MEAT 5.0 LCD-Array Kit (Chipron GmbH, Berlin, Germany) was strategically selected. This commercially available and rigorously validated system is specifically engineered for food authenticity testing and quality assurance applications, offering exceptional levels of analytical specificity and remarkably low detection thresholds, typically below 1% (as expressed by F_c -values exceeding 24). The key advantage of the LCD Array system lies in its capacity to simultaneously and unequivocally detect a broad spectrum of animal species within a single assay, making it a highly efficient and cost-effective tool for screening complex food matrices, such as processed meat products, for potential adulteration or mislabeling. The ability to simultaneously screen for a wide range of species is particularly valuable in situations where the nature of the adulterant is unknown or where multiple adulterants may be present.

The underlying principle of the LCD Array technology relies on the amplification and subsequent detection of a highly conserved, yet uniquely variable, 125–165 base pair (bp) fragment of the mitochondrial 16S ribosomal RNA (rRNA) gene. The strategic selection of the mitochondrial 16S rRNA gene as the primary target region is predicated on several key factors that contribute to the overall robustness and sensitivity of the assay. First and foremost, mitochondrial genomes are present in extremely high copy numbers within mammalian and avian cells, significantly amplifying the signal and enhancing the detection capabilities of the assay, even when dealing with highly processed or degraded DNA. Secondly, the 16S rRNA gene contains highly conserved flanking regions, which allow for the design of universal PCR primers that can amplify the target region across a broad range of animal species. Critically, the 16S rRNA gene also contains highly variable internal regions that exhibit unique species-specific sequence signatures, enabling the design of highly specific probes for unambiguous

species identification. This ingenious combination of conserved flanking regions and variable internal regions enables the LCD Array to achieve both broad-spectrum amplification and highly specific species discrimination within a single assay.

Prior to initiating LCD Array analysis, genomic DNA was meticulously extracted from the sausage samples using the well-established and commercially available DNeasy Mericon Food Kit (Qiagen GmbH, Hilden, Germany), following a modified cetyltrimethylammonium bromide (CTAB) protocol. This kit is specifically formulated and optimized for efficient DNA extraction from challenging food matrices, such as processed meat products, and is known for its exceptional ability to effectively remove PCR inhibitors, such as lipids, polysaccharides, and Maillard reaction products, that can commonly interfere with downstream enzymatic reactions and compromise the sensitivity and accuracy of PCR-based assays. To ensure the reproducibility and reliability of the extraction process and to minimize the potential for technical errors or variations, DNA extraction was performed in duplicate for each individual sausage sample. In brief, a precisely weighed 200 mg subsample was initially homogenized with one sterile stainless steel bead and 1 mL of a specialized tissue lysis buffer using a high-throughput TissuLyser II instrument (Qiagen GmbH, Hilden, Germany), operating at a frequency of 30 Hz for a duration of 30 seconds. This rigorous homogenization step ensured thorough disruption of the cellular matrix and efficient release of DNA from within the sausage tissue. Following homogenization, the resulting homogenate was subjected to enzymatic lysis with proteinase K (2.5 μ L) at a carefully controlled temperature of 60 °C for a duration of 30 minutes. This enzymatic digestion step facilitates the efficient degradation of proteins and further promotes the release of DNA into the surrounding solution. Upon completion of the enzymatic lysis, the samples were carefully cooled to room temperature and subsequently centrifuged to effectively remove any residual cellular debris or particulate matter. The resulting supernatant (700 μ L), now enriched with the extracted DNA, was then combined with 500 μ L of chloroform to selectively remove lipids, cellular debris, and other potentially inhibitory contaminants from the DNA preparation. Following thorough mixing by vortexing and subsequent centrifugation at a high speed of 14,000 \times g for 15 minutes, the aqueous phase (350 μ L), now highly enriched in DNA and relatively free of contaminants, was carefully transferred to QIAquick spin columns for further purification and concentration. This QIAquick spin column purification step relies on the selective

binding of DNA to a silica membrane under specific buffer conditions, allowing for the efficient removal of any remaining contaminants. The bound DNA was then rigorously washed with a specialized Buffer AW2 to further eliminate any residual impurities. Finally, the purified and concentrated DNA was eluted from the spin column with a precisely measured volume of 150 μL of Buffer EB, yielding a highly purified and concentrated DNA extract that was optimally suited for downstream PCR amplification and subsequent analysis by the LCD Array system. Throughout the entire DNA extraction process, stringent precautions were implemented to minimize the risk of cross-contamination, including the exclusive use of sterile filter tips, the consistent use of disposable gloves, the meticulous cleaning and disinfection of all work surfaces, and the strict adherence to designated work areas dedicated solely to DNA extraction procedures.

The polymerase chain reaction (PCR) was performed using a Topical Gradient 96 thermocycler (Biometra GmbH, Göttingen, Germany), a state-of-the-art instrument that provides precise temperature control and gradient optimization capabilities for enhanced PCR performance. Each PCR reaction was assembled in a final volume of 25 μL , consisting of 12.5 μL of a pre-optimized 2 \times Master Mix (specifically formulated for use with the MEAT 5.0 kit), 1.5 μL of the MEAT primer mix (containing a carefully designed blend of both universal and species-specific PCR primers), 6 μL of high-purity PCR-grade water (to provide the necessary reaction volume and maintain reagent concentrations), and 5 μL of the extracted DNA template (containing the target 16S rRNA gene sequences). The specific thermal cycling profile was carefully optimized to ensure efficient and specific amplification of the target 16S rRNA gene fragments from the various meat species of interest. The optimized thermal profile consisted of an initial denaturation step at 95 $^{\circ}\text{C}$ for a duration of 5 minutes (to ensure complete and thorough denaturation of the double-stranded DNA template), followed by 35 iterative cycles of denaturation (94 $^{\circ}\text{C}$ for 30 seconds), annealing (57 $^{\circ}\text{C}$ for 45 seconds), and extension (72 $^{\circ}\text{C}$ for 45 seconds), and a final extension step at 72 $^{\circ}\text{C}$ for an extended duration of 2 minutes (to ensure complete and full-length extension of all amplified DNA fragments).

Following PCR amplification, the resulting PCR products were verified by conventional 2% agarose gel electrophoresis to confirm successful amplification of the target 16S rRNA gene fragments and to assess the overall size distribution and integrity

of the amplified DNA. This electrophoretic analysis serves as a crucial quality control step, ensuring that only high-quality and specifically amplified PCR products are carried forward into the subsequent hybridization and detection phases of the LCD Array assay.

Hybridization, the key step in which the amplified DNA fragments specifically bind to complementary probes on the LCD Array, was carefully conducted at a controlled temperature of 35 °C for a duration of 30 minutes, precisely following the protocol recommended by the manufacturer. This controlled hybridization environment enables optimal binding kinetics and minimizes the potential for non-specific interactions or cross-hybridization.

Following the hybridization step, the LCD Array was thoroughly washed to remove any unbound or non-specifically bound DNA fragments, ensuring a clean and specific signal. The resulting hybridized array was then meticulously visualized and analyzed using the advanced PF3650u LCD-array scanner (Pacific Image Electronics Co., Ltd., Torrance, CA, USA) and the associated Slide Reader V12 software. The LCD Array scanner is specifically designed to capture the fluorescent signals emitted from the reporter molecules attached to the hybridized amplicons, providing quantitative measurements of the signal intensity at each probe location on the array.

The Slide Reader V12 software performs automated image analysis, quantifies the signal intensity at each probe location, and applies sophisticated algorithms to correct for background noise and spatial variations in signal intensity. To ensure accurate and reliable species identification, each PCR reaction and subsequent hybridization were performed in duplicate, and the resulting signal intensities were averaged across the duplicate spots. A stringent pixel intensity threshold of 2000, as explicitly defined by the MEAT 5.0 Manual (version 1-1-2014), was utilized as the primary cut-off value for determining positive detection of a specific meat species. This threshold was carefully determined and validated by the manufacturer through extensive testing and optimization to minimize the occurrence of false-positive results and to ensure the overall accuracy and reliability of the species identification data.

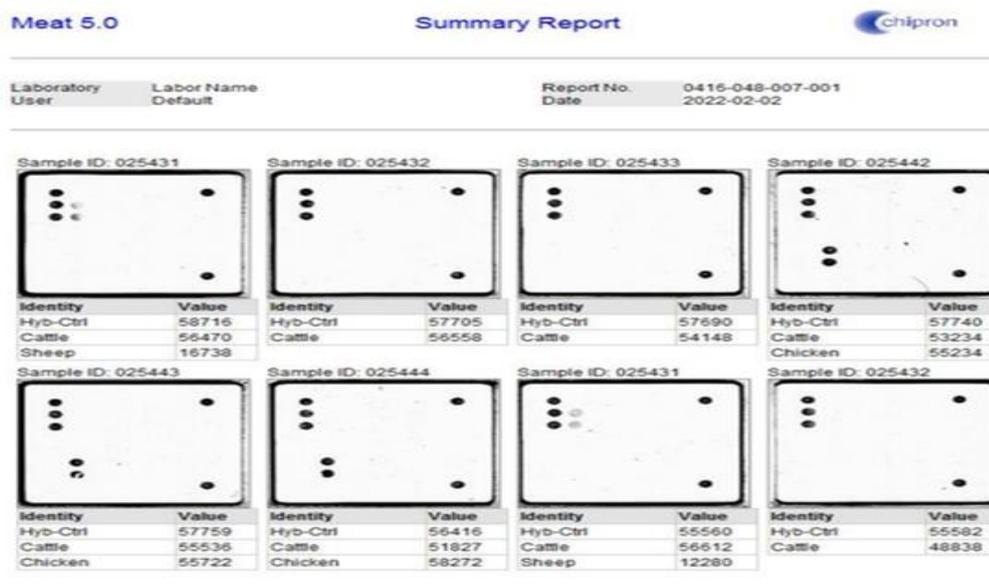


Figure 9. LCD chip during analysis with LCD Array

4.2.2. Real-Time PCR for the Detection of Soya DNA: Sensitive and Quantitative Soy Adulteration Assessment

To complement the meat species identification and to provide a more comprehensive and nuanced assessment of adulteration practices, real-time polymerase chain reaction (PCR) was strategically employed for the highly sensitive detection and accurate quantification of soy DNA within the beef sausage samples. While the LCD Array system offers broad-spectrum screening for meat species, real-time PCR offers unparalleled sensitivity and quantitative capabilities for specific targets such as soy DNA, allowing for the precise determination of soy content and the ability to detect even trace amounts of soy adulteration. This quantitative aspect is particularly valuable for assessing the economic motivations behind adulteration practices and for evaluating the potential impact of soy content on the overall nutritional profile of the sausages.

For real-time PCR analysis, DNA extraction was performed using the same DNeasy® mericon® Food Kit (QIAGEN, Cat. 69514, Hilden, Germany) that was used for LCD Array analysis, adhering to the manufacturer’s standardized protocol and utilizing 200 mg of the sample as the initial starting material. This consistent DNA extraction procedure ensured comparability of results across the two distinct molecular methods, minimizing potential biases arising from differences in extraction efficiency or sample preparation techniques. To enable accurate quantification of soy DNA copy numbers,

the Allergen RM 800 reference material (Hygiene, former Biotecon, Camarillo, CA, USA), which contains a known and certified concentration of soy DNA, was extracted concurrently with the sausage samples and served as an external calibration standard for quantitative analysis. This reference material allows for the construction of a precise calibration curve, enabling the accurate determination of soy DNA concentrations in the unknown sausage samples.

Real-time PCR for the detection and quantification of soya DNA was performed using commercially available, ready-to-use kits (food proof® Soya detection kit and food proof®, Hygiene, Camarillo, CA, USA), strictly following the manufacturer's detailed instructions. These kits are specifically designed and optimized for food allergen detection, providing all the necessary reagents, enzymes, primers, probes, and controls for achieving accurate and reliable soy quantification. The amplification reactions were conducted on the state-of-the-art Quant Studio 5 Real-Time PCR system (Applied Biosystems™, Thermo Fisher Scientific, Waltham, MA, USA), a highly sensitive and precise instrument that allows for real-time monitoring of PCR amplification and accurate quantification of target DNA sequences.

The following carefully optimized thermal profile was employed to maximize the efficiency and specificity of the soy DNA amplification: a pre-incubation step at 37 °C for 4 minutes (to allow for optimal reverse transcription, if necessary), followed by an initial denaturation at 95 °C for 10 minutes (to fully denature the DNA template), and a subsequent 50 amplification cycles consisting of a denaturation step at 95 °C for 5 seconds and an annealing/extension step at 60 °C for 60 seconds. During each amplification cycle, fluorescence data were collected on the FAM channel (specific for the soy DNA target) and the VIC channel (specific for the internal positive control). The use of an internal control is essential for normalizing any cycle-to-cycle variations in PCR efficiency and for ensuring the accuracy and reliability of the soy DNA quantification process (10). The cycle threshold (Ct) values, defined as the number of cycles required for the fluorescent signal to exceed a predetermined threshold, were automatically calculated by the QuantStudio 5 software and were used to generate a standard curve.

4.3. Gas Chromatography–Flame Ionization Detector (GC/FID) for Fatty Acid Profiling: Dissecting the Lipid Composition

To comprehensively investigate the potential impact of adulteration on the nutritional composition and authenticity of the beef sausages, detailed fatty acid profiling was performed using gas chromatography coupled with flame ionization detection (GC-FID). Gas chromatography is a powerful and widely used analytical technique that allows for the separation and quantification of volatile organic compounds, such as fatty acid methyl esters (FAMES). When coupled with a flame ionization detector (FID), GC becomes a highly sensitive and quantitative method for determining the fatty acid composition of complex lipid mixtures. By separating and quantifying the individual fatty acids present in the sausage samples, GC-FID provides invaluable insights into the authenticity, nutritional quality, and potential for adulteration within these processed meat products.

Before performing GC-FID analysis, it was essential to extract and chemically derivatize the fatty acids from the sausage samples into their corresponding fatty acid methyl esters (FAMES). This derivatization process is necessary to enhance the volatility and thermal stability of the fatty acids, making them more amenable to separation and detection by gas chromatography. The chemical derivatization procedure employed in this study was based on the well-established and widely used method of base-catalyzed transesterification of fatty acids, which involves reacting the extracted lipids with methanol in the presence of a strong base catalyst, such as sodium methoxide. This transesterification reaction effectively converts the fatty acids into their corresponding methyl esters, which are then readily separated and quantified by GC-FID. The specific derivatization protocol was carefully modified from a previously published method by Haifeng Sun and Suli Zhao in 2014 [83], incorporating key optimizations and procedural refinements to enhance the yield and efficiency of the transesterification reaction and to minimize the formation of unwanted by-products.

To ensure the accuracy, precision, and reliability of the GC-FID analysis, only the highest purity chemicals and reagents were used throughout the entire extraction, derivatization, and analytical processes. Specifically, the following high-purity chemicals were employed: hexane (99.9% purity) from Honeywell Riedel-de-Haën™ (Honeywell, Charlotte, NC, USA), methanol (99.9% purity, CHROMASOLV™ grade)

from Honeywell Riedel-de-Haën™ (Honeywell, Charlotte, NC, USA), ethyl acetate (99.7% purity, CHROMASOLV™ grade) from Honeywell Riedel-de-Haën™ (Honeywell, Charlotte, NC, USA), and a certified reference material (CRM) consisting of a Supelco® 37 Component FAME Mix from Sigma-Aldrich® (Burlington, MA, USA). This certified reference material, which is prepared and certified according to stringent ISO 17034 and ISO/IEC 17025 standards, was used to prepare calibration standards and to verify the accuracy and linearity of the GC-FID response. The utilization of certified reference materials and high-purity solvents is absolutely critical for minimizing the potential for systematic errors and ensuring that the resulting fatty acid profiles are accurate, reliable, and traceable to internationally recognized standards.

The extraction and derivatization procedure involved several key steps. First, sausage samples were homogenized using a Velp Scientifica™ OV5 homogenizer (Usmate Velate (MB), Italy) to ensure a representative and uniform sample for analysis. Following homogenization, 500 mg of the sample was accurately weighed into 15 mL conical tubes and mixed with 5 mL of hexane (99.9%) to extract the lipids. After thorough vortexing for 1 min to facilitate lipid solubilization, 1 mL of sodium methoxide (5.4 M) in methanol was added and mixed for 1 min by vortexing to initiate the transesterification reaction. The esterification reaction was allowed to proceed at room temperature under controlled conditions. After strong vortexing for 1 min and centrifuging for 5 min at 5000 rpm to separate the phases, the supernatant, containing the FAMES, was carefully transferred to a 2 mL glass vial and 2 μ L was injected in the GC/FID analysis. To minimize potential degradation or isomerization of the FAMES, samples were analyzed within an hour after esterification.

The determination of FAMES was conducted using an Agilent 8890 GC System, which features a split/splitless inlet and an FID detector, along with an Agilent 7693 A automatic liquid sampler (ALS). The operational parameters of the GC-FID system, including column type, temperature program, carrier gas flow rate, and detector settings, were carefully optimized to achieve optimal separation and quantification of the FAMES. The specific operational setup of the GC-FID system is detailed in Table 5. Precise control and monitoring of these parameters are essential for accurate fatty acid profiling and ensuring the reproducibility of the results. The operational setup of GC-FID is presented in Table 5.

Table 5. The operational setup of GC-FID (GC System 8890 GC).

| | |
|-------------------|--|
| GC system | 8890 GC |
| S/SL Inlet | 250°C, split ration 50:1, |
| Liner | Split, ultra inert, glass wool, low pressure drop (p/n 5190-2295) |
| Oven ramp program | 50°C (0.5minutes), 30°C/min to 194°C (3.5minutes), 5°C/min to 240°C (3minutes) |
| Carrier gas | Nitrogen, 13 psi, constant pressure mode |
| Column | DB-Fast FAME 30m x 0.250mm x 0.25um 40 to 250/260°C |
| Detector-FID | 260°C, H2: 40mL/min Air: 400mL/min Make up gas: 25mL/min |
| Injection volume | 1ul |

To ensure the reliability and validity of the GC-FID method, a comprehensive validation process was conducted following the guidelines outlined in the ICH guidance entitled “Text on Validation of Analytical Procedures (ICH Q2A)” (84). This validation process involved assessing key performance characteristics of the method, including linearity, sensitivity, accuracy, precision, and robustness. The calibration curve of the GC-FID method for fatty acids was established by analyzing a series of standard solutions with varying concentrations of FAMES. The linearity of the calibration curve was assessed by plotting the peak area of each FAME against its corresponding concentration and determining the correlation coefficient. The sensitivity of the method was determined by calculating the limit of detection (LOD) and limit of quantification (LOQ) for each FAME. The accuracy of the method was assessed by analyzing spiked samples with known concentrations of FAMES and calculating the recovery rate. The precision of the method was evaluated by performing replicate injections of the same sample and calculating the relative standard deviation (RSD) of the peak areas. The robustness of the method was assessed by making small, deliberate changes to key operating parameters (e.g., column temperature, carrier gas flow rate) and evaluating the impact on the FAME separation and quantification.

To further validate the GC-FID method in the context of sausage analysis, a series of calibration standards was prepared by mixing beef ground meat with known proportions of chicken mechanically deboned meat (MDM), ranging from 2% to 90%. Eleven

samples of traditional sausages from small family businesses, previously confirmed to be negative for chicken matter using the LCD Array, were used as the beef matrix for these validation experiments. These experiments allowed for the quantitative assessment of adulteration levels and their impact on the overall fatty acid profile of the sausage samples. The fatty acid composition of each standard was then determined using the GC-FID method, and the data were used to generate calibration curves for each fatty acid of interest. These calibration curves were then used to quantify the fatty acid content of the unknown sausage samples (Table 6).

Table 6. Fatty acids profile (C *) in different proportions of beef and poultry for validation of the GC-FID method.

| Chicken Meat (%) | Beef Sausage (%) | C14:0 | C14:1 | C15:0 | C16:0 | C16:1 | C17:0 | C18:0 | C18:1 | C18:2 | C18:3 | C20:0 | SFA | MUFA | PUFA | Total |
|------------------|------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 100 | 0 | 0.08 | 0.33 | 0.06 | 18.28 | 3.44 | 0.13 | 6.57 | 29.18 | 37.59 | 4.28 | 0.05 | 25.17 | 32.95 | 41.87 | 99.99 |
| 0 | 100 | 3.58 | 0.44 | 0.55 | 29.05 | 2.98 | 1.88 | 24.84 | 33.34 | 2.76 | 0.35 | 0.14 | 60.04 | 36.76 | 3.11 | 99.91 |
| 99 | 1 | 0.46 | 0.16 | 0.08 | 19.4 | 3.37 | 0.17 | 8.31 | 29.37 | 35.33 | 3.29 | 0.06 | 28.48 | 32.9 | 38.62 | 100 |
| 90 | 10 | 0.66 | 0.17 | 0.11 | 20.2 | 3.32 | 0.29 | 9.84 | 16.5 | 32.18 | 3.14 | 0.07 | 31.17 | 19.99 | 35.32 | 86.48 |
| 80 | 20 | 1.23 | 0.27 | 0.19 | 22.23 | 3.02 | 0.58 | 14.18 | 31.26 | 24.64 | 2.29 | 0.08 | 38.49 | 34.55 | 26.93 | 99.97 |
| 70 | 30 | 1.57 | 0.32 | 0.26 | 23.91 | 2.96 | 0.85 | 15.46 | 31.85 | 20.72 | 2.04 | 0.04 | 42.09 | 35.13 | 22.76 | 99.98 |
| 60 | 40 | 2.05 | 0.43 | 0.31 | 25.3 | 3.03 | 0.95 | 17.5 | 32.12 | 16.66 | 1.59 | 0 | 46.11 | 35.58 | 18.25 | 99.94 |
| 50 | 50 | 2.12 | 0.42 | 0.32 | 25.73 | 2.93 | 1.09 | 19.39 | 32.46 | 14.03 | 1.33 | 0.1 | 48.75 | 35.81 | 15.36 | 99.92 |
| 40 | 60 | 2.28 | 0.43 | 0.34 | 26.43 | 3.06 | 1.11 | 18.37 | 32.76 | 13.84 | 1.37 | 0 | 48.53 | 36.25 | 15.21 | 99.99 |
| 30 | 70 | 2.84 | 0.55 | 0.44 | 28.35 | 2.85 | 1.41 | 21.65 | 33.24 | 7.77 | 0 | 0.07 | 54.76 | 36.64 | 7.77 | 99.17 |
| 20 | 80 | 2.77 | 0.37 | 0.44 | 28.85 | 2.79 | 1.6 | 22.78 | 34.25 | 5.5 | 0.56 | 0.07 | 56.51 | 37.41 | 6.06 | 99.98 |
| 10 | 90 | 3.22 | 0.61 | 0.49 | 28.64 | 2.81 | 1.58 | 24.24 | 34.27 | 3.53 | 0.37 | 0.12 | 58.29 | 37.69 | 3.9 | 99.88 |
| 7 | 93 | 3.04 | 0.27 | 0.46 | 29.55 | 2.68 | 1.59 | 23.85 | 34.9 | 3.11 | 0.32 | 0.11 | 58.6 | 37.85 | 3.43 | 99.88 |
| 2 | 98 | 3.57 | 0.66 | 0.56 | 31.06 | 2.9 | 1.87 | 23.7 | 33.15 | 2.05 | 0.27 | 0.11 | 60.87 | 36.71 | 2.32 | 99.9 |

* C14:0 Myristolein, C14:1 Myristin, C15:0 Pentadecane, C16:0 Palmitin, C16:1 Palmitolin, C17:0

Heptadecan, C18:0 stearic, C18:1 oleic, C18:2 Linol, C18:3 Linolen alfa, C20:0 Arachin, SFA- Saturated fatty acids, MUFA- Monounsaturated fatty acids, PUFA-polyunsaturated fatty acids.

4.4. Quality Control and Data Validation

Throughout the entire analytical process, from sample collection and preparation to instrumental analysis and data processing, stringent quality control (QC) measures were implemented to ensure the accuracy, reliability, and traceability of the data. These QC measures included the use of certified reference materials (CRMs) for calibration, the analysis of blank samples to monitor for contamination, the inclusion of internal standards to correct for instrument drift and matrix effects, and the performance of replicate analyses to assess precision and reproducibility. All data were carefully reviewed and validated by experienced analysts, and any outliers or anomalies were thoroughly investigated and resolved.

4.5. Statistical Analyses

Following data collection and validation, appropriate statistical analyses were performed to identify significant differences and correlations between the presence of soy, meat species, and fatty acid profiles in the analyzed sausage samples. Data were analyzed using established statistical software packages, such as SPSS or R. A one-way analysis of variance (ANOVA) test was used to assess differences in mean values among different groups of samples (e.g., industrial vs. traditional sausages, sausages with high soy content vs. sausages with low soy content). Post-hoc tests, such as Tukey's HSD or Bonferroni correction, were applied to determine which specific groups differed significantly from one another.

To investigate potential relationships between soy DNA content (as determined by real-time PCR) and fatty acid profiles (as determined by GC-FID), correlation analyses were performed using the correlation analysis tool within Analysis ToolPak in Microsoft Excel 2016. Pearson correlation coefficients (r) were calculated to quantify the strength and direction of the linear association between each fatty acid and the soy DNA content. The statistical significance of the correlation coefficients was assessed using p -values, with p -values less than 0.05 considered to be statistically significant. The results of these statistical analyses provided valuable insights into the impact of adulteration on the nutritional composition of the beef sausages and helped to identify potential biomarkers for detecting adulteration.

5. RESULTS AND DISCUSSION

This section presents an analysis of the findings related to the detection of DNA from various species and soy in beef sausages, and the correlation between soy content and fatty acid profiles. The study encompasses both industrial and traditional sausage production methods prevalent in Kosovo.

5.1. Detection of DNA of Species in Beef Sausages

After analyzing the samples for the detection of DNA and other species in local industrial and traditional sausages, it was found that 32 (47.05%) samples contained only beef DNA, 31 (45.55%) contained chicken DNA, 3 (4.41%) samples contained mutton, and 2 (2.9%) samples contained turkey DNA. None of the 68 samples contained the DNA of other mammalian species like horse, goat, camel, buffalo, pig, kangaroo, hare, rabbit, reindeer, roe deer, red deer, fallow deer, springbok, dog, or cat, or bird species like goose, ostrich, mallard duck, Muscovy duck, or pheasant (Table 7). These results highlight the prevalence of meat adulteration in beef sausages within the studied region, potentially driven by economic factors or mislabeling practices.

Table 7. DNA detection and declaration status in declared beef sausage samples (n = 68).

| Category | Industrial | | Traditional | | Total | |
|--|------------|----------|-------------|----------|-------|--------|
| | n | % | n | % | n | % |
| Sample distribution | 43 | 63.23% | 25 | 36.76% | 68 | 100% |
| Samples with only beef DNA | 15 | 34.88% | 17 | 68.00% | 32 | 47.05% |
| Samples with DNA of other species | 28 | 65.12% | 8 | 32.00% | 36 | 52.94% |
| Containing chicken DNA | 23 | 82.14% * | 7 | 87.50% * | 31 | 45.55% |
| Containing mutton DNA | 3 | 10.71% * | 1 | 12.50% * | 3 | 4.41% |
| Containing turkey DNA | 2 | 7.14% * | 0 | 0.00% | 2 | 2.94% |
| Containing DNA of other species ** | 0 | 0.00% | 0 | 0.00% | 0 | 0.00% |
| Declared the added species meat | 15 | 41.60% | 0 | 100.00% | 15 | 41.46% |
| Did not declare the added species meat | 13 | 46.42% | 8 | 100.00% | 21 | 58.33% |

* Percentages in rows for chicken, mutton, and turkey DNA are calculated within the subgroup of samples with other species DNA; ** No DNA from other mammalian (e.g., pig, horse, goat) or bird species (e.g., duck, goose, pheasant) was detected in any sample.

Regarding industrial sausages (n = 43), it turns out that 15 of them (34.8%) contained only beef DNA, while 28 (65.2%) contained DNA of other species. Of the 28 samples that contained other species' DNA, chicken DNA was detected in 23 (82.14%). In comparison, mutton DNA was detected in three samples (10.71%) (two samples contained beef and one sample also contained chicken DNA), and two samples (7.14%) tested positive for turkey DNA (both samples in beef sausages, which also contained chicken DNA). Regarding the homemade beef sausages (n = 25), it turns out that 17 (68%) contained beef DNA, while 8 (32%) contained DNA from other species. Of the eight samples, seven of them (87.5%) contained chicken DNA, while one sample (13.5%) contained mutton DNA. This discrepancy between declared and actual meat content emphasizes the need for stricter quality control and labeling regulations.

Of all the beef sausage samples that tested positive for the presence of DNA from other species (n = 36), 28 of them belonged to industrial sausages, and of these, 15, or 41.66%, were declared, compared to 13 samples, or 46.42%, which were not declared. On the other hand, although 8 samples of traditional beef sausage tested positive for the presence of DNA from other species, none of those samples contained any detectable amount of those species.

The results of sausage analysis, using the Chipron LCD Array Analysis System, show that 36 out of 68 samples, or 52.94%, tested positive for the DNA of other species, indicating the potential intentional addition of meat from other species. The presence of poultry DNA (45.55%) is a reference to deliberate addition since, from an economic point of view, it is more profitable to add meat of cheaper species. When it comes to the presence of mutton DNA (4.41%), which has a higher price than beef itself, this does not surprise us since, in some parts of Kosovo, mutton meat is added to beef sausage to improve its sensory properties. The high presence of poultry DNA in beef sausage, for economic reasons, will be the ongoing focus of our work.

Compared to works by other authors, these values are somewhat lower than a study focusing on the substitution of meat species in Poland, which found that 60% of the foods analyzed contained an undeclared ingredient or the substitution of an expensive ingredient with a cheaper option [85], and that 17 (60%) of 28 beef sausages contained added poultry. Their results showed that 112 (78.3%) samples were mislabeled, attributed to the false declaration of species and/or presence of undeclared meat species [86]. On the other hand, our results are higher than those of other authors, such as beef

sausages containing 33% chicken meat [87], where the results indicate that 15 (14.7%) of the total samples were found to contain undeclared species, with poultry meat detected in 7 (21.8%) and 2 (6.06%) of 32 salami and 33 sausage samples, respectively [88]. In another study, undeclared animal species were detected in 27% of the meat products tested [89]. These comparisons provide context for the current study's findings, demonstrating the variability of meat adulteration across different regions and studies.

The sampling strategy was designed to provide a preliminary overview of meat species usage and potential adulteration in sausages available in the Kosovar market. Although the sample size is relatively modest, it reflects the local availability and accessibility of sausage products. The higher proportion of industrial sausages in the sample (43 vs. 25) corresponds to their wider market presence and availability across the region rather than an intentional sampling bias. We acknowledge that this imbalance, along with the limited geographic scope confined to Kosovo, may reduce the statistical power for generalizing findings to broader regional or global contexts. Additionally, the lower number of traditional sausages may under-represent the variability inherent in artisanal production practices. Therefore, the study should be interpreted as an exploratory assessment of local practices rather than a comprehensive evaluation of international meat adulteration trends. Future studies with larger and more geographically diverse samples are recommended to validate and expand upon these findings.

It is worth noting that the addition of meat from other species to industrial sausages was perhaps expected, but such findings in 32% of homemade sausages are somewhat of a surprise, considering that traditional recipes for homemade sausage-making in Kosovo consist of 100% beef only. Consumer acceptance is crucial, as the inclusion of other meat species in beef sausages, despite economic benefits, may face resistance without sensory studies on the impact of added poultry. In addition, traditional food authenticity is highly valued in Kosovo, making undisclosed additions potentially controversial.

The LCD Array has proven to be a reliable and sensitive method for detecting species' DNA, with this study confirming its high accuracy in food testing. Other authors also found that PCR was associated with a commercial DNA macro-array on pure meat samples, spiked samples, proficiency test samples, and processed samples,

and showed high specificity on the targeted species, with sensitivity down to 1% (w/w) [90]. In addition, the Meat 5.0 LCD-Array kit is highly specific and allows easy identification of animal species, is sufficiently sensitive, and provides repeatable results. These methods of analysis are recommended for comprehensively monitoring the presence of animal species in food samples, regardless of the degree of heat treatment or mechanical processing, as an effective tool for detecting food adulteration [91]. However, the high sensitivity of the LCD Array may result in the detection of trace DNA from cross-contamination sources, such as animal-derived casings, processing equipment, or environmental exposure. This introduces a limitation, as the assay cannot definitively differentiate between low-level incidental contamination and deliberate adulteration. Therefore, when species are detected at or near the threshold of detection, caution is warranted in interpreting the results, especially in the absence of quantitative DNA measurement.

5.2. Prevalence of the Fatty Acid in Sausages Made with 100% Beef and Added Poultry Meat

From the literature and numerous papers, it is emphasized that the fatty acid profile is different in beef and poultry meat. Raising the hypothesis that the high % presence of poultry DNA cannot be just accidental cross-contamination, we took the next step to analyze the fatty acid profile through GC-FID analysis of the differences in the fatty acid profile of sausages containing 100% beef and sausages with the presence of poultry DNA and revealed several key findings (see Figure 10).

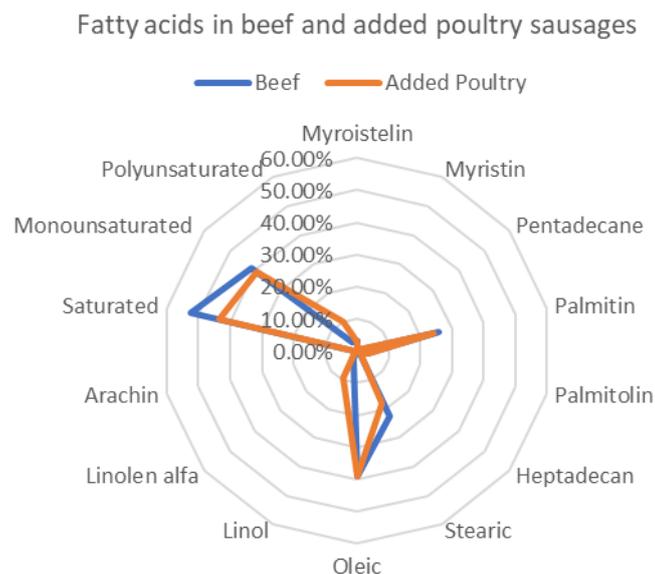


Figure 10. Fatty acids in beef and added poultry sausages

Figure 10 provides a comparison of the levels of various fatty acids between 100% beef sausages and sausages with added poultry meat. The results show that beef sausages have higher levels of saturated fatty acids compared to sausages with added poultry. This is typical as beef fat generally contains more saturated fats. Sausages with added poultry have higher levels of polyunsaturated fatty acids. This aligns with the leaner profile of poultry, which typically contains more unsaturated fats. Monounsaturated fats seem slightly higher in beef sausages compared to the poultry-added ones. Specific fatty acids like Myristin, Palmitin, and stearic acids (components of saturated fats) are more prevalent in beef sausages. Linolenic acid and other polyunsaturated fats (like oleic) are elevated in sausages with added poultry meat, a fact that is also supported by the works of other authors who emphasize that sausages with chicken meat had lower stearic (C18:0) and higher linoleic (C18:3) fatty acid contents than those made with beef [92]. Similar results emphasize that turkey and chicken sausages presented a higher content of polyunsaturated fatty acids than the Chester and common sausages, which presented a low saturated fatty acids content [93]. It is important to note that the fatty acid profile of sausages can be influenced not only by the animal fat content but also by the possible addition of unmeasured ingredients such as vegetable oil. In relation to raw beef sausage, frying with oil substantially increased the amount of MUFA, PUFA, n-6, and PUFA/SFA [94], and the substitution of animal fat with canola oil in sausage resulted in nutritional improvements due to an increase in mono- and polyunsaturated fatty acids in the lipid fraction that specifically decreased lipid oxidation [95]. In addition to vegetable oils, in our previous study, we demonstrated that the fatty acid composition of sausages is also influenced by the amount of added soy [96]. These components were not quantitatively assessed in this study and may act as confounding variables when interpreting differences in fatty acid composition between industrial and traditional sausages. Future research incorporating direct analysis of added fats would improve the accuracy of fatty acid profiling.

The above results verify the general differences in fatty acids in 100% beef and added poultry sausages. The results are also consistent with the research of other authors. However, we analyzed and verified through the ANOVA statistical program whether the differences expressed above between fatty acids are statistically significant (Table 8).

Table 8. Statistical differences in fatty acids (%) in beef and added poultry sausages.

| Fatty Acids | Beef Sausage | Beef Sausage with Poultry DNA | p-Value |
|--------------------|---------------------|--------------------------------------|---------------------------|
| Myroistelin | 3.17 ± 12.28 | 2.56 ± 35.82 | 0.000893 * |
| Myristin | 0.39 ± 58.87 | 0.30 ± 87.46 | 0.156974 |
| Pentadecane | 0.57 ± 24.95 | 0.43 ± 42.26 | 0.000555 * |
| Palmitin | 25.66 ± 5.45 | 24.14 ± 7.33 | 0.000238 |
| Palmitolin | 3.17 ± 26.05 | 3.55 ± 23.26 | 0.066677 |
| Heptadecan | 1.34 ± 17.90 | 1.09 ± 38.45 | 0.003113 * |
| Stearic | 22.76 ± 16.73 | 18.11 ± 32.46 | 0.000305 * |
| Oleic | 39.18 ± 9.79 | 39.00 ± 7.24 | 0.828574 |
| Linol | 2.72 ± 23.96 | 9.46 ± 80.19 | 3.28 × 10 ⁻⁶ * |
| Linolen alfa | 0.41 ± 43.26 | 0.81 ± 56.59 | 1.15 × 10 ⁻⁵ * |
| Arachin | 0.58 ± 35.28 | 0.51 ± 59.36 | 0.250436 |
| Saturated | 54.12 ± 7.53 | 46.59 ± 18.58 | 0.002973 * |
| Monosaturated | 42.75 ± 10.38 | 41.70 ± 17.43 | 0.425882 |
| Polyunsaturated | 3.13 ± 25.26 | 10.27 ± 77.80 | 7.64 × 10 ⁻⁶ * |

* Statistically significant differences.

Fatty acids that showed statistically significant differences in levels between the two groups and were higher in sausages in 100% beef meat include C14:0 Myristolein, C15:0 Pentadecane, C16:0 Palmitin, C17:0 Heptadecan, C18:0 Stearic, and saturated acids. On the other hand, fatty acids that showed statistically significant differences in levels between the two groups and were higher in sausages with added poultry meat include C18:2 Linoleic, C18:3 alpha-linolenic, and Polyunsaturated Acid. These differences highlight the compositional shifts caused by adding poultry meat, particularly in increasing the levels of polyunsaturated fatty acids while decreasing saturated fatty acids. Fatty acids that did not show statistically significant differences include C14:1 Myristin, C16:1 Palmitolin, C18:1 oleic, C20:0 Arachin, and monounsaturated acids. This indicates that the inclusion of poultry meat has a minimal impact on these specific fatty acids, suggesting a level of consistency in these profiles regardless of the meat source.

As a summary of the above results, we can emphasize that the inclusion of poultry meat in beef sausages results in significant compositional shifts, particularly reducing saturated fatty acids and increasing polyunsaturated fatty acids, which may improve the nutritional profile of the sausages. However, some fatty acid levels (e.g., C14:1 Myristin, C18:1 oleic, and monounsaturated acids) remain stable across formulations, suggesting that these components are less sensitive to the addition of poultry meat.

5.3. The Fatty Acid Profile Depends on the % of Chicken Meat Addition

By observing the fatty acid profile and statistically significant variations in some specific fatty acids, we performed an analysis for the detection of the amount of poultry meat in 33 samples of beef sausage. This resulted in detecting the presence of poultry DNA (31 chicken and 2 turkey) through the GC-FID validation method, allowing us to observe the impact of the increased amount of poultry meat on the values and profile of fatty acids. Based on the GC-FID validation method, the results from the 33 samples analyzed show that 8 samples (24.24%) had poultry meat added to the amount of 10%, 10 samples (30.30%) to the amount of about 20%, 5 samples (15.15%) to the amount of 30%, 1 sample (3.03%) to the amount of 50%, 2 samples (6.06%) to the amount of 60%, 2 samples (6.06%) to the amount of 60%, 2 samples (6.06%) to the amount of 70%, 2 samples (6.06%) to the amount of 60%, 2 samples (6.06%) to the amount of 80%, and 3 samples (9.09%) to the amount of 90%. Using the GC-FID validation method, we found that about 70% of the analyzed samples had up to 30% poultry meat added to the beef sausage, while the other 30% of the samples had additions of 50-90%.

Comparing the % of poultry addition in industrial and traditional beef sausage results, out of a total of 33 samples of sausages with added poultry meat, 25 samples (75.7%) belong to industrial sausages, and 8 samples (24.4%) belong to traditional sausages. Of the samples of sausages with the presence of poultry meat of 10%, it turns out that five of them are industrial and five of them are traditional. Of the sausages with the addition of 10-20% chicken meat, six belonged to industrial sausages while three belonged to traditional sausages. The sausages with more than 20% of added poultry meat belong only to industrial sausages, including five samples with 30%, one sample with 50%, two samples with 60%, 70%, and 80%, and three samples with 90%. From the above results, it is observed that industrial sausages, in addition to having a higher

frequency of added poultry meat (75.7%), also have amounts of added meat reaching up to 90%. While traditional sausages have a lower frequency of added poultry (24.4%), at the same time they include smaller amounts of poultry meat, not exceeding 20%.

Although it is evident that, based on the average fatty acids, in sausages with added chicken meat, the values of saturated fatty acids decrease and the values of unsaturated and semi-saturated fatty acids increase. However, we have analyzed the profile of these fluctuations according to the added amount in % of chicken meat in beef sausage (Table 9).

Table 9. Profile of fatty acids in different % of added poultry meat in beef sausage.

| Added Meat Poultry % | Samples (No) | C14:0 | C14:1 | C15:0 | C16:0 | C16:1 | C17:0 | C18:0 | C18:1 | C18:2 | C18:3 | C20:0 | SFA | MUF A | PUFA |
|----------------------|--------------|--------|--------|--------|---------|--------|--------|---------|---------|--------|--------|--------|---------|---------|--------|
| 10% | 1 | 2.95 | 0.21 | 0.43 | 23.77 | 1.94 | 1.24 | 28.63 | 36.73 | 3.29 | 0.34 | 0.47 | 57.49 | 38.88 | 3.63 |
| | 2 | 2.91 | 0.81 | 0.47 | 24.97 | 3.32 | 1.08 | 24 | 38.3 | 3.1 | 0.39 | 0.67 | 54.10 | 42.43 | 3.49 |
| | 3 | 3.35 | 0.41 | 0.71 | 26.56 | 2.37 | 1.52 | 28.25 | 31.75 | 3.94 | 0.61 | 0.53 | 60.92 | 34.53 | 4.55 |
| | 4 | 3.08 | 0.24 | 0.59 | 26.14 | 3.3 | 1.26 | 21.65 | 39.29 | 3.47 | 0.48 | 0.51 | 53.23 | 42.83 | 3.95 |
| | 5 | 3.96 | 0.23 | 0.7 | 27.68 | 3.46 | 1.41 | 21.21 | 37.42 | 3.13 | 0.35 | 0.43 | 55.39 | 41.11 | 3.48 |
| | 6 | 3.17 | 0.32 | 0.58 | 24.4 | 2.69 | 1.38 | 22.62 | 40 | 3.6 | 0.57 | 0.67 | 52.82 | 43.01 | 4.17 |
| | 7 | 3.02 | 0.28 | 0.49 | 24.19 | 2.95 | 1.24 | 22.65 | 40.01 | 3.73 | 0.71 | 0.73 | 52.32 | 43.24 | 4.44 |
| | 8 | 2.72 | 0.24 | 0.5 | 24.27 | 3.67 | 1.26 | 17.58 | 44.99 | 3.74 | 0.39 | 0.63 | 46.96 | 48.9 | 4.13 |
| | MEAN ± | 3.15 ± | 0.34 ± | 0.56 ± | 25.25 ± | 2.96 ± | 1.30 ± | 23.32 ± | 38.56 ± | 3.50 ± | 0.48 ± | 0.57 ± | 54.16 ± | 41.79 ± | 4.05 ± |
| | SD * | 11.25 | 54.50 | 17.55 | 5.14 | 18.72 | 9.65 | 14.70 | 9.04 | 8.18 | 26.62 | 18.12 | 7.52 | ±9.78 | 9.02 |
| 20% | 1 | 3.24 | 1.19 | 0.56 | 24.32 | 5.05 | 1.24 | 15.33 | 43.73 | 3.61 | 0.5 | 1.23 | 45.92 | 49.97 | 4.11 |
| | 2 | 3.06 | 0.64 | 0.51 | 26.26 | 3.94 | 1.15 | 19.73 | 40.03 | 3.68 | 0.47 | 0.52 | 51.23 | 44.61 | 4.15 |
| | 3 | 2.72 | 0.4 | 0.43 | 27.06 | 2.7 | 1.39 | 24.25 | 37.76 | 2.6 | 0.37 | 0.33 | 56.18 | 40.86 | 2.97 |
| | 4 | 2.92 | 0.47 | 0.56 | 25.4 | 2.56 | 1.65 | 24.34 | 37.97 | 3.22 | 0.45 | 0.47 | 55.34 | 41 | 3.67 |
| | 5 | 3.04 | 0.35 | 0.53 | 25.5 | 3.73 | 1.28 | 19.81 | 38.59 | 5.78 | 0.68 | 0.71 | 50.87 | 42.67 | 6.46 |
| | 6 | 2.88 | 0.22 | 0.6 | 23.99 | 2.97 | 1.31 | 23.72 | 38.12 | 4.79 | 0.55 | 0.86 | 53.36 | 41.31 | 5.34 |
| | 7 | 3.39 | 0.2 | 0.57 | 26.01 | 2.43 | 1.36 | 24.52 | 35.7 | 4.88 | 0.43 | 0.51 | 56.36 | 38.33 | 5.31 |
| | 8 | 4.09 | 0 | 0.48 | 24.07 | 4.22 | 1.29 | 15.29 | 43.4 | 5.9 | 0.53 | 0.7 | 45.92 | 47.62 | 6.43 |
| | 9 | 3.19 | 0.26 | 0.5 | 23.24 | 3.13 | 1.22 | 21.14 | 41.37 | 4.12 | 0.41 | 1.43 | 50.72 | 44.76 | 4.53 |

| | | | | | | | | | | | | | | | |
|-----|--------|--------|--------|--------|---------|--------|--------|---------|---------|---------|--------|--------|---------|---------|---------|
| | 10 | 3.02 | 0.12 | 0.5 | 25.52 | 3.87 | 1.56 | 17.13 | 43.62 | 3.82 | 0.4 | 0.45 | 48.18 | 47.61 | 4.22 |
| | MEAN ± | 3.15 ± | 0.39 ± | 0.52 ± | 25.14 ± | 3.46 ± | 1.34 ± | 20.53 ± | 40.03 ± | 4.24 ± | 0.48 ± | 0.72 ± | 51.41 ± | 43.87 ± | 4.72 ± |
| | SD | 11.42 | 82.84 | 9.08 | 4.50 | 23.08 | 10.89 | 17.02 | 6.78 | 24.13 | 18.04 | 47.15 | 7.23 | ± 7.98 | 23.05 |
| | 1 | 3.34 | 1.03 | 0.53 | 25.53 | 4.69 | 1.02 | 13.27 | 41.18 | 7.66 | 0.95 | 0.77 | 44.46 | 46.9 | 8.61 |
| | 2 | 2.62 | 0.28 | 0.5 | 23.9 | 3.92 | 1.16 | 16.35 | 40.84 | 8.61 | 1.1 | 0.71 | 45.24 | 45.04 | 9.71 |
| 30% | 3 | 2.36 | 0.2 | 0.46 | 24.43 | 2.72 | 1.24 | 22.7 | 34.81 | 9.67 | 0.98 | 0.43 | 51.62 | 37.33 | 10.65 |
| | 4 | 2.77 | 0.37 | 0.59 | 25.19 | 2.66 | 1.61 | 23.03 | 35.65 | 7.07 | 0.52 | 0.55 | 53.74 | 38.68 | 7.59 |
| | 5 | 2.57 | 0.22 | 0.48 | 22.38 | 3.71 | 1.34 | 14.77 | 44.24 | 8.68 | 0.89 | 0.72 | 42.26 | 48.17 | 9.57 |
| | MEAN ± | 2.73 ± | 0.42 ± | 0.51 ± | 24.29 ± | 3.54 ± | 1.27 ± | 18.02 ± | 39.34 ± | 8.34 ± | 0.89 ± | 0.64 ± | 47.46 ± | 43.22 ± | 9.23 ± |
| | SD | 12.12 | 73.97 | 8.86 | 4.57 | 21.67 | 15.54 | 22.59 | 9.08 | 10.77 | 22.11 | 19.93 | 9.31 | 10.17 | 11.30 |
| 50% | 1 | 2.42 | 0.16 | 0.21 | 22.57 | 3.43 | 0.71 | 15.16 | 37.87 | 15.86 | 1.35 | 0.26 | 41.33 | 41.46 | 17.21 |
| | 1 | 1.52 | 0.47 | 0.26 | 21.03 | 3.78 | 0.63 | 11.4 | 40.83 | 17.99 | 1.58 | 0.51 | 35.35 | 45.08 | 19.57 |
| 60% | 2 | 1.51 | 0.11 | 0.23 | 23.09 | 4.13 | 0.57 | 14.84 | 38.85 | 15.28 | 1.2 | 0.18 | 40.42 | 43.09 | 16.48 |
| | MEAN ± | 1.52 ± | 0.29 ± | 0.25 ± | 22.06 ± | 3.96 ± | 0.60 ± | 13.12 ± | 39.84 ± | 16.64 ± | 1.39 ± | 0.35 ± | 37.89 ± | 44.09 ± | 18.03 ± |
| | SD | 0.33 | 62.07 | 6.12 | 4.67 | 4.42 | 5.00 | 13.11 | 2.48 | 8.15 | 13.67 | 47.83 | 6.69 | ± 2.26 | 8.57 |
| | 1 | 1.37 | 0 | 0 | 23.32 | 4.13 | 1.41 | 10.16 | 38.52 | 19.74 | 1.35 | 0 | 36.26 | 42.65 | 21.09 |
| 70% | 2 | 1.68 | 0.14 | 0.31 | 23.2 | 3.26 | 0.81 | 13.43 | 36.62 | 19.07 | 1.18 | 0.3 | 39.73 | 40.02 | 20.25 |
| | AV | 1.53 ± | 0.07 ± | 0.16 ± | 23.26 ± | 3.70 ± | 1.11 ± | 11.80 ± | 37.57 ± | 19.41 ± | 1.27 ± | 0.15 ± | 38.00 ± | 41.34 ± | 20.67 ± |
| | | 10.16 | 100.00 | 100.00 | 0.26 | 11.77 | 27.03 | 13.86 | 2.53 | 1.73 | 6.72 | 100.00 | 4.57 | ± 3.18 | 2.03 |
| | 1 | 1.16 | 0.15 | 0.18 | 20.93 | 4.24 | 0.42 | 13.35 | 36.64 | 21.81 | 0.99 | 0.14 | 36.18 | 41.03 | 22.8 |
| 80% | 2 | 1.93 | 0.04 | 0.17 | 22.39 | 4.77 | 0.4 | 11.16 | 36.95 | 20.51 | 1.57 | 0.11 | 36.16 | 41.76 | 22.08 |
| | MEAN ± | 1.55 ± | 0.10 ± | 0.18 ± | 21.66 ± | 4.51 ± | 0.41 ± | 12.26 ± | 36.80 ± | 21.16 ± | 1.28 ± | 0.13 ± | 36.17 ± | 41.40 ± | 22.44 ± |
| | SD | 24.92 | 57.89 | 2.86 | 3.37 | 5.88 | 2.44 | 8.94 | 0.42 | 3.07 | 22.66 | 12.00 | 0.03 | ± 0.88 | 1.60 |
| | 1 | 0.67 | 0.15 | 0.21 | 22.04 | 4.53 | 0.26 | 7.77 | 37.12 | 24.49 | 1.6 | 0.16 | 31.11 | 41.8 | 26.09 |
| 90% | 2 | 0.51 | 0.01 | 0.09 | 20.46 | 4.61 | 0.16 | 7.25 | 39.01 | 26.45 | 1.02 | 0.1 | 28.57 | 4.63 | 27.47 |
| | 3 | 0.54 | 0.16 | 0.08 | 21.54 | 5.22 | 0.13 | 6.57 | 39.26 | 24.19 | 2.19 | 0.12 | 28.98 | 44.64 | 26.38 |
| | MEAN ± | 0.57 ± | 0.11 ± | 0.13 ± | 21.35 ± | 4.79 ± | 0.18 ± | 7.20 ± | 38.46 ± | 25.04 ± | 1.60 ± | 0.13 ± | 29.55 ± | 30.36 ± | 26.65 ± |
| | SD | 12.11 | 64.20 | 46.63 | 3.09 | 6.44 | 30.32 | 6.83 | 2.48 | 4.00 | 29.79 | 19.69 | 3.77 | 60.05 | 2.23 |

SD *—Standard deviation.

From the results, we can notice a clear decrease in the total percentage of saturated fatty acids as the percentage of added poultry meat increases. For instance, it drops from 54.15% at 10% poultry meat to 29.55% at 90% poultry meat. Specifically, components like C16:0 Palmitin and C18:0 stearic acid show significant reductions, reflecting that poultry meat has fewer saturated fats than other sausage mixture components. Monounsaturated acids remain relatively stable between 10% and 70% of added poultry meat, ranging between 41 and 44%. However, there is a notable drop to 30.36% when the poultry meat content reaches 90%. C18:1 oleic acid is the most prominent MUFA and shows a slight fluctuation but no dramatic trend until the 90% level. Meanwhile, polyunsaturated fatty acids increase substantially as the percentage of added poultry meat rises. At 10% poultry meat, PUFAs are at 3.98%, while at 90%, they increase to 26.64%.

The most significant contributor is C18:2 linoleic acid, which grows consistently from 3.50% at 10% to 25.04% at 90%. This indicates poultry meat's high content of essential fatty acids. Focusing on individual fatty acid trends, it is noted that C16:1 Palmitolin and C18:2 linoleic acid increase with higher poultry meat content, suggesting poultry meat is richer in these unsaturated fatty acids. C18:0 stearic acid and C16:0 Palmitin, as major SFAs, decline, emphasizing the lower saturated fat profile of poultry meat compared to other fats used in the sausages.

A horizontal 100% stacked bar chart illustrating the averages of various fatty acids depending on the percentages of added meat and poultry in beef sausage (Figure 11).

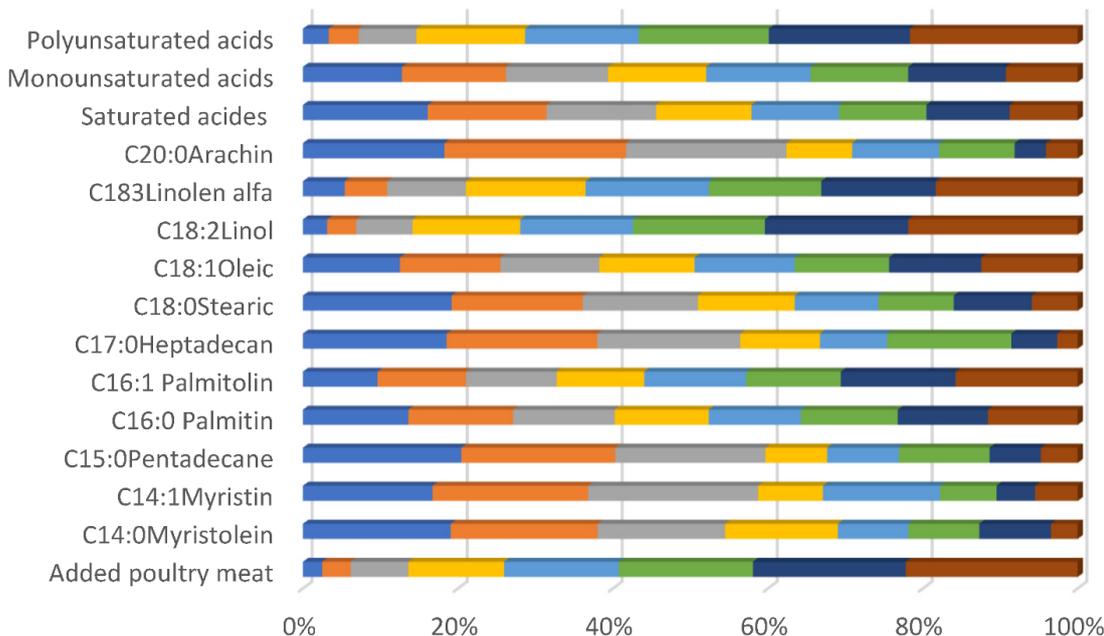


Figure 11. Averages of fatty acids depended on different % of added poultry meat.

Figure 11 shows that samples with higher percentages of added poultry meat show noticeable variation in the composition of fatty acids. Saturated fatty acids (SFAs) such as Palmitic (C16:0) and stearic (C18:0) acids tend to remain consistently high across all samples but show slight increases in formulations with a lower percentage of added meat. Monounsaturated fatty acids (MUFAs), including oleic acid (C18:1), appear more prevalent in samples with moderate added meat content, indicating a potential optimization point for healthier fat profiles. Polyunsaturated fatty acids (PUFAs) such as Linolenic (C18:3) and linoleic (C18:2) acids increase in samples with higher added meat percentages, which may be attributed to a higher proportion of poultry meat, known for its richer PUFA content. Added Meat/Poultry % increases in the lower section of the bars, correlating inversely with certain saturated fatty acid components. Overall, the graph suggests that increasing the percentage of added poultry or meat significantly influences the fatty acid composition of the final sausage product. Higher poultry content appears to be associated with a more favorable fatty acid profile (i.e., higher PUFA and lower SFA levels), which could have positive nutritional implications.

Based on the results of this study, it is argued that there is a difference in the fatty acid composition of beef meat and fat compared to poultry meat and fat. Undoubtedly, this difference in fatty acid composition is also reflected in meat products, such as sausages. The fatty acid content of beef is also argued by other authors who report that

meat fat comprises mostly monounsaturated and saturated fatty acids, with oleic (C18:1), palmitic (C16:0), and stearic acid (C18:0) being the most ubiquitous [97], and that beef sausage has the dominant fatty acids of palmitic acid (42.31%), oleic acid (20.19%), stearic acid (10.92%), and myristic acid (7.66%) [98]. Regarding poultry meat, it is reported that fat in broiler breast contained 29% saturated FAs (SFAs), 36% monounsaturated FAs (MFAs), and 35% polyunsaturated FAs (PUFAs). Meanwhile, legs and thigh meat had 28% SFAs, 38% MFAs, and 33% PUFAs [99]. In addition, poultry products showed a high content of linoleic (19.54%) and low content of stearic (8.22%) acids [100].

This study highlights the technical, nutritional, cultural, and economic impacts of adding poultry to beef sausages in Kosovo. While cost-effective, unclear labeling and deviations from traditional recipes may hinder consumer acceptance, as unfamiliarity can lead to negative perceptions.

5.4. The Presence of Soy DNA in Industrial and Traditional Sausages

In total, 63 samples were tested for the presence of soy DNA by real-time PCR, and 54 out of 63 samples were positive for soy DNA. A total of 41 out of 42 industrial sausage samples were positive for soya DNA, with an average ct value of 22.60 and a standard deviation (SD) of 4.288, whereas 13 out of 21 traditional sausage samples were positive for soya DNA, with a mean ct value of 23.36 and a standard deviation (SD) of 4.56. For a suitable statistical calculation, negative samples were adjusted to the value of the last ct = 40 (Figure 12). There was a statistically significant difference in means between industrial sausage and traditional sausage, with $p < 0.001$ based on ct values (ANOVA test).

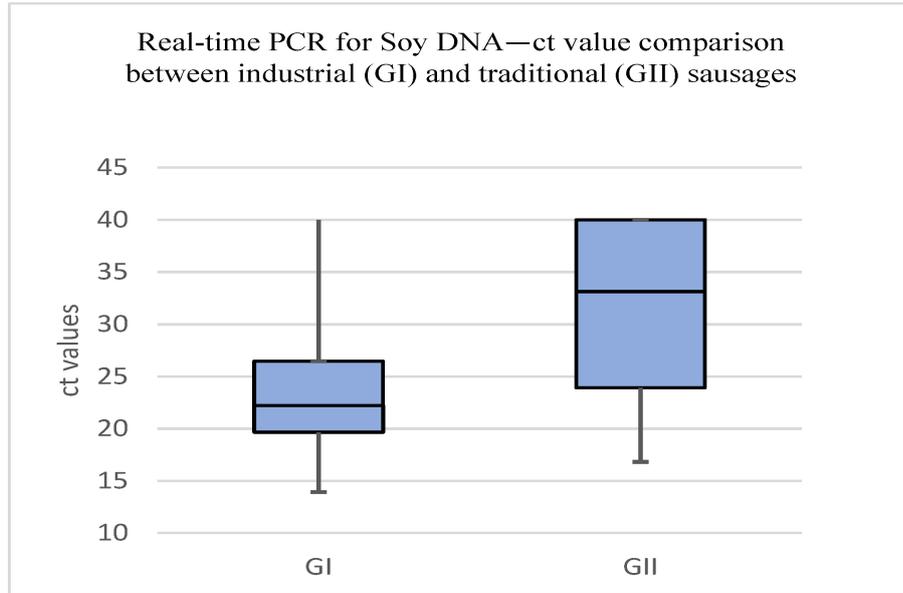


Figure 12. Real-time PCR for Soy DNA-ct value comparison between industrial (GI) and traditional (GII) sausages

The high proportion of positive samples (85.7%) indicates that the majority of sausage products in the sample set contain soy. This suggests that soy is commonly used as an ingredient or additive in sausage production in Kosovo. The widespread use of soy might be due to its cost-effectiveness, functional properties (such as water retention and texture improvement), or protein extender characteristics. The high incidence of soy presence might reflect economic strategies employed by producers to reduce costs while maintaining product quality. Similar results for the presence and values of soy in sausages can also be found in research by other authors. From one research paper, the results showed that of 100 samples of meat products, the presence of soybean was detected in 29%. This research has shown that the control of soybean protein and gluten presence in meat products is necessary because in 29.6% of cases, the presence of these allergens was identified in meat products but not indicated in their declarations, which poses a high risk for consumers [101]. During the investigation of the presence of soya proteins in 131 meat product samples such as salamis or sausages from the Czech Republic market, soya proteins were detected in 84% of the investigated samples without any declaration on the package of the product [102]. A survey of 38 Turkish processed meat products found only six samples to be negative for the presence of soybeans. In 32 (84%) positive samples, 13 (34%) contained levels of soy above 0.1% [103].

The research findings indicate that 41 out of 42 industrial sausage samples (97.6%) and 13 out of 21 traditional sausage samples (61.9%) tested positive for the presence of soy in Kosovo, providing significant insights into the practices and trends within the sausage production industry. The nearly ubiquitous presence of soy in industrial sausages suggests that soy is a standard ingredient in the production process of these products. Industrial manufacturers likely use soy for its functional properties, such as improving texture, water retention, and protein content, as well as for economic reasons. These factors play a critical role in consumer acceptance. While soy inclusion in Kosovo sausages may provide economic benefits, it remains to be seen whether local consumers will embrace these changes, especially in the absence of sensory studies that evaluate the impact of soy on product quality.

The finding that 61.9% of traditional sausage samples contain soy indicates a significant, though not universal, use of soy in traditional sausage production. This may reflect variations in traditional recipes or the adoption of cost-saving measures by some traditional sausage producers. The lower prevalence compared to industrial sausages suggests that some traditional producers may adhere more closely to historical recipes that do not include soy. However, many consumers in Kosovo place a high value on the authenticity and purity of traditional foods, which are often seen as emblematic of cultural heritage. Therefore, the inclusion of soy in these products could be viewed with skepticism, especially if it is not clearly labeled.

Regarding the labeling statement, no product in Group II (homemade sausages) declared the presence of soy proteins or plant proteins, while when it comes to Group I (industrial sausages), 11 of 42 of them declared the presence of plant proteins without quantitative information, while 18 of them declared the presence of soy without quantitative information. In total, 13 out of 42 sausages from Group I did not declare the presence of soy or vegetable protein. The declaration of soy, as well as vegetable protein, is reflected in the CT value of real-time PCR for soy, where an average ct was 21.54. On the other hand, the average CT of Group I, which did not declare the soy, was 26.72, and Group II was 31.36. Moreover, four sausages from Group I did not declare the presence of soy or vegetable proteins, and five sausages from Group II had a value of >1000 ppm for soy. The lack of a labeling declaration should be addressed as non-compliance with existing food regulations.

The use of real-time PCR in detecting soy DNA is a reliable and sensitive method, capable of identifying even trace amounts of soy. The high detection rate in this study underscores the method's effectiveness and reliability in food testing and allergen detection. However, the detection of soybean proteins in meat products presents difficulties related to the composition (meat species, meat quality, soybean protein source, presence of other non-meat proteins, etc.) and the processing of the meat products, and, although these analytical methods have tried to overcome all these difficulties, there is still no method enabling quantitative assessment of soybean proteins in all kinds of meat products.

5.5. The Prevalence and Levels of the Presence of Soy DNA in Industrial and Traditional Sausages

Allergen RM-800 standard (Hygiene, former Bioteccon, Camarillo, CA, USA) was used to quantify the allergens in the sausages. To date, there are no official permitted levels of soy in the food. It is considered an allergen. Overall, 19 out of 63 samples had less than 10 ppm of soy. Additionally, 9 samples had between 11 and 100 ppm, 11 samples between 101 and 1000 ppm, and 25 samples > 1001 ppm (higher levels above the quantification range). From the GI (industrial sausages), 35 samples (85.3%) exceeded a threshold of 10 ppm, whereas 5 samples had between 11 and 100 ppm, 11 samples had between 101 and 1000 ppm, and 20 samples had >1001 ppm. On the other hand, 12 out of 21 traditional sausages (GII) exceeded a threshold of 10 ppm; 4 samples had between 11 and 100 ppm; 3 samples had between 101 and 1000 ppm; and 5 samples had >1001 ppm (Table 10).

Table 10. Soy content comparison between GI and GII.

| Soy Content | GI (Industrial Sausages) | GII (Traditional Sausages) |
|--------------------|-------------------------------------|---------------------------------------|
| <10 ppm | 6 (14.63%) | 9 (42.90%) |
| 11 to 100 ppm | 5 (12.2%) | 4 (19.05%) |
| 101 to 1000 ppm | 11 (26.83%) | 3 (14.28%) |
| >1001 ppm | 20 (48.7%) | 5 (23.81%) |

The high prevalence of soy in industrial sausages (85.3%) highlights the common practice of using soy as an ingredient. The distribution of soy levels shows a significant number of samples (20) with very high soy content (>1001 ppm). This suggests that soy is being used not just as a minor additive but potentially as a major component in some products. The presence of soy at such high levels can be attributed to its cost-effectiveness, functional properties (e.g., emulsification and moisture retention), and its role as a meat extender. This practice helps manufacturers reduce costs while maintaining product characteristics.

The lower prevalence of soy in traditional sausages (57.1%) compared to industrial sausages suggests a more varied approach to soy usage. Traditional sausage producers may be less reliant on soy, possibly due to adherence to traditional recipes or consumer expectations. The fact that five traditional samples exceeded 1001 ppm indicates that some traditional producers also heavily rely on soy. This might be driven by similar economic reasons or attempts to improve texture and yield.

Overall, the GI had an average concentration of 12,555 ppm, whereas the GII had an average concentration of 3650 ppm. This is a clear indication that industrial sausages are more likely to contain a larger amount of soy compared to traditional sausages. However, the ANOVA test failed to show statistical significance ($p = 0.43$). These results indicate that soya could be used extensively in industrial sausages and could be one of the factors contributing to the difference in price between industrial and traditional sausages.

5.6. Concentration of Soya and Fatty Acid Profile

Furthermore, the concentration of soya in the sausage is reflected in the fatty acid profile. We investigated the correlation between ct values in real-time PCR for soy DNA with each fatty acid content. There is a moderate correlation of soy DNA ct values with C16:0 palmitin (decrease), C18:0 stearic acid (decrease), C18:1 oleic acid (increase), and overall saturated fatty acids (decrease) (Figure 13).

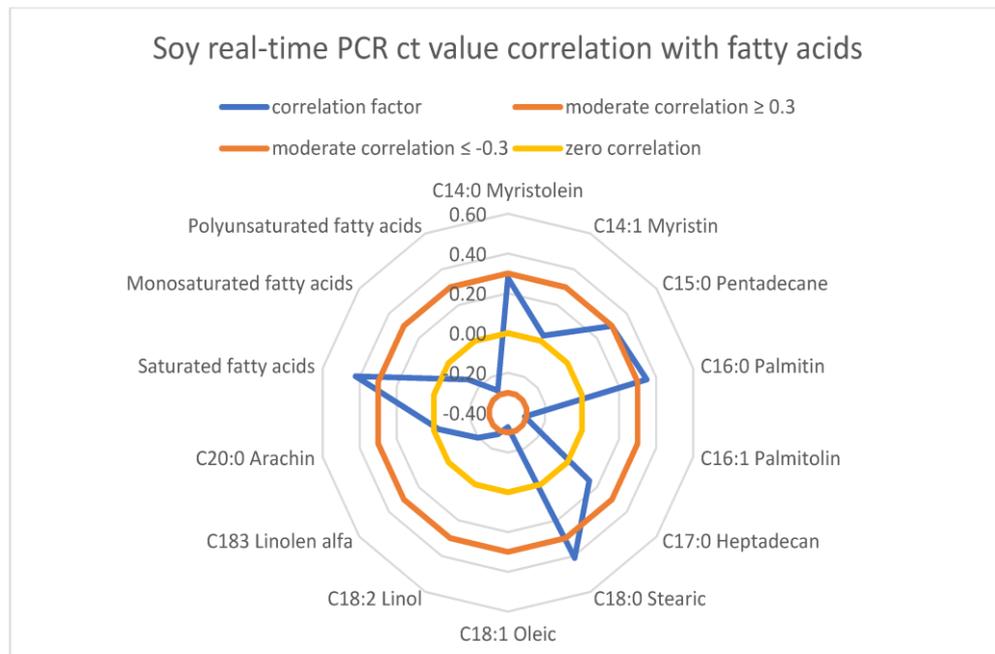


Figure 13. Correlation of real-time PCR ct values of soy DNA with the fatty acid profile.

A correlation factor above 0.3 and below -0.3 is considered moderate. Negative values of the correlation factor indicate an increase in the particular fatty acid. There is a moderate correlation of soy DNA ct values with C16:0 palmitin (decrease), C18:0 stearic acid (decrease), C18:1 oleic acid (increase), and saturated fatty acids overall (decrease).

Figure 4 shows a correlation factor above 0.3 and below -0.3 is considered moderate. Negative values of the correlation factor indicate an increase in the particular fatty acid. There is a moderate correlation of soy DNA ct values with C16:0 palmitin (decrease), C18:0 stearic acid (decrease), C18:1 oleic acid (increase), and saturated fatty acids overall (decrease).

The correlations between the amounts of soybean DNA in sausages and the fatty acid profile are as follows:

There is a moderate negative correlation between soy DNA ct values and the content of C16:0 palmitic acid. This suggests that as the amount of soy in the sausage increases, the content of palmitic acid decreases. Similarly, a moderate negative correlation is observed between soy DNA ct values and C18:0 stearic acid content. This indicates a reduction in stearic acid levels with higher soy content in sausages. The overall content of saturated fatty acids decreases as soy content increases, reflecting the specific trends observed for palmitic and stearic acids. Soy products typically contain lower levels of saturated fatty acids compared to animal fats. Soy oil, for example, has a higher proportion of unsaturated fatty acids. This substitution of animal fat with soy-derived ingredients in sausage formulations likely leads to a reduction in saturated fatty acids such as palmitic and stearic acids. The decrease in palmitic and stearic acids can be beneficial from a health perspective, as high levels of saturated fats are associated with an increased risk of cardiovascular diseases.

Conversely, there is a moderate positive correlation between soy DNA ct values and oleic acid content. This implies that as the soy content increases, the level of oleic acid also increases. Oleic acid is a monounsaturated fatty acid predominantly found in plant oils, including soy oil. The increase in oleic acid content with higher soy levels is consistent with the fatty acid profile of soy, which is rich in oleic acid. Oleic acid is considered heart-healthy and can help reduce bad cholesterol levels, providing a potential health benefit when soy replaces animal fat in sausages.

These data are also supported by the research of other authors, where the inclusion of modest amounts of soya protein (ca. 25 g) into the diet of adults with normal or mild hypercholesterolemia resulted in small, highly significant reductions in total and LDL cholesterol, equivalent to ca. 6% LDL reduction [104], and that all studies on SP and endurance performance suggested the potential beneficial effects of SP supplementation (10-53.3 g) on exercise performance by improving high-intensity and high-speed running performance, enhancing maximal cardiac output, delaying fatigue, improving isometric muscle strength, improving endurance in recreational cyclists, increasing running velocity, and decreasing accumulated lactate levels [106].

Correlation and Real-Time PCR ct Values: The ct (cycle threshold) value in real-time PCR indicates the cycle number at which the fluorescence of the PCR product crosses a threshold, reflecting the amount of target DNA present. Lower CT values indicate higher amounts of soy DNA. A moderate correlation between CT values and fatty acid

content suggests a significant but not exclusive influence of soy on the fatty acid profile, indicating that other factors may also play a role in determining the fatty acid composition of the sausages.

In the present study, it is unclear to what extent the fatty acid profile is influenced by other ingredients. However, an increase in oleic acid and a decrease in stearic and overall saturated fatty acids are expected.

5.7. The Means of Each Fatty Acid between GI (Industrial Sausages) and GII (Traditional Sausages) Using the ANOVA Test

Here, we also investigated the differences in means of each fatty acid between GI (industrial sausages) and GII (traditional sausages) using the ANOVA test, which can effectively identify overall differences between groups; we are aware that future analyses should include post hoc tests to determine specific pairwise differences among subgroups. There is a significant difference in means between groups of most fatty acids, except C14:0 Myristin, C18:1 oleic, and C20:0 Arachidic acids, as well as monounsaturated fats (Table 11).

Table 11. The differences in the means of each fatty acid between GI and GII in the ANOVA test.

| Fatty Acid | GI | GII | p Value |
|-------------------|-----------|------------|----------------|
| C14:0 Myristolein | 2.68% | 3.18% | * 0.013 |
| C14:1 Myristin | 0.37% | 0.27% | 0.162 |
| C15:0 Pentadecane | 0.45% | 0.56% | * 0.012 |
| C16:0 Palmitin | 24.42% | 25.77% | * 0.004 |
| C16:1 Palmitolin | 3.52% | 3.04% | * 0.012 |
| C17:0 Heptadecan | 24.42% | 25.77% | * 0.004 |
| C18:0 Stearic | 18.87% | 23.24% | * 0.002 |
| C18:1 Oleic | 39.81% | 38.35% | 0.104 |
| C18:2 Linol | 7.46% | 3.21% | * 0.011 |
| C183 Linolen alfa | 0.67% | 0.45% | * 0.039 |

| Fatty Acid | GI | GII | p Value |
|-----------------------------|-----------|------------|----------------|
| C20:0 Arachin | 0.53% | 0.56% | 0.705 |
| Saturated fatty acids | 48.14% | 54.66% | * 0.001 |
| Monounsaturated fatty acids | 42.76% | 41.67% | 0.518 |
| Polyunsaturated fatty acids | 8.13% | 3.66% | * 0.011 |

* p-value is significant.

The differences in means of each fatty acid between GI (industrial sausages) and GII (traditional sausages) by using the ANOVA are as follows:

Decrease in C16:0 Palmitic Acid and C18:0 Stearic Acid: These are major saturated fatty acids found in animal fats. Their decrease in sausages with higher soy content can be attributed to the substitution of animal fats with soy-based ingredients, which are naturally lower in these saturated fatty acids. Lower levels of saturated fats, such as palmitic and stearic acids, can be beneficial for cardiovascular health. Saturated fats are linked to increased levels of LDL cholesterol, which is a risk factor for heart disease. Thus, reducing these fats through the inclusion of soy can improve the nutritional profile of the sausages.

Increase in C18:1 Oleic Acid: This is a monounsaturated fatty acid commonly found in plant oils, including soy oil. The increase in oleic acid with higher soy content reflects the fatty acid composition of soy, which is rich in oleic acid. Oleic acid is considered heart-healthy as it can help reduce LDL cholesterol and increase HDL cholesterol. This shift towards higher oleic acid content makes the sausages potentially more beneficial for heart health.

Decrease in Overall Saturated Fatty Acids: The reduction in overall saturated fatty acids aligns with the decreases observed in palmitic and stearic acids. This is consistent with the replacement of animal fats by soy-based ingredients. The overall decrease in saturated fats and the increase in monounsaturated fats like oleic acid enhance the nutritional quality of the sausages. This is desirable from a public health perspective, given that the current dietary guidelines recommend a reduction in saturated fat intake.

On the other hand, regarding the content of polyunsaturated fatty acids in industrial sausages, linoleic acid C18:2 (7.46%) and a-linolenic acid C18:3 (0.67%) were more abundant, while traditional sausages had C18:2 (3.21%) and a-linolenic acid C18:3 (0.45%) as more abundant. The percentages of MUFAs in industrial sausages contained

an average of 41.67% and 8.13% of PUFAs in the total fatty acid content, whereas traditional sausages had 42.75% of MUFAs and 3.66% of PUFAs. Soybean fat has a uniquely high content of polyunsaturated fatty acids, such as linoleic C18:2 and linolenic C18:3 acids. Those data are correlated with our findings (Table 5) where industrial sausages' content has a higher concentration of soya.

The findings of this study highlight not only the technical and nutritional implications of soy inclusion in sausages but also the broader cultural and economic factors at play in Kosovo's food industry. While the use of soy can offer cost advantages, the lack of clear labeling and potential deviation from traditional sausage recipes may pose challenges to consumer acceptance. The lack of familiarity with these products may also engender negative consumer expectations and lower eventual acceptability.

6. CONCLUSIONS

Based on the results obtained, the following conclusions can be drawn:

1. This research has provided a comprehensive evaluation of the composition and authenticity of beef sausages in Kosovo, uncovering critical evidence of fraudulent meat substitution and undeclared soy incorporation within both industrial and traditional production sectors. The findings collectively demonstrate that meat adulteration represents a systemic and multifaceted issue with direct implications for food safety, consumer trust, and regulatory governance.
2. A substantial 52.94% of analysed samples contained undeclared meat species, with poultry, particularly chicken, being the predominant adulterant. The prevalence was significantly higher in industrial sausages (65.2%), yet the detection of adulteration in traditional sausages (32%) challenges long-held perceptions of their superior authenticity and adherence to traditional recipes. These results confirm the urgent necessity of rigorous control mechanisms, enhanced traceability systems, and stricter enforcement of labelling standards across the entire production and distribution chain.
3. The detection of undeclared meat species also bears significant religious and cultural implications, given that Kosovo's population largely observes dietary restrictions rooted in religious beliefs. Such mislabelling can lead to unintentional violations of dietary laws, emphasizing the ethical responsibility of producers and regulators to ensure transparent and truthful labelling that respects cultural and religious values.
4. From a nutritional perspective, the substitution of beef with poultry was associated with a more favourable fatty acid profile, characterized by reduced saturated fatty acids and increased polyunsaturated fatty acids such as linoleic (C18:2) and alpha-linolenic acid (C18:3). Despite these improvements, it must be unequivocally stated that potential nutritional benefits do not justify

undeclared or deceptive ingredient substitution, which remains both unethical and illegal under food authenticity standards.

5. The widespread presence of undeclared soy in both industrial and traditional sausages present another major concern. While soy inclusion was shown to influence the fatty acid composition positively, decreasing palmitic and stearic acids while increasing oleic acid, its hidden presence poses serious allergenic risks and further undermines consumer confidence. These findings highlight the pressing need for comprehensive allergen labelling, routine molecular screening, and reinforced regulatory oversight to ensure compliance with food safety standards.
6. This research advances understanding of adulteration practices in the Kosovar meat industry and identifies several priority directions for future investigation:
 - ✓ Economic analyses to quantify cost incentives driving adulteration and substitution practices.
 - ✓ Sensory evaluations to assess the effects of undeclared ingredients on product quality and consumer perception.
 - ✓ Consumer awareness studies to evaluate knowledge, attitudes, and behavioral responses toward adulteration and labeling accuracy.

 - ✓ Cost–benefit modeling to determine the economic and ethical implications of soy and poultry inclusion in beef sausages.

In summary, this study demonstrates that adulteration in beef sausages is not merely a technical or economic issue, but a complex ethical, cultural, and public health challenge. Addressing it requires a coordinated and multidisciplinary approach involving regulatory agencies, industry stakeholders, and academic institutions. Strengthening legal enforcement, implementing advanced molecular detection systems, and promoting transparency within the food supply chain are essential measures to safeguard food authenticity, protect consumer health, and preserve the integrity of traditional Kosovar food products.

7. LITERATURE

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