separations

MDPI

Article

Presence of Soya in Industrial and Homemade Sausage Production in Kosovo and Its Reflection on Fatty Acid Profile

Dafina Mehmetukaj [1,2], Xhavit Bytyçi [3,*], Armend Cana [1,3], Vlora Gashi-Zogëjani [1], Malbora Shandro-Zeqiri [1], Drita Bajraktari [1], Dean Jankuloski [2] and Zehra Hajrulai-Musliu [2]

[1] Kosovo Food and Veterinary Laboratory, Food and Veterinary Agency, Str. Lidhja e Pejes. No 241, 10000 Pristina, Kosovo; dafina.mehmetukaj@gmail.com (D.M.); armend.cana@ubt-uni.net (A.C.); vlora.zogejani@rks-gov.net (V.G.-Z.); malbora.shandro@rks-gov.net (M.S.-Z.); drita.bajraktari@rks-gov.net (D.B.)
[2] Faculty of Veterinary Medicine, Skopje, Ss. Cyril and Methodius University in Skopje, Lazar Pop-Trajkov 5–7, 1000 Skopje, North Macedonia; djankuloski@fvm.ukim.edu.mk (D.J.); zhajrulai@fvm.ukim.edu.mk (Z.H.-M.)
[3] Department of Food Sciences and Biotechnology, University for Business and Technology, Higher Education Institution, Kalabria, 10000 Pristina, Kosovo
* Correspondence: xhavit.bytyci@ubt-uni.net

Abstract: In the present study, we investigated to what extent soybean was used in industrial and homemade sausage produced in Kosovo, as well as its potential impact on the fatty acid profile. In total, 63 samples, 42 industrial and 21 traditional sausages, were collected either from the market or the production site. All samples were tested by means of real-time PCR for the detection and quantification of soy content, as well as the GC-FID to determine the potential reflection in the fatty acid profile. The presence of soy DNA was detected in 54 out of 63 samples. In total, 41 out of 42 industrial sausage samples were positive for soy DNA, with an average ct value of 22.60 and a standard deviation (SD) of 4.28, whereas 13 out of 21 traditional sausage samples were positive for soy DNA, with a mean ct value of 23.36 and a standard deviation (SD) of 4.56. There was a statistically significant difference in means between industrial sausage and traditional sausage, with $p \leq 0.001$ based on CT values (ANOVA test). 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). With the exception of C14:0 Myristin, C18:1 oleic, and C20:0 Arachin acids and monosaturated fats, the ANOVA test reveals a significant difference in means between groups for the majority of the fatty acids between industrial sausages and traditional sausages. The current study demonstrates that the fatty acid composition of sausages is influenced by the amount of soy present in them. The extent to which other components affect the fatty acid profile is unknown. However, an increase in oleic acid and a decrease in stearic and overall saturated fatty acids are expected.

Keywords: allergens; soya; fatty acid profile; sausage; real-time PCR; GC-FID

Citation: Mehmetukaj, D.; Bytyçi, X.; Cana, A.; Gashi-Zogëjani, V.; Shandro-Zeqiri, M.; Bajraktari, D.; Jankuloski, D.; Hajrulai-Musliu, Z. Presence of Soya in Industrial and Homemade Sausage Production in Kosovo and Its Reflection on Fatty Acid Profile. *Separations* **2024**, *11*, 302. https://doi.org/10.3390/separations11100302

Academic Editor: Gavino Sanna

Received: 24 September 2024
Revised: 16 October 2024
Accepted: 17 October 2024
Published: 21 October 2024

1. Introduction

The addition of non-meat protein sources in food products is widely used because of its capability to improve the product properties and reduce production costs. Soy protein fractions are preferred because of their higher protein content and functional properties [1]. The use of soy protein fractions in meat products is also widely applied for their properties such as water binding, fat binding, texture, and emulsification capability and for providing improved economy with increasing yield [1,2]. Moreover, soybeans are composed of proteins (35%), carbohydrates (31%), oil (17%), water (13%), and ash (4%). It is important to emphasize that the protein and lipid contents are the components of soybeans that are of greatest commercial interest [3].

Adding soy to sausages can contribute to increasing the amount of protein as well as reducing fat. Thus, fermented sausages with healthier characteristics can be produced without quality loss by reducing fat from 15 to 10% and by adding 1% soy fiber [4]. The addition of soy to salami is a legal action, and the European Commission establishes regulations regarding food additives and ingredients allowed in processed meats like salami. Soy protein and its derivatives are among the approved food additives used in meat products, subject to EU regulations and labeling requirements [5]. However, although it is allowed, the levels of soy must be determined. For this reason, the European Commission Regulation (EC) No. 1333/2008 sets maximum levels for food additives, including soy-based additives, in various food categories. Regulation (EU) No. 1169/2011 [6] on food information to consumers mandates the labeling of allergens, including soy; in the USA, prepackaged foods must use the name of the food source from which the major food allergen is derived (FDA, 2022) [7]. In Canada, if soy is part of the product formulation, must be declared in the list of ingredients or in a separate "contains" statement immediately following the list of ingredients [8]. However, Australia and New Zealand also allow the use of soy-based fillers in processed meats like sausages, similar to practices seen in the US (FSANZ, 2020) [9]. The food placed on the market of Kosovo must have a declaration in the list of prescribed ingredients, and the quantity and category of the certain ingredient, based on Law No. 03/L-016 [10].

On the other hand, food allergy is a serious health problem that affects between 3% and 5% of adults, and 8% of children [11,12]. Additionally, the statistics in developed countries show that up to 20% of people suffer from some kind of food sensitivity caused by the consumption of foodstuffs containing chemical, microbiological, or pharmacologically active contaminants. Soy is among the many allergens used in the production of salami. In research by many authors, it turns out that salami has soy content.

Fat is an important component of salami, which is reported to play a major role in the texture, juiciness, and flavor of comminuted meat products [13]. Mammalian fat is usually higher in saturated than unsaturated fatty acids, but this also depends on the type of animal from which the fat is derived. Fat in sausages is reported to be a reservoir for flavor compounds and contributes to the texture and juiciness of the products [14]. The absence of fat in beef sausages therefore contributes to the products becoming "dry and tough", as described by some consumers. There is a need to find alternative fats that are less saturated but have the potential to improve the sensory qualities of beef sausages. In addition, the addition of soy to sausages can play the role of enriching the sausage with unsaturated fatty acids since mammalian fat contains more saturated fatty acids. For this reason, recent studies are focusing on increasing the unsaturated fatty acid contents of meat products, by substituting beef fat with non-beef fats in beef sausages [15–17].

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. [18] 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. 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 validated in-house. 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 estimated amounts of soybean. 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 [19–23].

2. Materials and Methods

2.1. Sampling

In total, 63 samples, 42 industrial and 21 traditional sausages, were collected either from the market or the production site. The collection was carried out without taking into consideration whether the soy was declared on the label. Each sample containing 250 g was divided into two parts for molecular and chemistry testing. Samples were kept at 4 °C until further testing. This sample size allows for initial exploration and provides valuable insights into the use of soy in sausages, although we are aware that a larger and more diverse database would provide more representative results.

2.2. Real-Time PCR for the Detection of Soya DNA

Different methods can be used to quantify the exact amount of soy present in the samples; however, we have focused exclusively on the use of the real-time PCR method. DNA extraction was performed using the DNeasy® mericon® Food Kit (QIAGEN, Cat. 69514, Hilden, Germany), according to the manufacturer's standard protocol, using 200 mg of the sample as a starting material. For quantification, the Allergen RM 800 reference material (Hygiene, former Biotecon, Camarillo, CA, USA) was extracted simultaneously with samples. Real-time PCR for the detection and quantification of soya DNA was performed in all samples as well as the reference material by using commercially available kits (food proof® Soya detection kit and food proof®, Hygiene, Camarillo, CA, USA). The amplification was carried out according to the manufacturer's instructions. All reactions were performed on the Quant Studio 5 Real-Time PCR system (Applied Biosystems™, Thermo Fisher Scientific, Waltham, MA, USA), with the following thermal profile: pre-incubation step at 37 °C for 4 min, initial denaturation at 95 °C for 10 min, and 50 amplification cycles with denaturation at 95 °C for 5 s and annealing at 60 °C for 60 s. Data were collected on the FAM channel for soy and the VIC channel for internal control.

2.3. Gas Chromatography–Flame Ionization Detector (GC/FID)

Analytical determination was achieved using the MIX FAME-s fatty acid standard. The procedure is based on base-catalyzed transesterification of fatty acids, forming methyl esters (FAME-s), and modified from Haifeng Sun and Suli Zhao, 2014 [24]. The following chemicals were used: hexane 99.9% from Honeywell Riedel-de-Haën™ (Honeywell, Charlotte, NC, USA), methanol 99.9% CHROMASOLV™ from Honeywell Riedel-de-Haën™ (Honeywell, Charlotte, NC, USA), ethyl acetate 99.7% CHROMASOLV™ from Honeywell Riedel-de-Haën™ (Honeywell, Charlotte, NC, USA), and sodium methylate from Merk and Supelco® 37 Component FAME Mix Sigma-Aldrich®, Burlington, MA, USA, as a certified reference material (CRM) by ISO 17034 and ISO/IEC 17025.

Extraction Procedure and Derivatization of Fatty Acids

Samples were homogenized with a Velp Scientifica™ OV5 homogenizer (Usmate Velate (MB), Itlay). After homogenization, 500 mg of the sample was placed into 15 mL conical tubes and mixed with 5 mL of hexane (99.9%). After vortexing for 1 min, 1 mL of sodium methoxide (5.4 M) in methanol was added and mixed for 1 min by vortexing. Esterification was performed at room temperature. After strong vortexing for 1 min and centrifuging for 5 min at 5000 rpm, the supernatant was transferred to a 2 mL glass vial and 2 uL was injected in the GC/FID analysis. 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 setup of GC-FID is shown in Table 1.

Table 1. The operational setup of GC-FID (9).

GC System	8890 GC
S/SL inlet	250 °C, split ration 50:1
Liner	Split, ultra inert, glass wool, low pressure drop (p/n 5190-295)
Oven ramp program	50 °C (0.5 min) 30 °C/min to 194 °C (3.5 min) 5 °C/min to 240 °C (3 min)
Carrier gas	Nitrogen, 13 psi, constant pressure mode
Column	DB-FastFAME 30 m × 0.250 mm × 0.25 um 40 °C to 250/260 °C
Detector-fid	260 °C, H_2: 40 mL/min Air: 400 mL/min Makeup gas: 25 mL/min
Injection volume	1 uL

2.4. Statistical Analysis

The difference in means between groups was calculated by the ANOVA test. The correlation factor between ct values in real-time PCR for soy DNA with each fatty acid content was calculated by the correlation analysis tool, Analysis Tool Pak Excel, 2016.

3. Results and Discussion

3.1. 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 1). There was a statistically significant difference in means between industrial sausage and traditional sausage, with $p < 0.001$ based on ct values (ANOVA test).

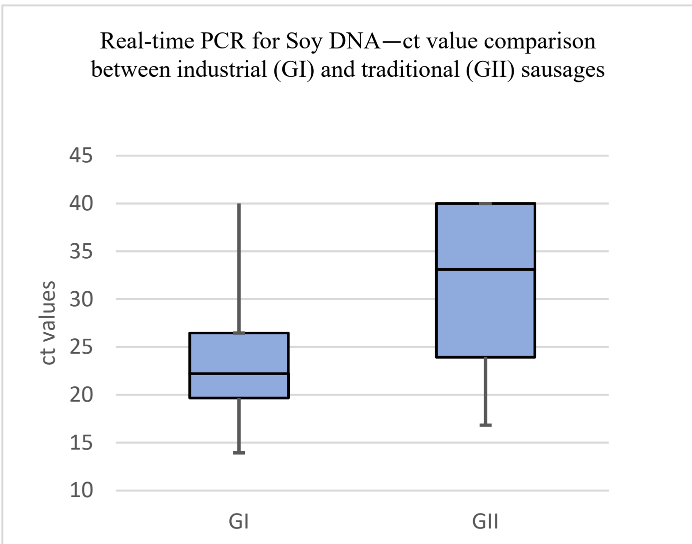

Figure 1. Box plots showing the Ct value comparison between industrial (GI) and traditional (GII) sausages obtained by real-time PCR for soy DNA.

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 [25]. 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 [26]. 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% [27].

The research findings indicating 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 provide 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.

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

Allergen RM-800 standard (Hygiene, former Biotecon, 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 2).

Table 2. 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.

3.3. Concentration of Soya and Fatty Acid Profile

Furthermore, the concentration of soy 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 2).

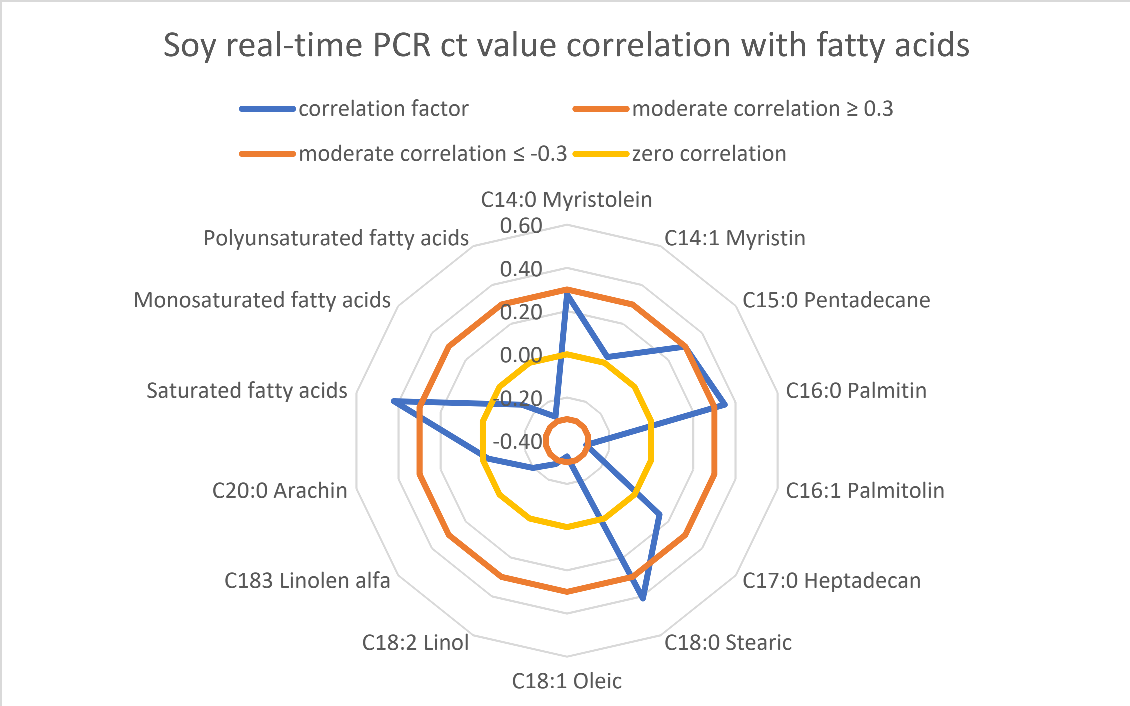

Figure 2. Correlation of real-time PCR ct values of soy DNA with the fatty acid profile. A correlation factor above 0.3 and below −0.3 is considered moderate. Negative values of the correlation factor indicate an increase in the particular fatty acid. There is a moderate correlation of soy DNA ct values with C16:0 palmitin (decrease), C18:0 stearic acid (decrease), C18:1 oleic acid (increase), and saturated fatty acids overall (decrease).

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 [28], 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 [29].

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.

3.4. 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 Arachin acids, as well as monosaturated fats (Table 3).

Table 3. 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
C20:0 Arachin	0.53%	0.56%	0.705
Saturated fatty acids	48.14%	54.66%	* 0.001
Monosaturated 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 α-linolenic acid C18:3 (0.67%) were more abundant, while traditional sausages had C18:2 (3.21%) and α-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 3) 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.

4. Conclusions

The research highlights the significant presence of soy in both industrial and traditional sausages in Kosovo, with higher levels predominantly found in industrial products. These findings have important implications for regulatory compliance, consumer safety, and industry practices. Ensuring accurate labeling and enhancing regulatory oversight are critical steps to address these issues, protect consumer health, and maintain trust in food products.

The moderate correlations observed between real-time PCR ct values for soy DNA and fatty acid profiles indicate significant changes in the fatty acid composition of sausages with increased soy content. Specifically, the decrease in palmitic and stearic acids and the increase in oleic acid reflect the fatty acid profile of soy, offering potential health benefits by improving the nutritional quality of sausages. These findings underscore the importance of considering both nutritional and sensory aspects when incorporating soy into sausage formulations. Future research should focus on comprehensive cost–benefit analyses, including both direct and indirect savings, to better quantify the economic impact of soy inclusion.

Author Contributions: Conceptualization, X.B. and D.M.; methodology, D.M. and A.C.; formal analysis, D.M., V.G.-Z. and M.S.-Z.; investigation, D.M. and A.C.; writing—original draft preparation, D.M. and X.B.; review and editing, D.B. and Z.H.-M.; supervision, D.J. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: Data is contained within the article.

Acknowledgments: The authors would like to thank the staff of the chemistry and molecular biology laboratory of the Kosovo Food and Veterinary Laboratory.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Kurt, Ş.; Kılınççeker, O. The effects of cereal and legume flours on the quality characteristics of beef patties. *Kafkas Univ. Vet. Fak. Derg.* **2012**, *18*, 725–730. [CrossRef]

2. Belloque, J.; Garcia, M.C.; Torre, M.; Marina, M.L. Analysis of soybean proteins in meat products: A review. *Crit. Rev. Food Sci.* **2002**, *42*, 507–532. [CrossRef] [PubMed]
3. Norman, A.G. *Soybean Physiology, Agronomy, and Utilization*, 1st ed.; Academic Press: New York, NY, USA, 1978.
4. Campagnol, P.C.B.; Santos, B.A.D.; Wagner, R.; Terra, N.N. The effect of soy fiber addition on the quality of fermented sausages at low-fat content. *J. Food Qual.* **2013**, *36*, 41–50. [CrossRef]
5. *Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on Food Additives*; European Commission: Brussels, Belgium, 2008.
6. *Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the Provision of Food Information to Consumers*; European Commission: Brussels, Belgium, 2011.
7. FDA. *Guidance for Industry: Questions and Answers Regarding Food Allergen Labeling*, 5th ed.; FDA: White Oak, MD, USA, 2022.
8. Health Canada. *Soy—A Priority Food Allergen*; Health Canada: Ottawa, ON Canada, 2017.
9. Food Standards Australia New Zealand (FSANZ). *Approval Report—Application A1186: Soy Leghemoglobin in Meat Analogue Products*; FSANZ: Majura, ACT, Australia, 2020.
10. Official Gazette of the Republic of Kosovo. Law No. 08/L-120 on food. *Off. Gaz. Repub. Kosovo* **2022**, *23*, 12.
11. Blom, W.M.; Michelsen-Huisman, A.D.; van Os-Medendorp, H.; van Duijn, G.; de Zeeuw-Brouwer, M.L.; Versluis, A.; Castenmiller, J.M.; Noteborn, H.; Kruizinga, A.; Knulst, A.; et al. Accidental food allergy reactions: Products and undeclared ingredients. *J. Allergy Clin. Immunol.* **2018**, *142*, 865–875. [CrossRef]
12. Parker, C.H.; Khuda, S.E.; Pereira, M.; Ross, M.M.; Fu, T.J.; Fan, X.; Wu, Y.; Williams, K.; DeVries, J.; Pulvermacher, B.; et al. Multi-allergen quantitation and the impact of thermal treatment in industry-processed baked goods by ELISA and liquid chromatography-tandem mass spectrometry. *J. Agric. Food Chem.* **2015**, *63*, 10669–10680. [CrossRef]
13. Crehan, C.M.; Hughes, E.; Troy, D.J.; Buckley, D.J. Effects of fat level and maltodextrin on the functional properties of frankfurters formulated with 5, 12 and 30% fat. *Meat Sci.* **2000**, *55*, 463–469. [CrossRef]
14. Troutt, E.S.; Hunt, M.C.; Johnson, D.E.; Claus, J.R.; Kanstner, C.L.; Kropf, D.H.; Stroda, S. Chemical, physical and sensory characterization of ground beef containing 5 to 30 percent fat. *J. Food Sci.* **1992**, *57*, 25–29. [CrossRef]
15. Martín, D.; Ruiz, J.; Kivikari, R.; Puolanne, E. Partial replacement of pork fat by conjugated linoleic acid and/or olive oil in liver pâtés: Effect on physico-chemical characteristics and oxidative stability. *Meat Sci.* **2008**, *80*, 496–504. [CrossRef]
16. Teye, M.; Teye, G.A.; Odoi, F.N.A. The potential of soya oil and egg-yolk as sources of fat in beef sausages. *Sci. J. Anim. Sci.* **2012**, *1*, 81–89.
17. Panea, B.; Ripoll, G. Substituting fat with soy in low-salt dry fermented sausages. *NFS J.* **2021**, *22*, 1–5. [CrossRef]
18. Soares, S.; Amaral, J.S.; Oliveira, M.B.P.P.; Mafra, I. Quantitative detection of soybean in meat products by a TaqMan real-time PCR assay. *Meat Sci.* **2014**, *98*, 41–46. [CrossRef] [PubMed]
19. Muratoğlu, K.; Yilmaz-Eker, F.; Ozgen-Arun, O. PCR detection of soy protein in ready-to-eat meat doners. *Kafkas Univ. Vet. Fak. Derg.* **2016**, *22*, 557–560.
20. Kim, J.H.; Song, J.Y.; Hong, Y.; Kim, H.Y. Monitoring of genetically modified soybean events in sausage products in South Korea. *Food Control* **2016**, *67*, 63–67. [CrossRef]
21. Kesmen, Z.; Sahin, F.; Yetim, H. PCR assay for the identification of animal species in cooked sausages. *Meat Sci.* **2007**, *77*, 649–653. [CrossRef]
22. Guntarti, A.; Ahda, M.; Kusbandari, A. Determining fatty acids and halal authentication of sausage. *Food Res.* **2020**, *4*, 495–499. [CrossRef]
23. Pereira, N.R.; Tarley, C.R.T.; Matsushita, M.; de Souza, N.E. Proximate composition and fatty acid profile in Brazilian poultry sausages. *Food Compos. Anal.* **2000**, *13*, 915–920. [CrossRef]
24. Sun, H.; Zhao, S. *Determination of Fatty Acid Methyl Esters (FAMEs) in Milk Matrix Using an Agilent 5977E GC/MS*; Agilent Technologies: Shanghai, China, 2014.
25. Jankovic, V.; Matekalo-Sveraka, V.; Lakicevica, B.; Spirica, D.; Petronijevica, R. Soybean and gluten in meat products—Consumer protection strategy. *Procedia Food Sci.* **2015**, *5*, 121–124. [CrossRef]
26. Renčová, E.; Tremlová, B. ELISA for detection of soya proteins in meat products. *Acta Vet.* **2009**, *78*, 667–671. [CrossRef]
27. Ulca, P.; Balta, H.; Senyuva, Z.H. A survey of the use of soy in processed Turkish meat products and detection of genetic modification. *Food Addit. Contam. Part B Surveill.* **2014**, *7*, 261–266. [CrossRef]
28. Harland, J.I.; Haffner, T.A. Systematic review, meta-analysis and regression of randomized controlled trials reporting an association between an intake of circa 25 g soya protein per day and blood cholesterol. *Atherosclerosis* **2008**, *200*, 13–27. [CrossRef]
29. Zare, R.; DevriM, L.A.; Guazzotti, S.; Redha, I.I.; Prokopidis, K.; Spadaccini, D.; Cannataro Cione, E.; Henselmans, M.; Aragon, A.A. Effect of soy protein supplementation on muscle adaptations, metabolic and antioxidant status, hormonal response, and exercise performance of active individuals and athletes: A systematic review of randomized controlled trials. *Sports Med.* **2023**, *53*, 2417–2446. [CrossRef]