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Detection of adulteration in meat products by histological methods

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Abstract

According to the Communiqué on Turkish Food Codex - Meat and Meat Products, it is forbidden to add any internal organs, bones, cartilage, and other tissues and organs into the meat products. However, some companies have mixed these internal organs into meat products such as sausages, salamis, and fermented sausages. These commercial tricks cause these products to deteriorate before their expiry date, and endanger consumers' health. However, it is possible to detect the different organs within these products by histological analysis. Nevertheless, since these products are crushed and heated during the production phase, normal organ structure is impaired. It is, therefore, very important to recognise the histological structure of the cell in the smallest cellular dimension, and this requires expertise. The present work thus aimed to provide information on the microscopic appearance of these organs and tissues with impaired integrity. For this purpose, a total of 1,000 meat products, including 300 salamis, 350 sausages, and 350 fermented sausage samples, belonging to different brands, and purchased from all provinces in Turkey, were examined in Konya Food Control Laboratory, Turkey. Routine histological techniques were used, prepared slides were stained with the triple staining method, and studied under the light microscope. It was determined that more than half of these samples were below the prescribed standards. It was also found that histological examination was an efficient method in the detection of these tissues and organs, and the triple staining method yielded the best results.

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Introduction

Meat and meat products have great importance in human nutrition because they are rich in high-value proteins, certain minerals, B complex vitamins, and especially iron (Sadeghinezhad *et al.*, 2016). Production and consumption of meat products such as salami, sausage, and fermented sausage have been increasing rapidly in recent years; however, some manufacturers have mixed low-quality and degraded meats with connective tissues, tendons, ligaments, bones, cartilages, internal organs, and very high amount of soybean into their products to obtain higher profits.

Counterfeit meat and meat products can be defined as substituted products instead of genuine products, while adulteration can be defined as the substitution of a high-value or high-quality meat species for less expensive or low-quality meat, or the inclusion of organ meats (such as the heart), at the expense of skeletal muscle in ground meat (Çetin *et al.*, 2011; Kozan *et al.*, 2013; Johnson, 2014). According to the 'Turkish Food Codex, Notification No. 2018/52 on raw and processed meat, and poultry meat and their compounds', it is forbidden to add any internal organs, bones, cartilages, and other tissues and organs, except muscle and adipose tissue, and fat, into the meat products (Turkish Food Codex, 2019). The reason for this is that the shelf life of such meat products would be adjusted based on the muscle tissue's shelf life; the shelf life of muscle tissue is between six to eight months, and the shelf life of offal is at most a few days. Therefore, when offal are added to processed meat products, this would cause them to spoil in a short time, and deteriorate earlier than their actual shelf life, and consumers would eat the spoiled products unintentionally, thus putting their health at risk. Besides, sausages with high proportion of soybeans present significant risk of GMOs. Muscle tissue is extremely important in nutrition because it contains large amounts of exogenous amino acids. On the other hand, connective tissues, tendons, fascia,

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ligaments, and cartilages do not contain some of these amino acids, or only contain a very small amount. So, almost all people who think that they purchase meat, actually purchase little or no meat at all (Bansal *et al.*, 2017; Rout *et al.*, 2018).

Food adulteration contributes to many diseases ranging from mild to life-threatening conditions like vision problems, liver problems, skin diseases, and several stomach disorders such as diarrhoea and cancer (Bansal *et al.*, 2017). On the other hand, mechanically deboned meat (MDM) products are completely mixed into the sausage paste without separating the bone and cartilage, which has high calcium content. The consumer is unknowingly forced to buy foods containing high levels of calcium. Excessive and long-term consumption of calcium increases the risk of kidney stones, and decreases zinc and iron absorption. It collapses on the vessel walls, and causes arteriosclerosis. This is a precursor to cardiovascular diseases (Migaldi *et al.*, 2016; Bansal *et al.*, 2017).

In order to identify adulteration in meat products, many physical, sensorial, anatomical, chemical, serological, biochemical, chromatographic, spectrophotometric, electrophoretic, immunological, immuno-diffusion, immunoelectrophoretic, immunoenzymatic, and DNA-based methods have been developed and employed (Çetin *et al.*, 2011; Özşensoy and Şahin, 2016; Rout *et al.*, 2018; Schieber, 2018; Omran *et al.*, 2019). However, none of these methods provide for the detection of foreign tissues and organs involved in these products. Histological examination is the only method that can be used to detect tissues and organs added for adulteration purposes.

Haematoxylin eosin, triple staining, and Paphenhaim's panoptic staining methods are used in histological examinations (Sağlam, 1987; Öznurlu *et al.*, 2007; Moghtaderi *et al.*, 2019). Among the staining methods, the triple staining technique provides better results than the others in the differentiation of epithelial, muscle, connective, and cartilaginous tissue types (Crossman, 1937; Junqueira *et al.*, 1998; Öznurlu *et al.*, 2007; Bancroft *et al.*, 2013).

The present work thus aimed to microscopically detect by histological methods the foreign tissues and organs in meat products such as salami, sausage, and fermented sausage belonging to different brands, and produced by different companies.

Materials and methods

In the present work, a total of 1,000 sausage, salami, and fermented sausage samples belonging to different brands sold in the markets in all provinces in Turkey were examined in Konya Food Control Laboratory, Turkey. Samples were randomly purchased from markets in their original packaging, immediately transported to the laboratory, and histological examinations performed in compliance with the 'Communiqué on Turkish Food Codex meat and meat products' (Turkish Food Codex, 2017).

The samples were divided into 12 parts, and nine pieces were taken from each part for the histological study. These tissues were kept in a formaldehyde solution for 1 d. The next day, after formaldehyde was removed, routine histological techniques were applied, and received parts were immobilised with cryo-matrix to the apparatus of cryostat device, and 5 - 10 µm sections were taken on the slide. These slides were dried at room temperature for 1 d, or 1 - 2 h in the oven, stained with Crossman's Mallory modification triple staining method (Crossman, 1937; Culling *et al.*, 1985; Ayaz *et al.*, 2012; Migaldi *et al.*, 2016; Moghtaderi *et al.*, 2019), examined under the Leica DM 7500 brand light microscope, and detected tissues were photographed.

Results and discussion

Histological analysis was carried out on a total of 1,000 sausages, salami, and fermented sausages belonging to different brands. It was determined that the organs and tissues that were crushed and shredded could be identified by histological tissue analysis. For this purpose, the tissue pieces were stained with the triple staining method. Different types of tissues stained with different colours by the triple staining method. Therefore, it can be concluded that this staining method is superior to other methods, and is the most suitable staining method because this method gives the clearest results by dyeing the bone, muscle, and cartilage in different colours (Crossman, 1937; Junqueira *et al.*, 1998; Öznurlu *et al.*, 2007).

According to the Communiqué on Turkish Food Codex Meat and Meat Products, it is forbidden to add any tissues or organs except skeleton muscle (Figure 1a), and adipose and connective tissues (Figure 1b) into meat products (Turkish Food Codex, 2017; 2019). Internal organs have smooth muscle structures. These smooth muscle structures belonging to the internal organs were observed intensively in the samples. Bone (Figure 1c) and hyaline cartilage (Figure 1d) were detected intensively in the products. This is because MDM products are completely mixed into the sausage paste without separating the bone and cartilage, which has intensive calcium content.

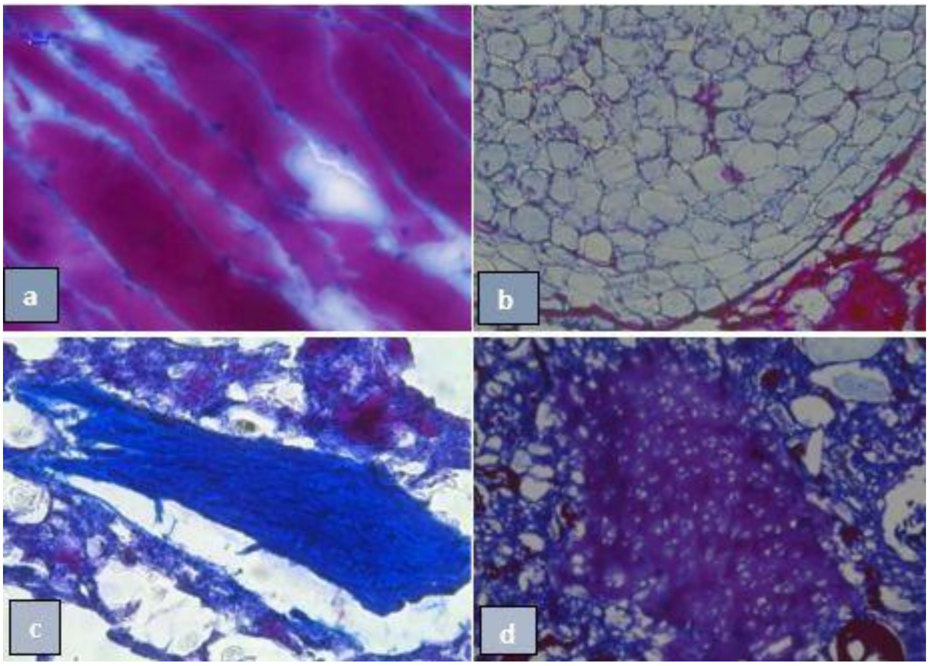

Figure 1. (a) Skeleton muscle T.B. (200× magnification); **(b)** Adipose tissue T.B. (200× magnification); **(c)** Bone T.B. (100× magnification); and **(d)** Hyaline cartilage T.B. (100× magnification).

The tissue types detected are shown in the figures. Among the 1,000 studied samples, the observed unauthorised tissues were hyaline (Figure 1d) and fibrous cartilage (Figure 2a), heart muscle (Figure 2b), lung and respiratory system organs (Figure 2c), and very dense connective tissue and skin (Figure 2d). The presence of very little amount of muscle tissue, and very dense connective tissue and skin, in almost all microscope areas, indicated that the connective tissue was involved in adulteration. Normally, connective tissue is not separated from the muscle. Therefore, connective tissue is present with muscle tissue, but sometimes producers can accumulate connective tissues and ligaments, especially ligamentum nuchae (Figure 3a), to increase weight, and mix them into sausage paste at very high amount. So, these samples consisted of very high amount of connective tissue. Mammary tissue (Figure 3b), testicle (Figure 3c), and uterus (Figure 3d) were also found in sausages, fermented sausages, and salami. Apart from this, it was also determined that a high percentage of soy (Figure 4a) was added instead of meat. High amount of vascular formation (Figure 4b) was found in some preparations. This indicated that all unconsumed parts was mixed into the sausage paste, such as ligament, tendon, *etc*., which were collected and mixed into the sausage paste to increase the weight of the paste and reduce the cost. In this way, consumers would have been deceived, and this situation causes unfair competition with companies making quality products. Apart from these, tongue (Figure 4c), ren (Figure 4d), spleen (Figure 5a), and liver (Figure 5b) were among the detected tissues.

Overall, in the ratio of 90 - 95% different degrees of adulterations were detected in the samples taken from the markets. Meat and meat products are very important for healthy nutrition. Therefore, although its cost is high, its consumption is increasing day by day. However, some manufacturers have started to cheat to make more profit by reducing the cost of manufacturing; incorporating low-quality meat, various tissues, and organ pieces into their products, contrary to Turkish Food Codex Notification No. 2018/52 on raw and processed meat, and poultry meat, and their compounds (Turkish Food Codex, 2017; 2019). This situation causes unfair competition against firms that produce high-quality products by the standards, as well as public health threats (Kozan *et al.*, 2013; Migaldi *et al.*, 2016; Bansal *et al.*, 2017).

Food adulteration is the alteration of food quality by addition of ingredients to modify different

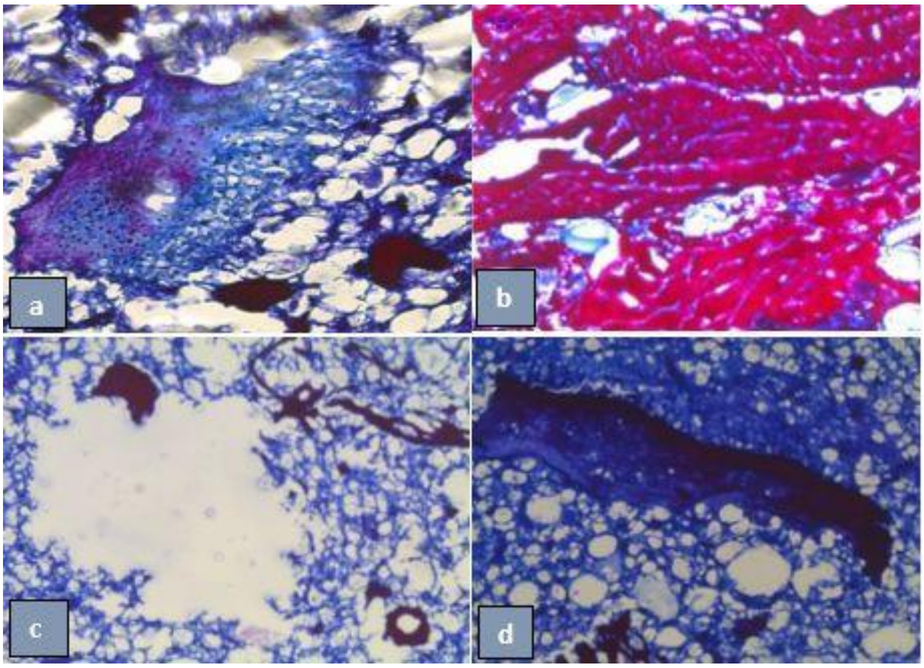

Figure 2. (a) Fibrous cartilage T.B. (100× magnification); **(b)** Heart muscle (top), skeleton muscle (bottom) T.B. (200× magnification); **(c)** Lung T.B. (100× magnification); and **(d)** Skin and connective tissues T.B. (100× magnification).

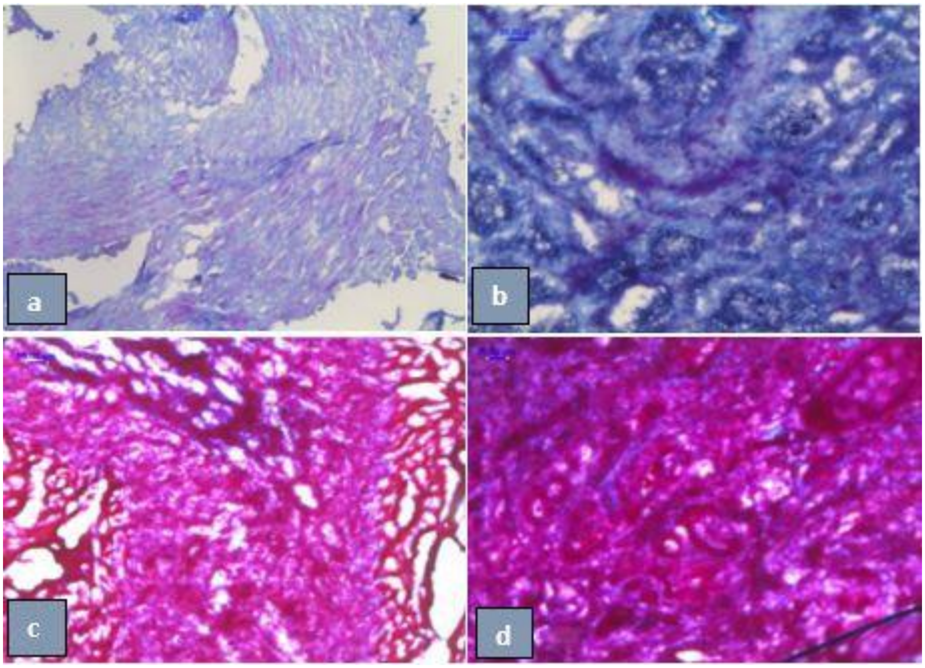

Figure 3. (a) Ligamentum nuchae T.B. (100× magnification); **(b)** Mammary tissue T.B. (200× magnification); **(c)** Testicle T.B. (100× magnification); and **(d)** Uterus T.B. (200× magnification).

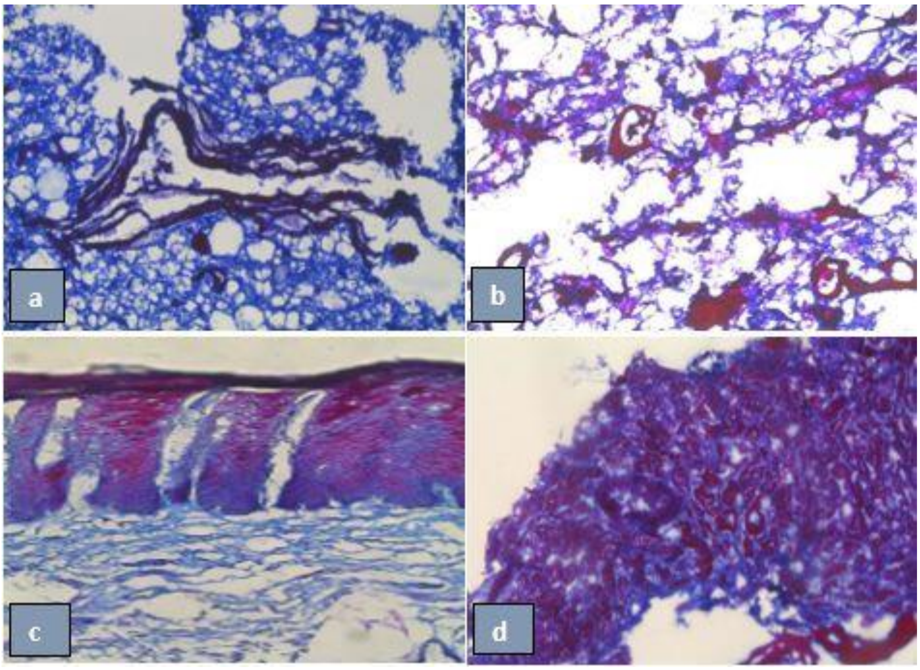

Figure 4. (a) Soy T.B. (100× magnification); **(b)** Blood vessels T.B. (100× magnification); **(c)** Tongue T.B. (100× magnification); and **(d)** Ren T.B. × (100× magnification).

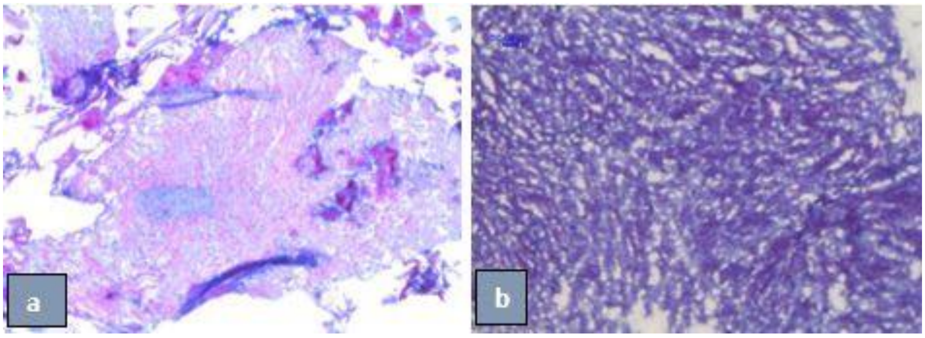

Figure 5. (a) Spleen T.B. (100× magnification); and **(b)** Liver T.B. (100× magnification).

properties (colour, weight, volume, shelf life, taste, and appearance) of food products for economic advantage (Momtaz *et al*., 2023). Food adulteration is usually done to make a profit by selling low-quality foods at a higher price. Poor socio-economic structure and low legal standards may play a significant role on this. These products are responsible for various health problems as well as financial damage. Research has shown that substituted meat products may cause allergic reactions, diabetes, diarrhoea, constipation, food poisoning, cardiovascular diseases, and various chronic diseases. These are frequently observed illnesses upon consumption of adulterated food (Everstine *et al.*, 2013; Ali *et al.*, 2014; Karabasanavar *et al.*, 2014; Ayza and Belete, 2015; Inbaraj and Chen, 2016; Choudhary *et al.*, 2020; Rees, 2020). In the present work, among the processed meat products examined, internal organs with a very short shelf life and waste products with no nutritional value have been detected. And these organs in processed meat products are risk factors for causing serious poisoning (Derbew *et al.*, 2013).

The risks of spoiled meat consumption include an increase in bacterial proliferation that can cause gastrointestinal infections, conditions that can range from mild to complex, and that may cause chronic intestinal problems. Intestinal infection causes abdominal pain, nausea, vomiting, and diarrhoea, and eventually these toxins in the intestine pass into the blood poisoning it, thus causing more severe symptoms ranging from mild gastroenteritis to systemic illness, such as septicaemia and other longer-term conditions (FDA, 2000; Seo and Bohach, 2007).

The dense bone and cartilage added to MDM products increase the calcium content of these products. Excessive and long-term consumption of calcium causes development of kidney stones, arteriosclerosis, and cardiovascular diseases. It also disrupts the mineral balance in the body by reducing the absorption of zinc and iron (Migaldi *et al.,* 2016; Bansal *et al.,* 2017). In the present work, bone and cartilage containing high amounts of calcium were detected in approximately 75% of all products. Additionally, it was determined that soy protein was used instead of muscle protein in the examined products. Soy is poor in exogenous amino acids, and poses a significant risk of GMOs. This creates serious health problems from nutritional deficiency to cancer (Bansal *et al.,* 2017).

Therefore, physical, chemical, microbiological, and histological analysis methods are used to determine the product quality and adulterations (Çetin *et al.,* 2011; Rout *et al.,* 2018; Schieber, 2018). According to Turkish Standards (TS 13511/2012) and Turkish Food Codex (2012/74 and 2018/52), meat and meat products offered to people's consumption must not have any other organs and tissues, except skeletal muscle, adipose, and connective tissues (Turkish Standard, 2012; Turkish Food Codex, 2017; 2019).

In the current 10-year literature, there are many physical, chemical, and microbiological studies on the quality of meat products in Turkey, but studies including histological examinations were very limited. Sensorial, chemical, serological, and microbiological examination methods; and proline and hydroxyproline analyses, are performed mostly in quality control of meat and meat products. Sensory examination gives an idea about subjective criteria; chemical examination gives information about the presence of substances of vegetable origin; microbiological criteria are determined by microbiological examination; and serological examination is performed to determine which animal species the meats belong to. Proline and hydroxyproline analyses determine the amount of connective tissue. However, histological tissue analysis is the only analysis that enables the detection of different tissue types present in meat products (Sağlam, 1987; Disbrey and Ruck, 2000; Öznurlu *et al.,* 2007; Prayson *et al.,* 2008; Özşensoy and Şahin, 2016).

Any analysis other than histological tissue analysis (including gene analysis) cannot detect the tissues and organs in meat products. Histological tissue analysis is the only analysis that enables the detection of different tissue types present in meat products. It is not possible to detect tissues and organs in meat products in Real Time PCR or another gene analysis. Species determination can be made in meat products with Real-Time PCR and gene analysis, but the only method by which we can detect the tissues and organs in meat products is histological tissue analysis. Therefore, the only and most appropriate method for this study is histological tissue analysis. The present work is not about species detection, but about searching for foreign tissues and organs.

There are many staining methods used in histological tissue analysis. Haematoxylin Eosin, Triple Staining, Paphenhaim's Panoptic Staining Methods, and Periodic Acid Schiff Reaction (PAS) are some of them. Among these staining methods, the triple staining technique has provided better results than the others in the differentiation of epithelial, muscle, connective, and cartilaginous tissue types. In the triple staining method, different tissue types (connective tissue, muscle, and core) are stained different colours. Therefore, it provides superiority to other staining methods (Crossman, 1937; Junqueira *et al.,* 1998; Öznurlu *et al.,* 2007). All scanned literature has shown that the triple staining method is the most suitable staining method for histological examination to detect organs and tissues (Öznurlu *et al.,* 2007; İnce and Özfiliz, 2008). So, triple staining method was used in the present work. İnce and Özfiliz (2018) detected connective tissue, adipose tissue, smooth muscle on muscle wall, and tendo-ligament in all sausage samples prepared by fermented and heat treatment methods. The nerve fibres were found to be 87.5% in fermented sausage samples, and 62.5% in heat-treated sausage samples. Bone tissue was seen in 100% of heat-treated sausage samples, and 75% of fermented sausage samples. Cartilaginous tissue was detected in 50% of all sausage samples. In the present work, we observed fibrous cartilage in 650 samples (65%), hyaline cartilage in 850 samples (85%), bone in 950 samples (95%), and skin tissue in 375 samples (37.5%). This indicated that MDM products are highly added to meat products, in addition to a large amount of tendon and ligament (especially ligament nuchae, 85%). Besides, it was observed that high amount of soy (95%) and connective tissue (100%) were present in all 1,000 samples examined. Since it is not possible to separate the connective tissue from the muscle, it is present in meat products. However, the fact that the microscope area is located in a large part indicates that it participated in the purpose of adulteration. So, its amount is important. The percentage of connective tissue can be determined by hydroxyproline analysis.

Çetin *et al.* (2011) detected an excessive amount of fascia (dense fibrous and connective tissue) cartilage, either hyaline or fibrocartilage, in all samples. Sezer *et al.* (2013) evaluated the quality characteristics of fermented sausages and sausage-like products sold in Kars. They found epithelial tissue in 13 (32.5%) of the 40 samples, and 27.5% (11 samples) contained glandular epithelial tissue, which was mostly seromucous in nature, and five (12.5%) contained cartilaginous and bone tissues with smooth muscle tissue. In the present work, we observed 35% gland epithelium, 95% bone tissue, 75% cartilaginous tissue, 60% lung tissue, 75% nerve tissue, and 45% other internal organs such as genital organs, liver, and kidney. İlhak and Güran (2015) investigated the presence of horse, donkey, chicken, turkey, and pig meat in 50 sausages (30 beef and 20 beef + poultry) collected from markets using the Real-Time PCR method, and reported that 23.3% of 30 beef sausage samples contained poultry meat, None of the 50 sausage samples contained pig meat, but one (2%) of the samples generated horse fragments. In the present work, high amounts of MDM products in sausages belonging to beef were detected. This indicated that the products labelled as beef had poultry (chicken, turkey) meats in them. Sadeghinezhad *et al.* (2016) detected oesophagus, heart muscles, cartilage, bone, lymph nodes, and spleen in sausage, *kofta*, and *hawawshi* samples. Similar findings were obtained in the present work.

Food adulteration which has so many risks to public health is monitored by law in our country, and manufacturing companies that do not produce in accordance with the standards are punished by law, and announced on the Ministry's website. According to Law No. 5996 article 21/5; “Foods and materials and articles contact with food which does not comply with the food codex shall not be produced, processed, and placed on the market” (Official Gazette, 2010). The objective of Law on Veterinary Services Plant Health Food and Feed No:5996 is to protect and ensure food and feed safety, public health, plant and animal health, animal improvement, and welfare and consumer interests, taking into consideration environmental protection. Those who violate paragraph five of Article 21 of this Law shall be charged an administrative fine of ten thousand Turkish Liras. If products pose risks to human health, they are withdrawn from the market with expenses borne by the responsible person, and their property shall be transferred to the public. If violation is only related to labelling requirements, an administrative fine of five thousand Turkish Liras shall be charged. If imitation in food products creates unfair competition, in the case, it will constitute a crime according to Turkish Commercial Law No. 6102 the regulation in 62/1-a. According to Turkish Commercial Code No. 6102 article 62/1-a; those who intentionally committed one of the unfair competitive proceedings written in the Article 55, unless the proceeding is another crime that requires more severe punishment; by Article 56, upon complaint, they are punished with imprisonment or a judicial fine of up to two years. If the life or body integrity of the consumer is damaged due to the products produced and sold by making adulteration, people who suffer damage will be able to file material and moral damages (Official Gazette, 2011). In addition, accurate labelling is important to support fair trade (Ballin, 2010). It is a challenging task to ensure the quality of meat, and help in law enforcement in different countries (Singh and Sachan, 2009).

According to the food law, the species’ names of meats used to prepare the meat products have to be presented on the label of the product. Moreover, selling other meat species with false labels to get more profit is held as imitations, and is prohibited according to the foodstuff laws (Hassanin *et al.*, 2018). Despite all this, in Turkey, as in many other countries, the fine is very low, and there are no serious restrictions on food adulteration in the law. The lack of proper legislation and its strict enforcement is one of the primary reasons for the increase in food adulteration. Therefore, it is necessary to take action against adulteration which may help consumers to live healthy life.

Conclusion

Based on histological examination, more than half of the sausage, salami, and fermented sausage samples (almost all of them) were found not to comply with Communiqué on Turkish Food Codex Meat and Meat Products. The samples were detected to contain unconsumed tissues and organs belonging to respiratory and digestive systems, genital organs, tendons, ligaments, and bones.

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