

# Histological Investigation Of Fermented Sausages Sold In Sanliurfa Province

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**Abstract:** Whether meat products are produced in accordance with standards and in quality control; In addition to chemical, microbiological and organoleptic examinations, histological examinations are performed in order to give better results than obtaining good quality and healthy products. In this work, it was aimed to reveal the internal organs and tissues used in the construction of sausages which are sold in retail in Şanlıurfa province by histological diagnosis methods. Thirty-two sausages sold in retail were obtained from the butchers and grocery stores in the city center and histologically analyzed by hematoxylin-eosin staining method. Cartilage tissue, bone tissue, lymphoid tissue, connective tissue, skin glands, hair follicles, skin and internal organs were determined in fermented sausage samples. It has been determined that the analyzed sausage does not carry the characteristics specified by national standards. In the study, it was determined that sausage samples obtained from Şanlıurfa province were added with different animal tissues which should not be included except for muscle tissue. Control of whether meat products conform to standards and histological analysis in addition to other analyzes in order to protect consumer health has resulted in contributing to healthy food production.

**Index Terms:** Sausage, histological examination, tissue

## 1 INTRODUCTION

Sausage is a meat product that is very suitable for adulteration because of the substances added to it during manufacturing and the determination of the scams is very important in terms of quality control and human health. Adulteration is made by adding meat, different organs and tissues, herbal ingredients and chemical substances that should not be present in sausages, which are not allowed to be used in making sausages, for various purposes [1]. The skeletal muscles composed of the striated muscle cells and the connective tissue between them are defined as meat [2], [3], [4], [5]. Compared with meat, the nutritional value of the organs that contain connective tissue, epithelial tissue, and secretory gland cells in the structure is very low. Due to the chemical structure of the cells in them, the addition of organs to the sausages, which are more easily damaged than the skeletal muscles, causes the sausages produced to be deteriorated more quickly and the important risks in terms of human health arise. The use of skeletal muscles called meat in slaughter animals and other parts other than fat tissue in sausage production is prohibited by the provisions of Article 5-d of Turkish Food Codex Meat Products Communiqué. Accordingly, tendon and fascial pieces, genital organs of the male and female animals, eyes and ears, skin, tripe and bowel, spleen, lung and liver, breast tissue, cartilage, and bones cannot be used in sausage production [1], [6]. TS-1070 standard of the Turkish Standards Institute defines the Turkish sausage as "meat product obtained by stuffing dough prepared from slaughtered bovine meat into natural and artificial sheaths and matured for a while" [7]. According to the same communiqué, fermented sausage; the total meat protein ratio is at least 16% by mass, the collagen ratio is at most 20% by mass of total meat proteins, the ratio of moisture content to total meat protein content is less than 2.5, the ratio of fat content to total meat protein content is below 2.5, the value should be at most 5.4 [8].

In fermented sausages, the maximum amount of fat is 40%, the maximum amount of moisture is 40%, the maximum value is 5.4; In heat-treated sausages, the amount of fat should be maximum 40% and the pH value should be maximum 5.8 [9]. In the quality control of the Turkey sausage density chemical, microbiological and organoleptic testing methods are applied. However, many researchers [10], [11], [12], [13], [14] reported that the addition of histological examinations to the present examinations for this purpose would give better results in determining and increasing the quality they have. These researchers have emphasized that the tissue composition contained in sausages and the type of organ added can be determined only by histological analysis in the most precise manner. Many studies have been carried out on the chemical, microbiological and sensory quality of sausages produced in different countries in our country. The appropriateness of the sausages produced in the researches on the Turkish Food Codex and TSE Turkish Standard [7], [15], [16]. The use of high-quality raw materials in the production of foodstuffs is an important factor in the quality production of sausage products. Whether they are manufactured in accordance with standards or not and in the classification of qualities; chemical, microbiological and organoleptic examinations in addition to histological examinations [17]. In the histological studies performed, fat vacuoles in sausages are observed as vacuolar voids [13]. Flat arteries in the muscle layer and arterial walls of the internal organs are flattened and ring-shaped in the tissues of the arteries [2], [4], [12], lined up on the cell edges in the preparations stained with hematoxylin- cartilage tissue is reddish and chondrocytes are visible. Moreover, because of the typical alveolar structure, lung tissue can easily be distinguished in preparations [12]. Histological determination for detect the organs and tissues that are used for the purpose of adulteration has been concluded as useful method for good quality products [18]. In this study, it was aimed to determine the presence of tissues and internal organs in 32 sausage products sold in retail in Şanlıurfa province by histological analysis methods.

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## 2 MATERIALS AND METHODS

### 2.1 Sample Collection

In our study, 32 sausages belonging to different firms sold in Şanlıurfa city center were used. Experimental histological preparations prepared from various cattle tissues and beef cattle were used for the purpose of comparison with the preparations prepared from sausage samples. The method applied by Ayaz et al., [19] was used in the research.

### 2.2 Preparation of experimental specimens

Five pieces of tissue mixture consisting of lung, liver, lymph follicle, kidney and spleen components and tissue mince added. The sausage doughs to be fermented were stored in fermentation for a period of 10 days under a temperature of 18-22°C, a relative humidity of 75- 85% and an air flow of 0.5- 1 m/second.

### 2.3 Histological preparations of samples

150 g of each sausage sample was taken, cut 2-3 times, 5 mm in depth, and fixed, followed, paraffin-embedded, cut and stained. The samples were then fixed in 10% formol solution for 24 hours. The samples, which were detected in the formalin solution, were washed under tap water for 24 hours. Subsequently, paraffin impregnation was applied after 18 hours of semi-enclosed tissue monitoring device and water clarification steps as:

120 minutes in 70% alcohol,  
120 minutes in 90% alcohol,  
60 minutes in 100% alcohol,  
120 minutes in 100% alcohol,  
120 minutes in 100% alcohol,  
90 minutes in xylene  
90 minutes in xylene  
120 minutes in paraffin

Paraffin 180 minutes method was applied.

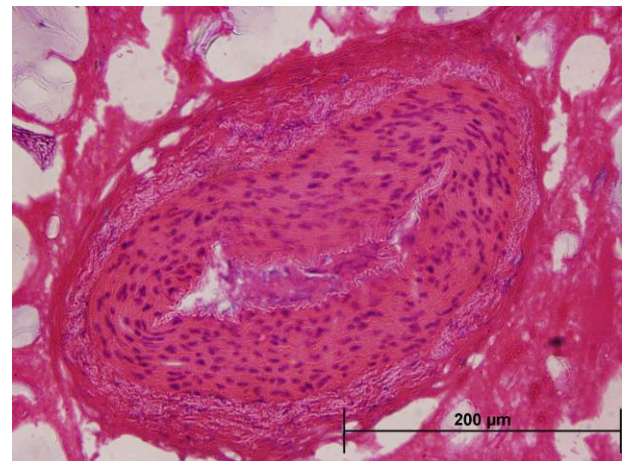
Subsequently, the samples were blocked in the tissue embedding device and sections with a thickness of 6 microns were taken in the microtome device. The sections were dried at room temperature and then rehydrated in distilled water by passing through 90%, 80%, 70% and 50% ethanol respectively after deparaffinization, rehydration in 100% ethanol and hydration, and the sections were stained with hematoxylin-eosin.

### 2.4 Hematoxylin-eosin staining

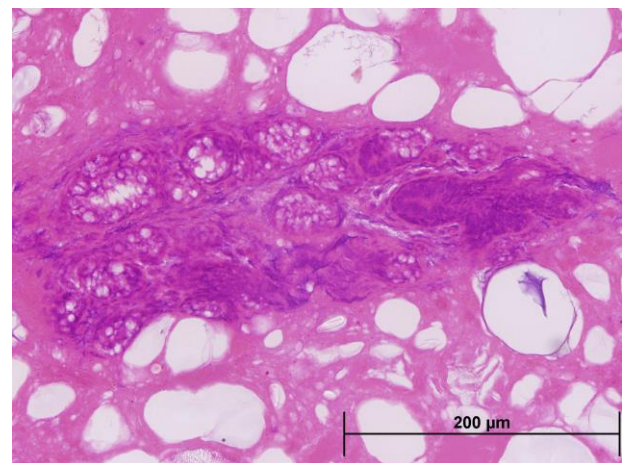
The preparations were held for 10 minutes with instant hematoxylin (Shandon Cat No: 9990107), then rinsed with tap water and removed from excess dyes. Subsequently, the preparations were decolorized in 0.25% HCl and 50% alcohol, rested in the ammonia water for 20-25 minutes to purple color, and stained with Instant Eosin Alcoholic (Shandon Cat No: 1900140) for 1 minute. For dehydration it was treated with 50%, 70%, 80%, 90%, 96%, 100% ethyl alcohol. The preparations were then clarified for 10-15 minutes. The dyed finished sections were examined in DP-71 (Olympos, Tokyo, Japan) research microscope coated with laminated xylene based adhesive.

## 3 RESULTS AND DISCUSSION

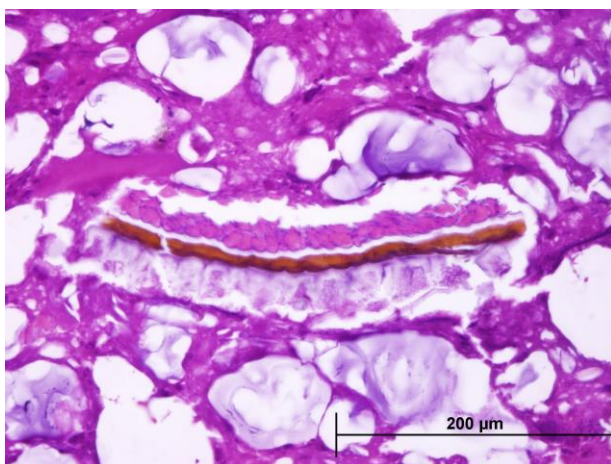
It was determined that the structural and staining properties of the tissues were better preserved in the preparations prepared by the paraffin blocking method in the histological examinations. According to the best preservation of the structural features, tissues are classified as cutaneous mucosa, skeletal muscle, glandular epidermal. In addition, blood vessels walls, cartilage tissue, lung tissue, peripheral nerve fibers are well preserved. The cross-sectional quality of the preparations prepared by the paraffin embedding method is particularly good in terms of the regular arrangement of the tissues in the cross-sectional area. While the hematoxylin-eosin staining resulted in cell cytoplasm of all tissue types and the intermediate material was painted in pink and red tones; cell nuclei are stained with cyano. In hematoxylin-eosin staining, fat cells were observed as gaps in all sausage preparations. The alveolar structure of lung tissue, thick-walled and ring-shaped arteries, multilamellar flat keratinized epithelium, skin glands and hair follicles, bone, cartilage and lymphoid tissue were clearly observed. In addition, nuclei of skeletal cells were observed lined up at the edges of the cell. As a result of the study, it was determined that 32 of the 22 specimens did not conform to the Turkish standards in the histological direction.



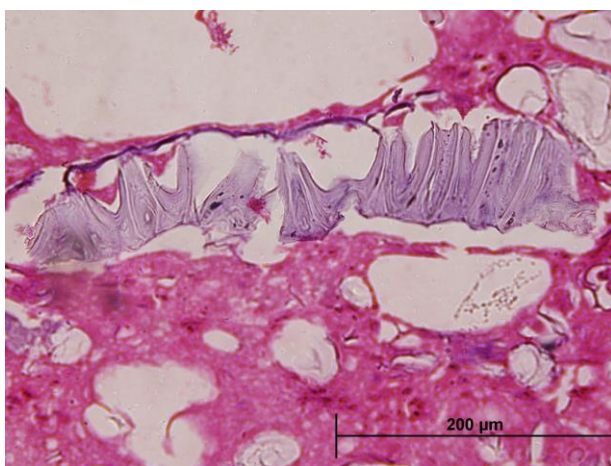
*Fig. 1. Muscular arter. H&E.*



*Fig. 2. Glandula. H&E.*



**Fig. 3.** *Cutis. H&E.*



**Fig. 4.** *Bone. H&E.*

Meat and meat products are very important groups of nutrients in people's adequate and balanced diet. The amount of animal protein consumption which is low in developing countries has increased in recent years. In the world, demand for meat and meat products, especially for animal origin, and production of these foods are higher than those for vegetable origin [20], [21]. Meat and meat products, which are starting to be consumed more often, are an important group of foods that have been cheated many times because of the high price, the difficulty of production and the inability to meet the demand. Meat and meat products; it is one of the frequent cheats to mix meat products that are cheaper or are not preferred by consumers. For this reason, it has become increasingly important to identify substances that should not be used in raw and heat processed meat and meat products [22], [23], [24], [25]. In order to determine these; many methods based on physical, sensory, anatomical, histological, chemical, biochemical, immuno-enzymatic and DNA have been developed and used [26]. In the sections stained with hematoxylin-eosin, the nuclei of skeletal muscle cells are seen as arranged on the cell edges as Sarigöl [12] reported. In the examined sections, the fat texture was seen as empty vacuoles in the case of the kernel pushed by the cellular as Uğurlu [13] showed. It is believed that xylenes are used for the preparation of the preparations, which are empty for the cells. Skeletal cells had the same characteristics as the muscular

structures that Artan [2] and Erençin and Sağlam [4] described with nuclei pushed to the edge of the cell in all sausage samples. The alveolar structure of lung tissue confirmed to the findings of Sarigöl [12]. Likewise, the cartilaginous tissue pieces were painted red and the chondrocytes were clearly visible. In a study conducted in Kahramanmaraş city, 24.0% of the samples of 16 sausages and 50 sausages had 31.2% of bone and cartilaginous tissues were detected [27]. Yıldız et al., [28] studied the preparation of ready-to-made meatballs in Istanbul and 4 (5.4%) of the samples had small amounts of tissues and 2 (2.7%) of the tissues were not to be consumed in very small quantities. In 2013, Sezer et al., [29] reported on fermented sausages and sausages purchased from traditional markets in Kars; epithelial tissue in 13 (32.5%) of the samples, gland epithelium in 11 (27.5%) sero-mucous character and smooth muscle, cartilage and bone tissue in 5 (12.5%). Atasever et al., [17] conducted a survey of 48 fermented sausages from the market in Konya and found that 18 of them (37%) had organ fragments that should not be present in sausages. In conclusion, in this study, it was determined that many different animal tissues were mixed in the meat products of different firms in Şanlıurfa province compared to muscle tissue, and it was determined that they could be determined reliably by histological methods. As a result, it has been concluded that meat products are in compliance with the standards, and that, in addition to other analyzes, the histological analysis is beneficial in the safe production of food by the systematic and controlled application of the histological analyzes. In accordance with the Turkish Food Codex, Meat Products Communiqué, it is prohibited to add sausage additives other than the additives specified in the related regulation and in the establishments producing and/or selling other meat products, the meat of different animal species are produced separately from each other and from other foods and sold separately [8]. Although the labeling of the products analyzed in this study is labeled as "100% beef", it is suggested that the structures of the internal organs found are not in accordance with the notification

#### 4 CONCLUSION

In this study, it was aimed to determine the presence of tissues and internal organs in 32 sausage products sold in retail in Şanlıurfa province by histological analysis methods.

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