

## Histological and Molecular Evaluation of Raw Meatball Products

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### Abstract

Meat is an important source of protein with high biological value. Due to this importance, the production and consumption of meat and meat products such as minced meat, salami, sausage, meatballs, and roasted meat have been increasing rapidly in recent years. In order to earn more money, some companies sell animal meats that are not consumed by society by mixing them into meat products without considering the health, habits, ethical and cultural values of people. In this study, it is aimed to examine whether there are undesirable tissues and muscle tissue belonging to different species in meat products by making histological and molecular evaluations in raw meatball products offered for sale. Evaluations were made by taking samples of raw meatball products offered for sale by 6 different well-known supermarkets. In the results we obtained, it was observed that the integrity of the muscle tissue was not impaired in sample 1, but the presence of cartilage fragments in places. In sample 2, different sizes of tendon fragments and nerve fiber bundles were detected, with less muscle tissue. In sample number 3, it was observed that the integrity of the muscle tissue was not impaired and the spice pieces were excessive. In sample number 4, it was observed that the integrity of the muscle tissue was partially damaged. In sample 5, large tendon fragments and abundant connective tissue were detected. In sample 6, an image was detected, suggesting that it was caused by too much use of the additive. In the RT-PCR results, no tissue belonging to different species was found in any of the 6 samples we received. These results, in addition to being inappropriate in terms of public health and consumer rights, also appear as attempts to gain unfair financial gain.

Key words: Raw meatball, histology, Rt-Pcr.

### Introduction

Meat has an important place among the nutrients of animal origin in terms of nutrition. First of all, meat is an important source of protein. Other than that, meat; contains a significant amount of lipids, mineral substances and, vitamins (A and B complex). After water, protein is the most important nutrient required for the body's growth, development, and protection from diseases. Because every living thing necessarily contains protein. While protein controls the water balance and acid-base balance of the organism, it also helps the formation of hormones. Another important task is to ensure the production of hemoglobin. Hemoglobin is the blood protein that carries oxygen to cells and takes up carbon dioxide.<sup>1,2</sup>

Meat protein is a protein with high biological value. The reason why the proteins in meat are of high quality is that they contain all of the exogenous amino acids necessary for human nutrition. The absorption of these proteins in the body is 97 – 98%. In other words, it is used almost completely in the body. Even if the person has taken the protein that should be taken daily with vegetable proteins, this does not mean that the protein needs of the body are met. Because vegetable proteins are poorer in terms of exogen-amino acids. The amino acids found in meat and their amounts are almost the same in the meat of all animal species.<sup>2,3</sup>

Generally; meat is defined as "edible animal tissues obtained from healthy animals (bovine, ovine, poultry, and

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aquatic animals) that have reached sufficient maturity in accordance with the technique". According to the Turkish Food Codex Meat Products Communiqué; it is possible to define meat as "all parts suitable for human consumption obtained from domestic ruminants, poultry, rabbits and pigs".<sup>4</sup> For meatball; it is a ready-to-cook red meat mixture or a cooked meat product prepared in various ways by adding the fats of the same animal, flavorings, and one or more of the other food ingredients, if desired, to the mixture of one or more minced bovine and ovine animals.<sup>4</sup>

Meat consumption in our country is incomparably lower than in developed countries (7 kg/p in 2020).<sup>5</sup> Protein deficiency brings with it developmental disorders and health problems. The reason for growth retardation problems in Turkey is the inability to be fed with protein-rich foods. Since 90% of brain development is completed by the age of 3, energy and protein deficiency also negatively affect mental development. After a certain age, it is necessary to take enough white meats together with lean red meats. However, children and young people should consume more red meat.<sup>1</sup>

Due to the importance of meat, the production and consumption of meat and meat products such as minced meat, salami, sausage, meatballs, hamburger meatballs, and roasting have been increasing in recent years. Although a significant increase has been achieved in the quality of these products thanks to the developing production technologies, there are significant problems in compliance with the standards and quality control. As a matter of fact, some manufacturers resort to various tricks in production in order to make more profit by reducing the production cost and use low-quality meats and internal organs with residues that should not be added to production, contrary to the Food Products Regulations. This situation not only leads to unfair competition among companies that make quality products following standards but also deceives consumers and threatens public health.<sup>3,6</sup>

Meat and meat products are highly priced due to animal production policies. Therefore, the demand of low-income consumers for cheap meat and meat products is increasing. Some companies, taking advantage of this opportunity, to earn more money, without considering people's habits, ethics, culture, and religious values, especially by obtaining animal meat, which is not consumed by society, in a very cheap way, mixed into meat products and offered for sale. Some problems arise with the introduction of such meats or meat products for consumption.<sup>7</sup> Considering these reasons, it is necessary to distinguish

the tissues or organs of different animal species added to meat products that may threaten public health. Especially in products such as meatballs, sausages, salami, etc. which are prepared from a mixture, the rate of cheating increases even more.<sup>8</sup>

To summarize the tricks made for this purpose;

- The use of animal meats that are not allowed to be used in meat products,
- The use of various internal organ parts in the production of meat products,
- Incorporation of meat product residues into new product dough,
- Addition of plant origin substances to meat products,
- The addition of chemicals that are not allowed to be used in meat products.

Apart from the muscle and fat tissue determined by the food product registrations, very different textures can be mixed into meat products. Undesirable but most frequently encountered and used textures; cartilage tissue, bone tissue, nervous tissue, large arteries and veins, salivary glands, internal organ parts (thymus, lung, etc.), mammary glands, hair.<sup>8,9</sup>

In our study, we aimed to examine the presence of undesirable tissue parts and the presence of muscle tissue of different animal species, by making histological evaluation technics and RT-PCR, in raw meatball products offered for sale by well-known supermarkets.

## Materials and Methods

Histological evaluation and RT-PCR were performed on samples taken from meatball products ready for consumption (6 different supermarkets), offered for sale by well-known and preferred big supermarkets. All histological procedures were carried out in Bursa Uludağ University Faculty of Veterinary Medicine Histology-Embryology Laboratory. RT-PCR evaluations were carried out in Bursa Food and Feed Control Central Research Institute, Animal Products Department Laboratory.

## Histological Analysis

To obtain a more homogeneous product, a mixture was made by combining the purchased raw meatballs (for each supermarket). Samples were taken from 5 different points of the meatball content (6x5=30 samples) and placed in the fixation solution. Buffered neutral formalin was used as a fixation solution. A routine histological procedure was applied to the samples, which were kept in fixation liquid for approximately 48 hours, and the tissues were embedded in paraffin. 5µ thick sections were taken from the obtained

paraffin blocks and adhered to the slide. Samples from different supermarkets were randomly numbered from 1 to 6. Crossman's Triple Staining was then applied to the tissues.<sup>10</sup>

Tissues whose histological procedure and staining was completed were evaluated using a Nikon Eclipse 80i microscope and photographs were taken. In the histological evaluation, the presence of undesirable tissues in meat products, therefore in meatball products, and the integrity of the existing muscle tissue were taken into consideration. Real Time-PCR Analysis

RT-PCR technique was used in raw meatballs to determine whether there is a tissue belonging to a different species other than beef, as specified in the label information. After DNA isolation from the samples, purity and amount controls were made with a spectrophotometer. Species-specific commercial kits were used to compare the DNA isolates obtained and the manufacturer's recommended protocol was followed. Evaluation was made for five different species. Horse species identification (SNP Biotechnology, 301R-10-01, Ankara, Turkey), pig species identification (SNP Biotechnology, 302R-10-01, Ankara, Turkey), donkey identification (SNP Biotechnology, 303R-10-01, Ankara, Turkey), and chicken species identification (SNP Biotechnology, 312R-10-01, Ankara, Turkey).

## Results

The presence of undesirable tissues was examined histologically, and the presence of tissues belonging to different animal species was examined by PCR technique, in raw meatball products offered for sale by 6 different well-known and preferred supermarkets.

Since the raw meatball products offered for sale are made from beef on the sales labels, evaluations have been made on animal meats such as chicken, which is not prohibited but should not be included in these products.

### Histology

In sample No: 1, histologically, the integrity of the muscle tissue was not impaired, the general appearance was good, but the presence of cartilage pieces in places (Figure 1).

In sample No: 2, less muscle tissue amount, different sizes of tendon fragments, nerve fiber bundles, and some kind of food additives were detected (Figure 2). It was not possible to determine the types of food additives, histologically.

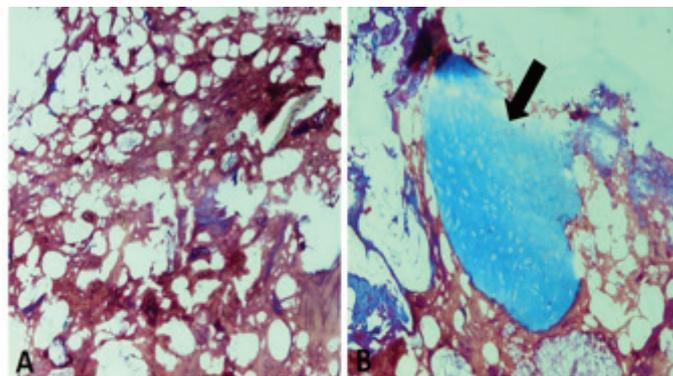


Figure 1. Histological section of sample No:1. (A) General view of muscle tissue. (B) big piece of cartilage tissue (arrow) (Triple Stain 100x).

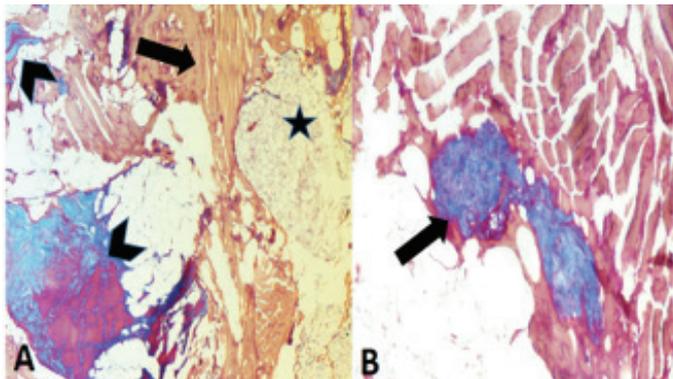


Figure 2. Histological section of sample No:2. (A) Muscle tissue (arrow), tendon pieces (arrowhead) and food additive (star). (B) Nerve fiber bundle (arrow) (Triple Stain 100x).

In sample No: 3, it was observed that the integrity of the muscle tissue was not impaired and the lots of spice pieces were excessive. Again in this sample, visceral parts that were suspected to be thymus or hemal nodes, but could not be determined histologically, were found (Figure 3).

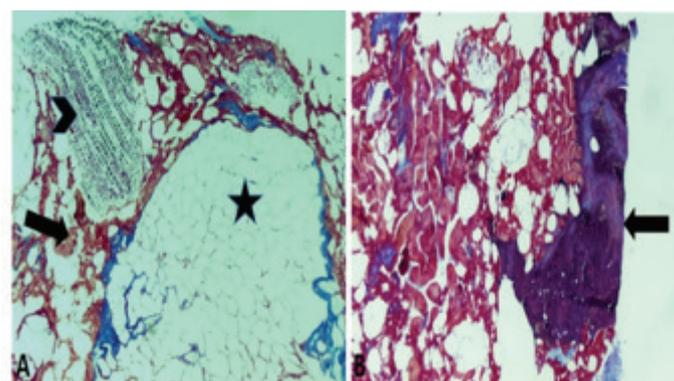


Figure 3. Histological section of sample No:3. (A) Muscle tissue (arrow), adipose tissue (star) and spice (arrowhead). (B) visceral part that was suspected to be thymus or hemal node (arrow) (Triple Stain 100x).

In sample No: 4, the integrity of the muscle tissue was partially damaged, nerve fiber bundles and large pieces of tendon were detected in large pieces of connective tissue (Figure 4).

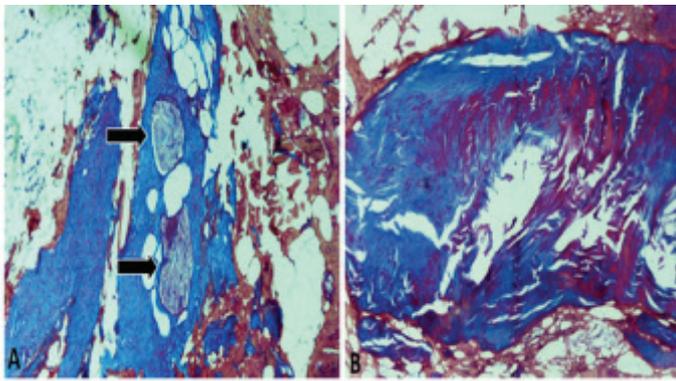


Figure 4. Histological section of sample No:4. (A) Nerve fiber bundles in a large connective tissue (arrow). (B) Large piece of a tendon (Triple Stain 100x).

In sample No: 5, large tendon fragments and abundant connective tissue were detected. In addition, the general appearance of the muscle tissue in this sample, as well as the staining intensity and quality, made us think that it might be an old-dated or expired product (Figure 5).

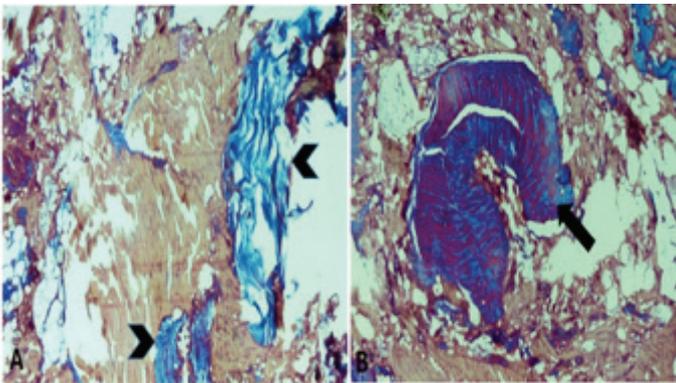


Figure 5. Histological section of sample No:5. (A) General view of muscle tissue and connective tissue pieces (arrowhead). (B) Piece of a tendon (arrow) (Triple Stain 100x).

In sample No: 6, we detected a result that we think is caused by using too much of the food additive(s). While the muscle tissue was seen in very small amounts, the additive(s) were detected in very large areas. It was not possible to determine the types of food additives, histologically (Figure 6).

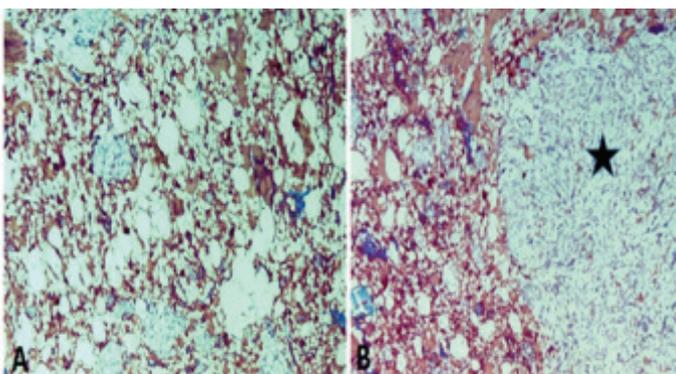


Figure 6. Histological section of sample No:6. (A) General view of muscle tissue. (B) Large amount of food additive (star) (Triple Stain 100x).

### Real Time - PCR

It was written on the sales labels of the raw meatball packages that we used in our study that they were made from beef. As a result of RT-PCR performed in our samples to determine species identification, it was determined that there was no mixture of horse, pig, donkey and, chicken meat in any sample (Figure 7).

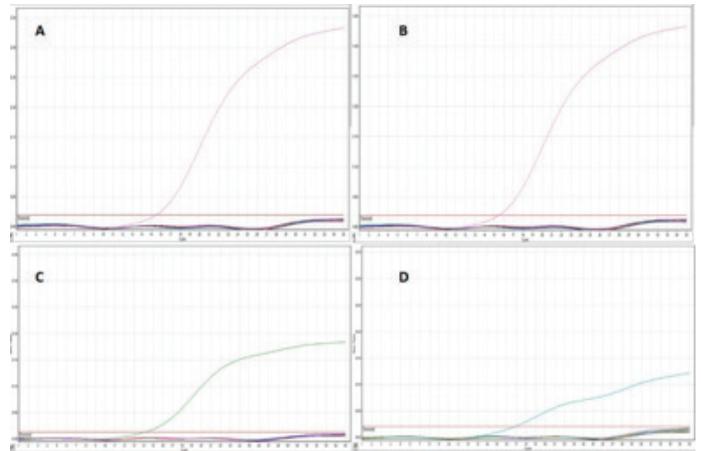


Figure 7. Readings registered by RT-PCR. (A) Horse DNA, (B) Pig DNA, (C) Donkey DNA, (D) Chicken DNA (detection limit 10<sup>-3</sup>).

### Discussion and Conclusion

To determine the quality of meat and meat products, physical, chemical, microbiological, and histological evaluations can be made. In the histological evaluations, the appropriate method should be selected and evaluations should be made by considering the structural integrity and staining characteristics of the tissues and organs in the meat products. In our study, raw meatball products were evaluated using the paraffin embedding method, which is frequently used and known to have more positive results.<sup>11</sup>

Although the label information of the samples we received stated that they were completely beef, the fact that we encountered tissues and organ parts other than muscle tissue in some meatball samples makes us think that these samples were not prepared in accordance with the 'Meat and Meat Products Communiqué'.<sup>4</sup> The cartilage, tendon pieces, visceral pieces, large connective tissue pieces, and nerve fiber bundles we encountered reinforce this idea. In addition, the morphological structure and staining of the muscle tissue seen in sample No: 5, suggest that the meat quality is low or that the product has completed its shelf life. It is possible to see spices or additives (soy, etc.) in such samples (meatballs, sausage, salami, etc.).<sup>4</sup> However, in some samples, especially in sample No: 6, images suggesting that it was used in excessive amounts were encountered. It is not possible to reveal a numerical ratio histologically. However, in terms of being the subject of future research, it would be appropriate to evaluate chemical tests

and samples from this point of view as well.

Similar situations are encountered not only in meatball products but also in meat products such as salami and sausages. Epithelial tissue, seromucous gland epithelial cells, cartilage, and bone tissues were found in salami produced locally and bought from markets in Kars city.<sup>12</sup> In another study conducted in Konya city, tissue and visceral parts that should not normally be found were found in 18 of 48 salami samples.<sup>13</sup> Yıldız et al.<sup>14</sup> reported that they found connective tissue pieces, inedible tissue, and offal pieces in meatball samples in another study they conducted in Istanbul city. Such samples can be increased especially for products such as salami and sausage. In our research, foreign tissues or visceral parts were found in all 6 samples, although at varying rates. Therefore, it is, unfortunately, possible to frequently see textures that should not be included in meat products, regardless of the type of product.

Another important issue in the evaluation of meat and meat products is which animal type muscle tissue is in the product. Although it is not possible to detect this with routine histological staining, it is possible to detect it with special immunologic staining, ELISA, or PCR method. For this purpose, species determination was made by the RT-PCR method in our study. It is a positive result that no tissue belonging to different species was found in any of the 6 samples we received.

Raw meatballs are among the preferred meat products due to their practicality. Although it is stated in the Meat and Meat Products Communique, cartilage, tendon, visceral parts, and similar tissues that are not suitable for human consumption and do not have nutritional value were found in the samples we examined histologically. These results, in addition to being inappropriate in terms of public health and consumer rights, also appear as attempts to gain unfair financial gain.

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