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Original Contribution

DETERMINING THE MORPHOLOGICAL CHARACTERISTICS OF FRANKFURTERS

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ABSTRACT

PURPOSE: Processed meat products in the form of various sausages are very popular worldwide. One of the most widely consumed processed meat products are frankfurters. The aim of the study was to apply a histological method to differentiate various components in the composition of frankfurters by describing their morphological characteristics.

METHODS: In this study, twenty-eight frankfurter samples from different brands were purchased from the retail network.

RESULTS: The histological evaluation distinguished clearly muscle fibers. Some of the frankfurter samples had larger oval voids than others. The voids appeared empty of content and that gave them a porous or spongy appearance. Monovacuole white adipose tissue was observed in some of the frankfurter samples studied. The histological analysis also found the presence of tendons and cartilage. Fragments of skin, nerve and cells of plant origin were also identified.

CONCLUSIONS: The histological method is a technique of practical importance for routine evaluation of the morphological composition of frankfurters.

Key words: frankfurters, histological analysis, morphological characteristics

INTRODUCTION

Processed meat products in the form of sausages are very popular worldwide (1, 2). Some of the most widely consumed processed meat products are frankfurters (3). Germany is considered to be their home country and it is the leading country in their production (4). Today, frankfurters can be purchased in the retail network, differing in shape, texture and taste. The great variety is a consequence of the diverse ingredients included in the production process (5). According to technological characteristics, frankfurters are boiled-smoked emulsion sausages (4) and are preferred by consumers due to their nutritional value, price and ease of handling (3). According to Liu and Lanier (6), their preparation begins with cutting the meat and adding spices, fat, salt and ice water, and mixing the components results in a fine homogenate (7). The dispersed phase, which is characterized by fat globules, is suspended in a continuous phase, which corresponds to a protein-water solution. In this way, a result similar to an emulsion is achieved. Technically speaking, since none of the phases is liquid, sausages are not true emulsions (8). According to Ruiz-Capillas et al. (9), there are possible differences in taste and aroma in various countries. Meat is the main raw material for their production. It can be chicken, turkey, pork, beef or a combination thereof (9). Recently, a change has been observed in the technological process of frankfurters. The shortage of animal protein and the changing dietary preferences of consumers have forced manufacturers to seek alternatives to meat. This has led to the inclusion of other types of meat, such as fish, ostrich, buffalo, goose and duck (10). Fat plays an important role in the stability of the emulsion, the smell, texture, juiciness and, last

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but not least, the shelf life of these products. Fat is a source of fatty acids and fat-soluble vitamins (3). It influences the stability of the sausage filling mass. Its participation positively affects the binding abilities of protein molecules (8). Processed meat products have a texture that is directly dependent on the resulting matrix. It is a combination of water content, solutes and protein gel (7). In this process, the formation of a protein network is key to the structural integrity of the matrix (8). When the components and subsequent heating are mixed, the free water available in the system binds to the protein network. Balancing the quantity and quality of protein with the nutritional value and processing functionality in the production of emulsified meat products is of great importance (7). By applying the histological technique and performing a specific staining, it is possible to highlight the structural components of plant and animal origin incorporated into meat products (11). The literature review shows that histological techniques have great potential in determining the composition of sausages (12). In this regard, we set ourselves the goal of applying a histological method to detect different components in the composition of frankfurters by describing their morphological characteristics.

MATERIAL AND METHODS

Twenty-eight samples of sausages were purchased from the commercial network and transported to a histology laboratory in a refrigerated bag. Samples from all sausages were fixed in 10% buffered formalin for 72 hours. After the fixation period, the samples were washed in running water, dehydrated through an ascending alcohol series and embedded in paraffin under a standard protocol. A total of 3 equal parts (1x1x0.5 cm) were obtained from each sample. A total of 84 paraffin-embedded blocks were processed, from which 252 sections were obtained, stained and observed with a light microscope. Sections with a thickness of 6 μm were obtained using a rotary microtome. The sections were stained under a standard protocol for staining using the hematoxylin-eosin method. Observation was performed using a light microscope Cambridge Instruments Galen III Microscope. Photo documentation was performed using a Levenhuk M500 BASE camera and LevenhukLite software.

RESULTS

Histological analysis presented intact muscle fragments of striated muscle tissue positioned amidst a non-homogeneous and amorphous eosinophilic stained mass. Cell morphology was moderately preserved. In addition to skeletal muscle fibers, their basophilic stained nuclei and their sarcolemma were also recognized **(Figure 1)**. Some of the frankfurter samples had larger oval voids than others. These voids appeared empty of content and gave them a porous or spongy appearance. In some frankfurter samples, the empty oval spaces were abundant, while in others they were observed in fewer places. The voids were found amidst the protein matrix. The different size of voids formed was clearly distinguished and visualized by a simple light microscope **(Figure 1).** The protein matrix was observed in all analyzed samples. In some it was determined to be coarser and non-homogeneous **(Figure 1).** In some of the studied frankfurter samples, monovacuole white adipose tissue was observed. Adipocytes were observed with preserved integrity in certain areas and did not cover the entire field of view **(Figure 2B).** In other samples, fragments of cartilage (hyaline cartilage tissue) were visualized. Single chondrocytes and those located in an isogenic group were recognized. They were all found scattered among amorphous intercellular substance **(Figure 2C).** The histological analysis also revealed the presence of formed fibrous tissue in the form of a tendon. The tendon was identified by rod-shaped basophilic nuclei, arranged in loci in typical parallel rows forming the boundaries of a primary tendon bundle, clearly demarcated in the longitudinal projection. In the transverse projection of the tendon, the boundaries of secondary tendon bundles were visible, surrounded by a thin layer of *peritenonium internum*. Tertiary tendon bundles were also clearly demarcated **(Figure 1A; 1C; 4C; 4D)**. In addition to preserved whole muscle fragments in the form of limited formations of skeletal muscle fibers, in the frankfurter samples, parts of skin with preserved epidermis and dermis were also visualized by a histological analysis **(Figure 2A).** It showed the presence of a fragment of spleen, which was recognized by the presence of a preserved Malpighian body with an eccentrically located arterial blood vessel and lymphocytes, part of the white splenic pulp **(Figure 2D).** The histological analysis showed the presence of a nerve with a connective tissue sheath, axons in the nerve fibers with a myelin sheath **(Figure 4 A; B).** Cells of plant origin were also recognized **(Figure 3).**

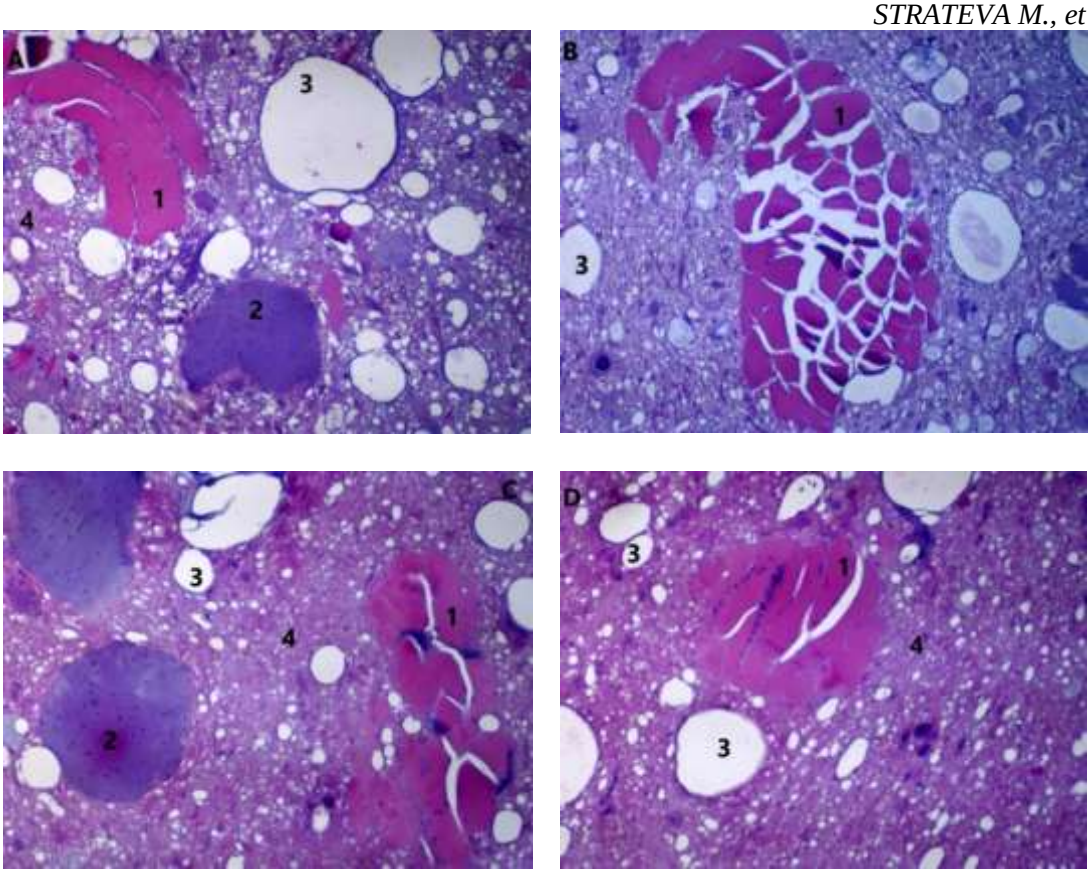

Figure 1 (A; B; C; D): Histological structure of frankfurter (scale bar: 100 μm; 10x; H-E staining).
Key: 1: preserved skeletal muscle fibers; 2: shaped fibrous connective tissue (tendon); 3: vacuoles of various sizes; 4: eosinophilic mass of protein nature

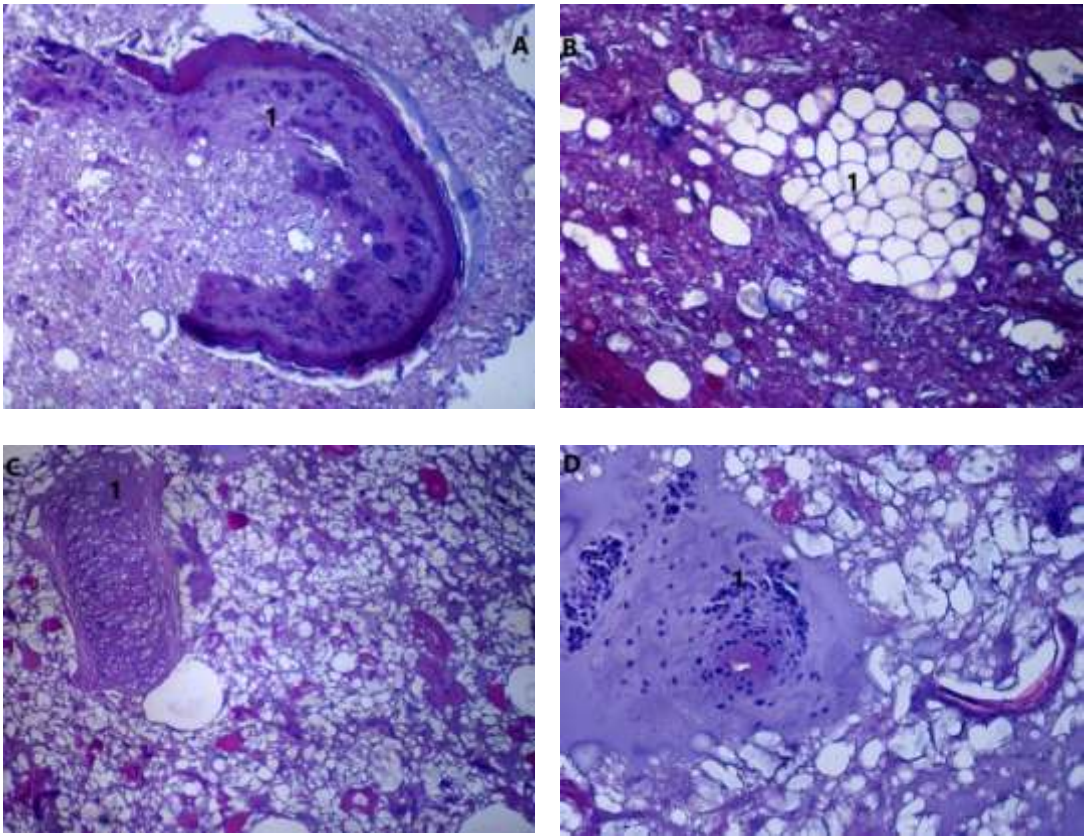

Figure 2 (A; B; C; D). Histological structure of frankfurter (scale bar: 100 μm; 10x; H-E staining).
Key: A 2: 1- skin fragment; B 2: 1- adipose tissue; C 2: 1- cartilage fragment; D 2: 1- part of spleen, preserved Malpighian body.

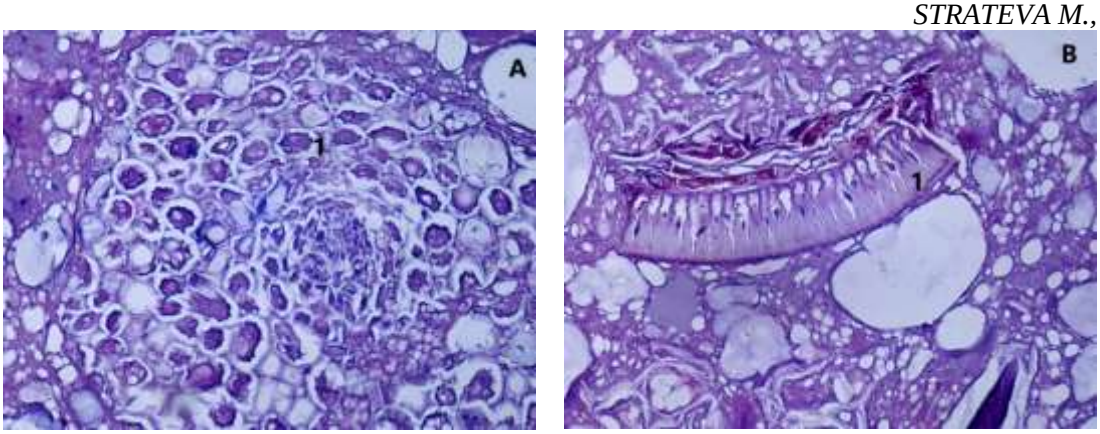

Figure 3 (A; B). Histological structure of frankfurter (scale bar: 100 μm; 10x; H-E staining)
Key: 1: cells of plant origin

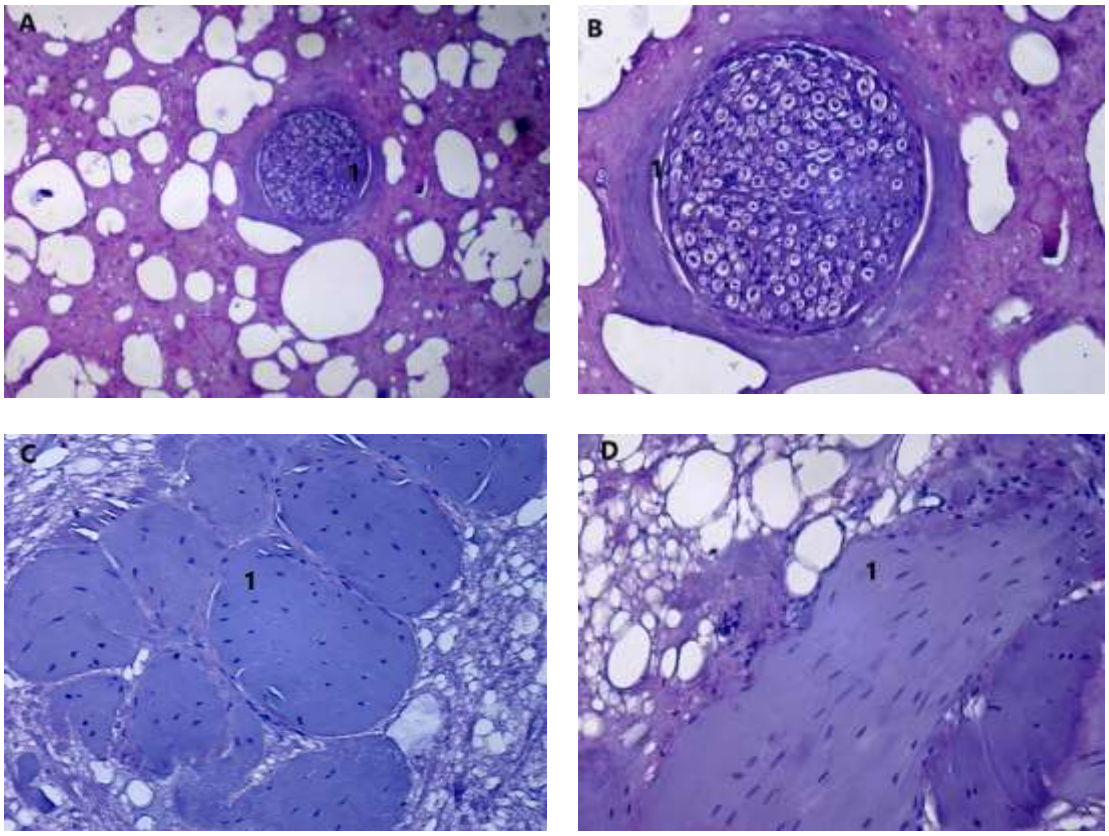

Figure 4 (A; B; C; D). Histological structure of frankfurter (H-E staining)
Key: A 4: 1- nerve (scale bar: 100 μm; 10x); B 4: 1- nerve (scale bar: 50 μm; 20x); C 4: shaped fibrous connective tissue (tendon) (scale bar: 50 μm; 20x, transverse projection); D 4: 1- shaped fibrous connective tissue (tendon) (scale bar: 50 μm; 20x, longitudinal projection)

DISCUSSION

The study aimed to investigate the histological features of sausages by indicating some morphological characteristics. Bal-Prylypko et al. (2) presented a microstructural analysis of sausages after staining samples using the hematoxylin-eosin method. They found a non-homogeneous mass with a pinkish-red color corresponding to the minced meat in sausages. We agree with Bal-Prylypko et al. (2) for this statement, since we also believe that the eosinophilic staining structure is obtained from the processing and mincing of meat (skeletal muscle fibers), necessary for the production of frankfurters. According to Morin et al. (8), individual muscle fibers and the surrounding connective tissue in the form of endomysium and perimysium can be found in the matrix. We confirm the finding of Morin et al. (8), since preserved muscle fibers and connective tissue were recorded in the studied areas. On the other hand, in processed meat sausages, according to

Doroudian et al. (13), the distinctive morphological characteristics of the muscles have been removed during sausage processing and meat mincing. In addition to the non-homogeneous mass, they found the presence of numerous vacuoles of various shapes and sizes. In the present review, similar heterogeneous cavities without any content were found. Regarding their presence, we are again in agreement with Bal-Prylypko et al. (2) and the finding that a common non-homogeneous mass with vacuoles is formed. Morin et al. (8) reported that the matrix obtained in the production of sausages is able to retain or not more water in its structure. On this basis, the matrix structure is considered to be either coarse with large pores or a fine, uniform matrix with numerous small spaces. A larger amount of water would be retained in the dense matrix with smaller pores, probably due to better absorption capacity. Delgado-Pando et al. (14) affirmed that the frankfurter matrix shows a spongy structure and numerous cavities. The expansion of a number of ingredients, such as air, water and fat is the reason for the formation of these cavities. This finding was also commented in a previous study by Jimenez-Colmenero et al. (15). Mousavi et al. (5) established that the shape of the air spaces varies. Some produce more small air spaces, while others produce large air spaces, in addition to smaller ones. These air spaces influence the texture of the product. According to Mousavi et al. (5), the reduced concentration of protein engaged in the formation of the emulsion matrix is the reason for achieving a softer and less dense matrix structure after the formation of small and large air spaces. The softer texture is obtained after a decrease in protein density, fat content and increased water content. This in turn is the reason for the reduced protein concentration.

In another study with frankfurters, Kowalczewski et al. (16) found the presence of fat globules as part of the histological structure. In the present histological examination, adipose tissue was visualized preserved in some of the frankfurter samples. The cells of the monovacuole white adipose tissue were recognized by their spherical shape (polygonal). It is defined as such due to the dense arrangement of the adipocytes relative to each other and the compressive deformation they exert. The fat cells appeared as empty cells due to the way the samples were processed for histological analysis with ethyl alcohol, which is an organic solvent and has the ability to extract fat from the cells. Kowalczewski et al. (16) proved that the fat particles are evenly distributed in the observed areas. The presence of surface-active compounds (e.g. hydrocolloids) has an influence on the size of the fat droplets. Jimenez-Colmenero et al. (15) provided an evidence that the fat added during the frankfurter preparation process plays a significant role in the texture of the final product. The texture is different when using animal fat and that of vegetable origin. Lee and Kim (17) believe that connective tissue, fat particles, muscle cells, myofibrillar proteins can be found as components of the sausage matrix. According to Morin et al. (8), during the sausage manufacturing process, in the stage of cutting and mixing the various components, the fat particles are covered with myofibrillar proteins. In addition, the fat particles remain embedded in the matrix intact, forming the dispersed phase. Polysaccharides, water and proteins enter in the continuous phase. Morin et al. (8) also comment on the role of fats in the matrix. It is related to the process of binding of lipophilic particles of proteins, which have the capacity to bind to fats. On the other hand, hydrophilic protein parts remain bound to the water part. Cetin et al. (18) stated that other components, such as cartilage may be found in processed products. The histological analysis of some of the samples in the present review showed the presence of cartilage fragments. Doroudian et al. (13) believe that skin and soy tissue can also be found in meat product samples. In this review, we also found the presence of similar components such as part of skin and cells of plant origin. According to Latorre et al. (19) and Isaconi and Militaru (12), the addition of plant-based additives may cause some allergic reactions. In previous studies by Moghtaderi et al. (20), Isaconi et al. (11) and Sami et al. (21) the presence of lung, salivary glands (meat used from the head area), bone, blood vessels, and smooth muscle have been established by the histological method. The results in this review did not identify such components in the studied sections. Fragments of cartilage, skin, nerve, spleen were found in single fields of view in a limited number of studied sections. In our opinion, these fragments have been accidentally found in the total mass of ingredients used in the production process of meat products. In this regard, we would like to recommend increased monitoring

in the production process in order to improve and avoid similar results in the future.

CONCLUSION

The study aimed to identify various tissue components in the composition of frankfurters by the histological method. The obtained histological results make it possible to expand and enrich the databases with this type of information. It should be noted that the histological study alone is not a sufficient analysis to specify the composition of this type of sausages. Morphological analysis is not able to determine the nutritional value of these products.

Conflict of Interest

The authors declare no conflict of interest.

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