

## Microstructural analysis of sausage quality

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Organic sausage products are those made from poultry meat of farm animals that are grown without the use of food additives and growth hormones. In today's market, increasing the number of producers of meat products and the withdrawal of mandatory certification creates an important problem associated with the identification of a specific type and name of the product, as well as its compliance with the stated regulatory documentation. The article provides information on the application of the microstructural method for meat products quality control. The structural composition of sausage products was investigated: top-grade uncooked smoked sausage "Moskovska", DSTU 4427: 2005; Class 1 half-smoked sausage "Hutsulska" TU U 15.1-33480284-015: 2007; Class 1 half-smoked sausage "Salami with beef" TU U 15.1-32566564-002: 2007. It has been determined that the sausage products include meat raw materials, including after mechanical boning, as evidenced by the inclusion of cartilage and connective tissue. The inclusion of starch grains, soy protein, or concentrate, gel-forming agents has been detected. It has been proved that histological methods of research allow evaluating the structure of the product as a whole, to differentiate the features of different tissue elements and cellular structures, to identify the replacement of high-quality raw materials with low-value dietary supplements, vegetable proteins, etc. The urgent task is to develop modern science-based methodological guidelines for determining the most objective and reliable indicators for the identification of goods of homogeneous groups.

**Keywords:** Histological examination; Sausage Products; Quality; Safety of meat products

### Introduction

In recent years, food safety has become particularly important in the context of man-made pollution. Of the total number of environmentally hazardous substances released from the environment into the human body, 30-95% comes from food. Although the content of these substances is regulated by regulatory and technical documentation, the issue of food safety and quality is one of the urgent issues. Providing the country's population with quality food has extremely social and epidemiological importance. It is the issue of the quality of life of citizens and their health. That's why the interest in clean and safe food is growing. This interest is present in the practical sphere and is analyzed by some scholars (Bondarenko & Guzenko, 2016; Hadzevych et al., 2019; Chalaya et al., 2019).

The trend for healthy lifestyles and, accordingly, healthy eating is combined in today's society with time savings and the purchase of ready meals and semi-finished products. In Ukraine, for example, traditionally sausage products are purchased. They occupy a large proportion in human nutrition. Buying a certain meat product, the consumer, first of all, evaluates its commodity qualities - appearance and freshness. However, in appearance, the buyer cannot judge the most important characteristic of sausage products - ecological and food safety, which characterizes the presence of toxic substances in the product, nitrosamines, pesticides, aflatoxin B1, hormonal drugs, radionuclides and the like. The control of the safety and quality of meat and meat products remains an urgent issue today. The safety of livestock products and raw materials is determined by the ability to effectively control the production and importation of food from abroad (Paliy et al., 2018). Despite the increased interest in the issue of food quality and safety, the situation in this area is becoming more and more complex and threatening each year. This is due to the intensive development of all areas of food chemistry and the desire of manufacturers to reduce the cost of finished products. Due to the entry into the consumer market of a large variety of meat and meat products, careful and rapid control of their quality and compliance with the current National Standards are required. The lack of effective control of the raw materials used leads to the fact that producers while maintaining acceptable levels of regulated raw materials put raw materials of lower grade into meat products or replace them with

offal and excessive quantities of plant components. Such products do not correspond to their name in composition, they are of poor quality and, in fact, are falsification. Today, the European market imposes strict requirements on the safety and quality of products before manufacturers in other countries. Europe's largest firms are consolidating efforts to select progressive forms and methods of product quality management. Such programs include stable technology, a proper system for maintaining the technological accuracy of equipment and facilities, metrological controls and product testing, an effective training system (Abdolghafour & Saghir, 2014). Today, Ukraine produces a large number of sausage products which, if they breach production technology, sanitary conditions at the enterprise, storage, transportation, and sale modes, may have risks to the health of consumers. Especially topical issues of veterinary-sanitary control of sausage products, if they are made at meat processing plants and entities that do not have a self-control system following the HACCP requirements (Dashkovskyy & Salata, 2016). The production of meat products is at the highest risk of biological hazards (Paliy et al., 2017; Ibrahim & Abu Salem, 2009). In this regard, veterinary and sanitary measures are of particular importance, which must be carried out following established technological steps and strictly controlled by the main indicators of their effectiveness (Paliy et al., 2020). It is established that a person without harm to health can do without full proteins, including meat, only 4 months a year. Besides, meat products include B and PP vitamins, potassium, phosphorus, and other essential minerals and biologically active substances (Flores, 2016). However, in current conditions, it is of concern to use soybean and corn in sausage production. Some meat market operators do not include GMO content information in their products. Therefore, there is now a need to control the production of sausage products and their clear labeling (Serdioucov & Kostenko, 2010; Gale, 2015). The falsification of sausage products is primarily through the partial replacement of high-grade meat with low-grade, by-products, pork skins, protein-collagen, and protein-fat emulsions, soybeans and even organs and tissues of animals that are not generally used in food industry. Also some manufacturers use low-value raw materials not provided for the recipe (larynx, trachea, esophagus, stomach, testes, uterus, etc.) (Pasechny & Zakhndrevich, 2008). The use in the manufacture of sausage meat, which is obtained when the bone is rolled, is common. It contains a significant number of microscopic bones that damage the mucous membrane of the gastrointestinal tract when consuming the product. The amount of meat that is added to the sausages is 40-76%, depending on the type and variety of sausages. However, there is often no appropriate marking on products (Kasyanchuk, 2000). Dry, smoked, half-smoked, and fried sausages, boiled sausages, meatloaves should be made following the National Standards of Ukraine (DSTU). Such a provision is provided after the Law No. 1782-V1 "On Amendments to Certain Laws of Ukraine on Supporting the Agro-Industrial Complex in the Conditions of the Global Financial Crisis" came into force on January 15, 2010. The requirements of DSTU - the raw material used in the production of these sausages, 85-100% (depending on the class) should consist of meat. As for boiled sausages, meatloaves, the rate of meat in them should be: for the highest grade - 100%, the first - 70%, the second - 60%. It is forbidden to assign well-known names of traditional assortment to new sausage products (Vinnikova, 2006). The taste and smell of the sausage products are closely linked to the quality of the main and auxiliary raw materials since the process of grinding and processing of meat slightly changes the direction of autolysis, which helps to reduce lactic acid in minced meat. Obviously, during these operations, lactic acid is partially involved in the aerobic process of converting carbohydrates, which contributes to its utilization. Eventually, lactic acid can produce aromatic substances that affect the taste and aroma of the finished product (Stangierski et al., 2019; Chattopadhyay et al., 2019). The assortment of meat products of both domestic and foreign manufacture on the consumer market of Ukraine is increasing every year. The official assessment of the quality of meat products is aimed primarily at determining its safety and almost does not involve determining the composition of the raw materials used and determining compliance with the regulations. A microstructural analysis is the only method that makes it possible to identify the composition of the finished meat products, that is why it requires continuous improvement and implementation (Mohamed et al., 2015; Lieshchova & Levchenko, 2015). The purpose of the study was to examine sausages for compliance with the specified composition by histological method of microstructural analysis in accordance with DSTU 4436:2005 "Sausages cooked, sausages, meatloaves. General Specifications" and DSTU 4427:2005 "Dry and smoked sausages. General Specifications".

## **Material and Methods**

The research was performed in the Laboratory of Veterinary Sanitation and Parasitology of the National Scientific Center "Institute of Experimental and Clinical Veterinary Medicine". Monitoring of off-the-shell products (sausages) were carried out to determine the quality of their raw meat. During our research the various types and classes of beef sausage products (half smoked, smoked) were investigated.

The following products were used in the experiments:

- Top-grade uncooked smoked sausage "Moskovska", DSTU 4427: 2005;
- First class half-smoked sausage "Hutsulska" TU U 15.1-33480284-015: 2007;
- First class half-smoked sausage "Salami with The purpose of the study was to examine sausages for compliance with the

specified composition by histological method of microstructural analysis in accordance with DSTU 4436: 2005 "Sausages cooked, sausages, meatloaves. General Specifications" and DSTU 4427: 2005 "Dry and smoked sausages. General Specifications". TU U 15.1-32566564-002: 2007.

Testing of sausages for freshness was started with a review of the shell of the products, established its appearance: integrity, contamination, dryness or presence of mucus, mold. Then the shell was removed, paying attention to its strength, adherence to the stuffing. Assessing the appearance of products, we noted the possible deformation of loaves, their contamination with fat, dirt, the presence of voids, swelling of fat or broth under the shell, the infusion of stuffing over the shell, its wrinkling. The smell and taste of the products were evaluated depending on their appearance at a temperature of 15-20 °C, in the heated form to enhance the smell. On the cut of the loaf, attention was paid to the color of the minced meat and the bacon, the consistency of the minced meat, the presence of gray spots, and exterior inclusions.

For the purpose of microstructural analysis, samples of sausage products with a size of 30 × 30 × 30 mm were taken from three sections of the product. For the manufacture of histological specimens used samples of size 15 × 15 × 4 mm, which were fixed in 10% neutral formalin solution for 48 hours. Paraffin filling was performed according to standard procedures, sections were made with a thickness of 5-7 μm, stained with hematoxylin and eosin.

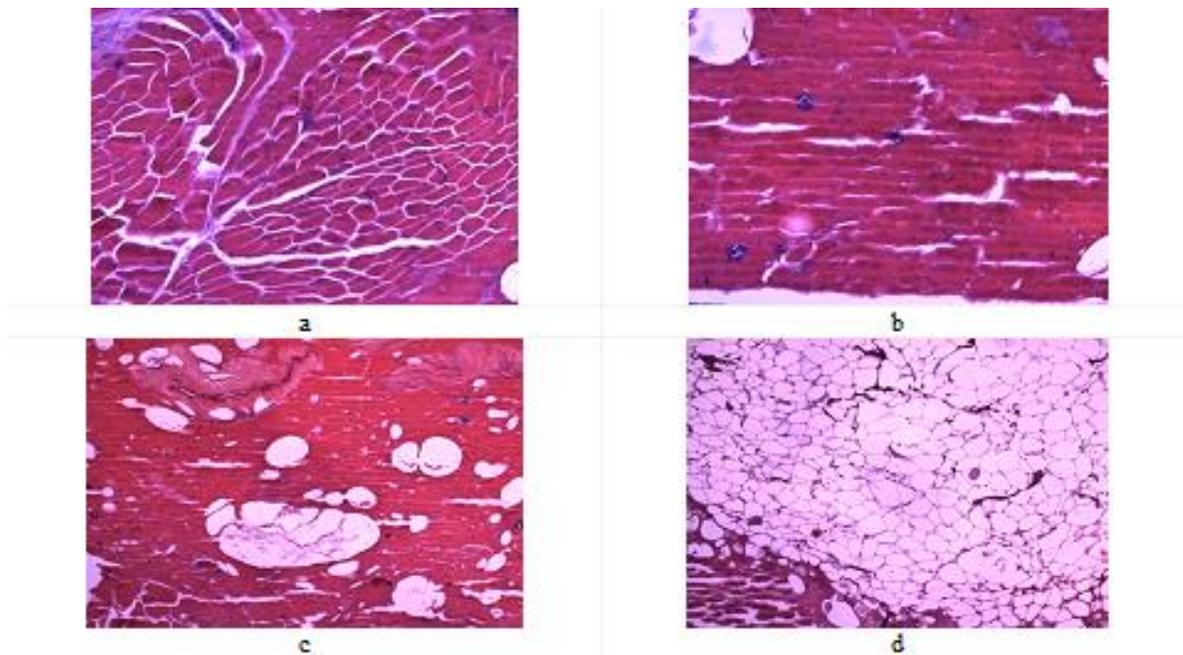
The research was guided by DSTU 7063:2009 "Semi-finished Meat and Meat-Vegetable Chopped Products. Determination of constituents by microstructural method". For the microbiological examination, two 15 cm samples were taken from each sausage product with the closed edge, from which the combined sample was prepared (according to GOST 9792-73). The experiments were carried out following the applicable methodological regulations (Kotsyumbas et al., 2011; Shutchenko et al., 2013).

## Results and Discussion

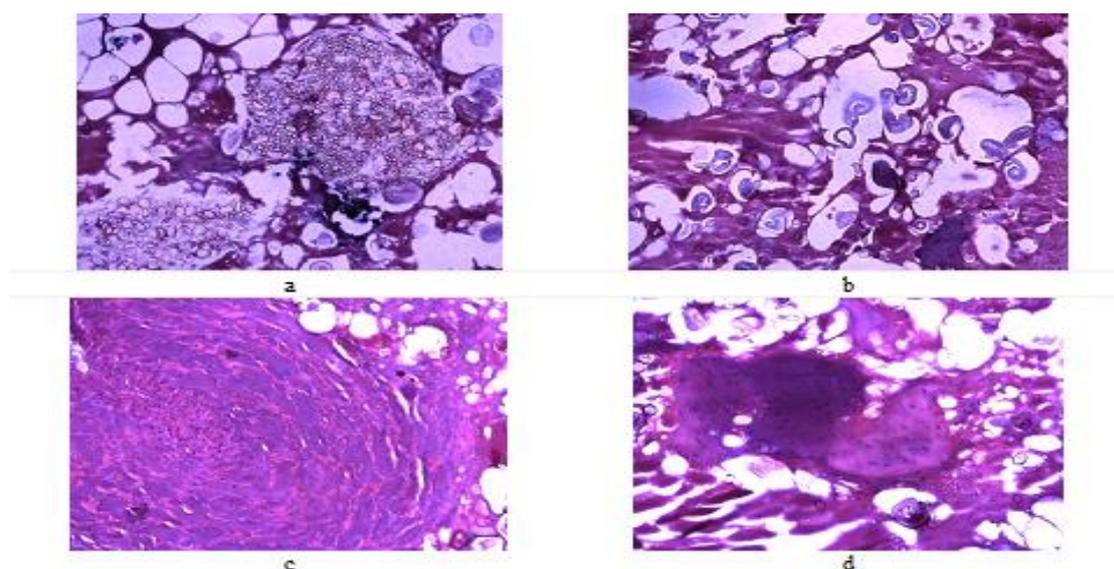
As a result of microstructural analysis of four samples of top-grade smoked sausage "Moskovska" (DSTU 4427: 2005), we verified that its composition includes raw beef (Figure 1a), and the actual composition corresponds to the trade data sheet: beef skimmed, sausage fat, ground black pepper, and nutmeg.

The muscle fibers and nuclei localized at the periphery below the sarcolemma are clearly expressed, moreover, among the finely grounded protein mass, the inclusions of spices are found which, when stained by hematoxylin-eosin, have a dark blue color and a granular structure (Figure 1b). Among the muscle fibers there were the inclusion of adipose tissue and fat droplets (Figure 1c). Thus, it was established that the tested sausage includes high-quality fresh raw meat, fat, spices. The four samples of the first class half-smoked sausage "Hutsulska" made according to TU U 15.1-33480284-015:2007 were tested. Ingredients: beef, vertebrate bacon, water, potato starch, dry multi-component moisture-retaining mixture "Pioneer Combi 4111" soy protein and other flavoring mixtures.

Microscopy of histological preparations of sausage "Hutsulska" revealed that a large area is occupied by adipose tissue (Figure 1d).



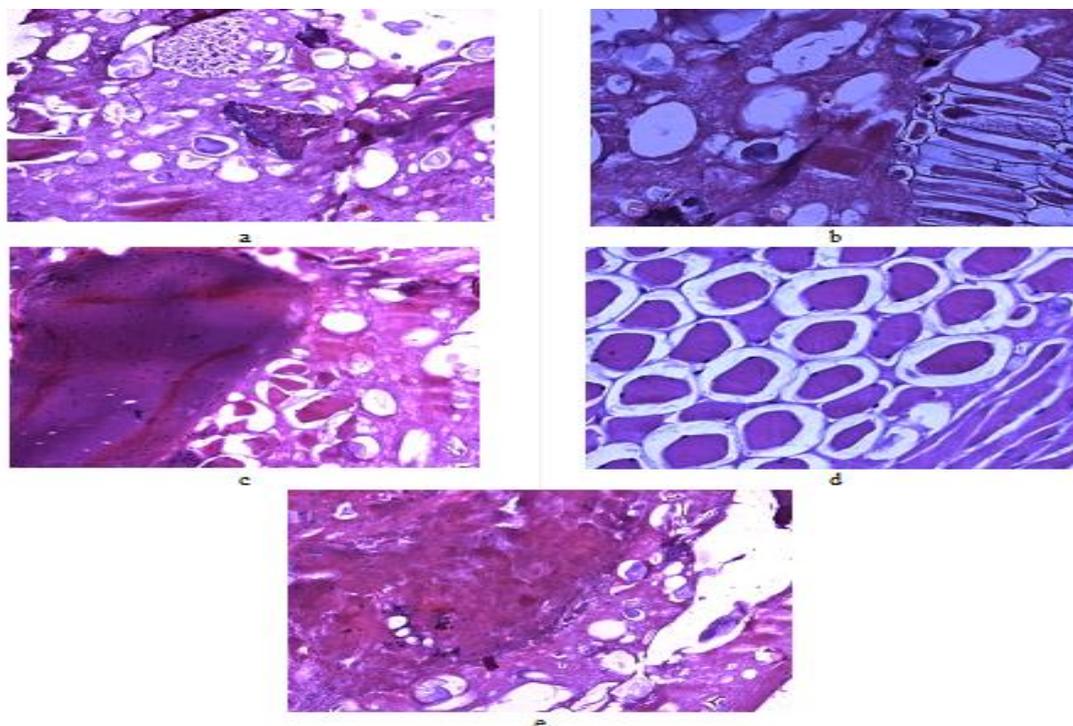
**Figure 1. Histological sections of some sausages. a)** histological section of a top grade smoked sausage "Moskovska", cross-striated muscle fibers (H + E,  $\times 100$ ); **b)** histological section of top-grade smoked sausage "Moskovska", the inclusion of spices (H + E,  $\times 100$ ); **c)** histological section of the high grade smoked sausage "Moskovska", inclusion of adipose tissue (H + E,  $\times 50$ ); **d)** histological section of a first half-smoked sausage "Hutsulska", inclusion of adipose tissue.(H + E,  $\times 50$ ).



**Figure 2. Histological sections of some semi-smoked sausages. a)** histological section of first class semi-smoked sausage "Hutsulska", starch-containing additives (H + E,  $\times 100$ ); **b)** histological section of first class half-smoked sausage "Hutsulska", inclusions of a gel-forming agent; **c)** histological section of a class 1 half-smoked sausage "Hutsulska", muscle fibers after freezing; **d)** histologic section of a class 1 half-smoked sausage "Hutsulska", cartilage tissue.

The slice plane shows a significant number of starch grains, as well as the inclusion of a gel-forming agent. Paying attention to the microstructure of the muscle fibers, it was found that the raw material contained both chilled and after freezing meat. The transverse striation of the muscle fibers is not expressed, no nuclei are found in the individual fibers, indicating the use of fresh raw meat and raw materials after freezing. On histological preparation, starch-containing additives (Figure 2a) are in the form of round particles 5-20  $\mu\text{m}$  in size, which are combined into large aggregates. Hematoxylin-eosin do not stain. Inclusions of carrageenan usually have an irregularly rounded shape (Figure 2b). They can be more or less uniformly colored in lilac color. The particle size of the carrageenan ranges from 60 to 140  $\mu\text{m}$ .

The manufacture of the sausage the meat was used as raw material after freezing, as evidenced by the specific coloration of the muscle fibers in blue, their homogeneity, loss of transverse striation (Figure 2c). We also observed the inclusion of cartilage tissue, which has a corresponding structure on the histological preparation: fibroblasts and chondrocytes, which form isogenic groups, nuclei located eccentrically, elongated (Figure 2d).



**Figure 3.** Histological sections of first class half-smoked sausage. **a)** histological section of a class 1 half-smoked sausage "Salami with beef", soy concentrate (H + E,  $\times 100$ ); **b)** histological section of a class 1 half-smoked sausage "Salami with beef", soy concentrate (H + E,  $\times 200$ ); **c)** histologic section of class 1 half-smoked sausage "Salami with beef", cartilage tissue (H + E,  $\times 100$ ); **d)** histologic section of class 1 half-smoked sausage "Salami with beef", cross section of muscles.(H + E,  $\times 200$ ); **e)** histologic section of class 1 half-smoked sausage "Salami with beef", smooth muscle (H + E,  $\times 100$ ).

Microstructural analysis of the "Hutsulska" sausage has revealed that it includes meat raw materials, including after mechanical boning, as evidenced by the inclusion of cartilage and connective tissue. The sample also contains a significant amount of adipose tissue and fat droplets, and the inclusion of starch grains and a gel-forming agent is detected. These ingredients are indicated on the label of the meat product.

Microstructural analysis of four samples of first class half-smoked sausage "Salami with beef" was carried out. It has been determined that the half-smoked sausage "Salami with beef" included high quality raw meat. Muscle fibers had clear contours, nuclei and transverse striation. However, in some sections of the slice it was possible to distinguish the soybean concentrate particles. There was also a small amount of a gel-forming agent.

In Figure 3a protein supplement can be seen, namely, particles of soy concentrate. They consist of cylindrical cells in a longitudinal section and rounded on a cross, surrounded by a cellulose sheath. They are colored in shades of red (from dark pink to bright red), surrounded by a narrow, even unpainted lumen - a cellulose sheath. In Figure 3b the inclusion of soy concentrate (longitudinal section) consisting of cylindrical cells is shown. Figure 3c shows the inclusion of cartilage tissue, which has a characteristic structure: chondrocytes and fibroblasts with elongated nuclei. We found that the half-smoked sausage "Salami with beef" included high-quality meat raw material, as evidenced by the clear structure of muscle fibers: the transverse striation is pronounced, the borders of the fibers are clear, the nuclei are rounded, well contouring (Figure 3d). We observed the sausage includes smooth muscle tissue that occupies large areas (Figure 3e).

Therefore, as a result of microstructural analysis of the samples of half-smoked sausage "Salami with beef", it was found that, in addition to meat and fat, it contained vegetable protein components, namely soy concentrate, as well as a gel-forming agent. It should be noted that their presence is indicated on the sausage product label. Thus, histological examination methods make it possible to evaluate the structure of the product as a whole, to identify the replacement of high quality raw materials with low-value additives, vegetable proteins and the like. In addition, it is possible to differentiate features of different tissue elements and cellular structures. It should be noted that working with raw materials in food has its own peculiarities, since, in this case, materials after mechanical, thermal and other types of technological processing are subject to research.

Issues of the veterinary and sanitary control of the livestock products are especially urgent in the current conditions when market relations are underway a period of formation, restructuring of the agro-industrial complex to increase the production of meat products. So far, meat products are manufactured at a large number of meat processing plants of different capacities and by individual entrepreneurs, which has led to a decrease in their sanitary quality and safety for the consumer. Besides, people with no

training are often involved in the production of meat products. Therefore, many products of poor sanitary quality, adulterated, etc., which can be a source of food toxicity, toxicosis, and other diseases in humans, enter the trading network (Ince & Özfiliz, 2018).

It is impossible to control the safety and quality of food products, including meat products (sausages, boiled sausages, smoked sausages, etc.) without observing the sanitary requirements and technological recipes provided for certain types of food. In Ukraine, to date, control is carried out using traditional methods of research: biochemical, physicochemical, and organoleptic, which do not always allow to identify the qualitative composition of the product. Against this background, the question of developing methods of identifying constituents or components in meat products and raw materials is becoming increasingly relevant to obtain a more complete assessment of their quality (Kuznetsova et al., 2018).

The main and integral part of obtaining high quality and safe livestock products is compliance with high standards of veterinary and sanitary measures at meat processing enterprises (Rodionova, 2017).

Nevertheless, the addition of mayonnaise and sauces with reduced fat and inulin is promising from a technological point of view. Dietary fibers that are part of inulin have the ability to organoleptically imitate the fat component in the formulations and could positively effect the texture of the product. They have a soft neutral taste and are easily combined with the recipe components, allowing to develop the formulations of new fat products without special changes in taste (Amaral et al., 2015; Silva et al., 2019). Other studies have also noted the effectiveness of microstructural analysis to detect adulteration of meat and meat and vegetable products. It has been found that the meat semi-finished products purchased from the Kyiv, Chernihiv, and Sumy trade networks in all samples of the tested products contain impurities not specified in the recipe (Senchenko, 2013).

Other researchers have carried out a veterinary-sanitary evaluation of five samples of minced meat products of different manufacturers for compliance with quality requirements. The use of raw meat of dubious freshness and undeclared carrageenan and soybeans in the production of minced meat has been detected (Tishkina & Dubska, 2015). Some non-compliance of sausages and canned meat with specified quality indicators have been identified (Tishkina et al., 2018).

## Conclusion

From data on microstructural analysis of sausage "Moskovska", we registered that its composition includes high quality fresh raw meat, fat, and spices, which corresponds to the composition indicated on the product label. No other constituents (vegetable or animal) or gel-forming agents have been identified. Microstructural analysis of the sausage "Hutsulska" has revealed that it includes meat raw materials, including after mechanical boning, as evidenced by the inclusion of cartilage and connective tissue. The sample also contained a significant amount of adipose tissue and fatty drops, and we detected the inclusion of starch grains and a gel-forming agent. These ingredients are indicated on the label of the meat product. By means of microstructural analysis of semi-smoked sausage "Salami with beef", we found that, in addition to meat, it contained vegetable protein components, namely soy concentrate and a gel-forming agent, and this must be reflected in the label or trade sheet.

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