

**APPLIED HISTOLOGICAL AND CHEMICAL ANALYSIS
FOR QUALITY ASSESSMENT OF MEAT AND MEAT PRODUCTS: REVIEW**
ANALIZA HISTOLOGICĂ ȘI CHIMICĂ APLICATĂ
PENTRU EVALUAREA CALITĂȚII CĂRNII ȘI PRODUSELOR DIN CARNE: RECENZIE

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ABSTRACT | REZUMAT

Meat and meat products are an important source of protein, vitamins, and minerals in the human diet. In order to improve the overall quality of foods and increase their shelf life, meat products are usually made from processed red meat and preservatives, such as nitrates. Histologically, the meat is composed of skeletal striated muscle fibres, water, adipose, and connective tissue. Histological investigation allows the identification of tissue structures in meat products and to some extent of the unauthorized content of plant and animal origin. For this reason, the histological examination was adopted in some developed countries as a complementary method of assessing the integrity of the products. The histological examination provides concrete images regarding the morphological integrity of the tissues in the composition of the meat. Chemical examination methods are commonly used to assess the quality of meat and meat products and to determine their content in proteins, fats, fatty acids, vitamins, and minerals. This brief review will present the benefits of using histological examination and physico-chemical determinations in analysing the composition and integrity of meat and meat products.

Keywords: physico-chemical analysis, histopathologic examination

Carnea și produsele din carne sunt o sursă importantă de proteine, vitamine și minerale în dieta umană. Pentru a îmbunătăți calitatea generală a alimentelor și a le crește perioada de valabilitate, produsele din carne sunt, de obicei, fabricate din carne roșie prelucrată și conservanți, cum ar fi nitrații. Din punct de vedere histologic, carnea este compusă din fibre musculare striate scheletice, apă, țesut adipos și conjunctiv. Investigația histologică permite identificarea structurilor tisulare din produsele din carne și într-o oarecare măsură a conținutului neautorizat, de origine vegetală și animală. Din acest considerent, examenul histologic a fost adoptat în unele țări dezvoltate ca metodă complementară de apreciere a integrității produselor. Examenul histologic oferă imagini concrete referitoare la integritatea morfologică a țesuturilor din componența cărnii. Metodele chimice de examinare sunt utilizate în mod curent pentru a aprecia calitatea cărnii și a produselor din carne și pentru a determina conținutul acestora în proteine, grăsimi, acizi grași, vitamine și minerale. Această scurtă recenzie va prezenta avantajele utilizării examenului histologic și a determinărilor și fizico-chimice în analizarea compoziției și integrității cărnii și produselor din carne.

Cuvinte-cheie: analiză fizico-chimică, examinare histopatologică

Meat and meat products are an important source of protein and other nutrients in the human diet, and their consumption depends on socio-economic conditions, cultural factors, ethics or religious beliefs as well as certain national traditions. Globally, meat consumption is constantly increasing, pork being the most consumed (15.8 kg / inhabitant / year) followed by poultry meat (13.6 kg / inhabitant / year), beef (9.6 kg / inhabitant / year) and sheep and goat meat (1.9 kg / inhabitant / year) (10, 21). Meat products are usually made from processed red meat. In order to improve the overall quality of food and increase the length of time it can be

consumed, preservatives such as salt and nitrates are added to meat products and preparations (37).

At present, a series of standardized procedures are applied to assess the integrity and quality of products intended for human consumption and new methods are being sought so as to bring additional information for the examination and control of food, such as chemical analysis, spectrometry techniques, immunochemistry and molecular biology (31).

Histological examination is one of the examinations used in the past to analyse food products in order to identify their contamination or falsification of products (41). The history of food microscopy dates from 1850 (32, 39). Therefore, this method is widely used to detect unauthorized content of plant and animal origin in meat products (21, 37).

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The topic of this study was approached by several researchers on products specific to their country in order to highlight and evaluate the quality of meat products as well as the accurate conformity with the recipe. The aspects related to pathology and the transport of some pathological agents in meat products (nerve tissue for prion diseases, plant additives for allergic reactions, protozoa localized in muscles) are not to be neglected (9, 21).

Sadeghi et al. used the histological method to examine 720 sausage samples, in which unauthorized tissues such as: adipose tissue, cardiac muscle, cartilage, oesophageal fragment, salivary glands were identified (40). Also by histological examination, Izadi et al. (2015) detected viscera fragments in 20 samples of frozen minced commercial meat (18). Abbasy-Fasarani et al. (2012) examined 44 industrial burgers containing 30% and 60% meat respectively. As methods of study, they used histological and chemical examination, which allowed the identification of unauthorized tissues in meat products (3). In another study, the histological investigation was applied and compared with the results of the chemical analysis to identify bone fragments and to determine the quantity of these fragments in 29 poultry products (41).

DETECTION OF UNAUTHORIZED ANIMAL TISSUE BY MEANS OF HISTOLOGICAL TECHNIQUES

The meat itself has a high economic value and the risk of adulteration or modification of some components of the meat products appears, for mere personal gain. Consequently, cases of adulteration are known by the use of the meat of other animal species than those used in the recipe of the respective product, the use of other animal tissues than those accepted, the substitution of animal fat with vegetable fat (5). Histological examination has also been used in the past in order to detect adulterated products or to identify structures of plant origin (wheat, soy, and flour) used to influence the percentage of protein in the product (22).

The histological method has been used in various studies to estimate the percentage of meat and also of the unauthorized tissues in meat products. Several experimental studies have emphasized the accuracy and the benefits that result from the application of the histological examination.

Tremlova and Starha (2003) applied the histological investigation to determine the size and number of bone tissue fragments in 26 samples of poultry meat products, including sausages, salami and minced meat. Following the analysis of the histological images, bone tissue fragments were detected in the examined samples. The study aimed to find the most objective method to evaluate the integrity of meat products, and

the histological examination proved to be extremely useful (41).

Numerous published works have presented the detection of unauthorized tissue, using histological techniques: Latorre et al. (2015) proposed to identify any unauthorized tissues in processed meat products. They collected 20 samples from different processed products (Kebab, sausages, hamburgers), from restaurants or supermarkets. The samples were applied the 10% formalin fixation routine technique, neutral solution, paraffin inclusion, and staining of sections by hematoxylin-eosin (HE) stain. Through this technique, the authors highlighted undamaged muscle tissue, degenerated muscle tissue, and fragments of tissues not authorized by Iranian law: soy, adipose tissue, ovary, hyaline cartilage, gizzard, bone, skin, peripheral nerves, lymph node, and glandular tissue (21). The researchers consider that the histological examination is extremely useful in assessing the content of meat products (7, 21).

Jahed-Khaniki and Rokni (2007) revealed a 10% presence of the following inadmissible tissues: mammary glands, lungs, soft tissues of the head, and visceral organs of cattle in prepared sausages, using Masson's trichrome stain and HE (19). The addition of some unauthorized abattoir tissues such as salivary gland and nodal ligament was reported using histopathological examination at different Iranian factories (35). In the study "Detection of unauthorized tissue in prepared sausages", by using the histological method, the same team discovered the use of unwanted organs from slaughtered animals, including visceral organs, hyaline cartilage, skin, fat, and bones to replace meat in meat products (11, 34).

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Avinee et al. (2010) examined six samples of sausages Merguez and Chipolatas. All samples contained fibrotendinous tissue fragments and parts of bone and cartilage. Although salivary glands were present in sausages and lymphoid tissue fragments in Merguez, however, nerve tissue was not found in the evaluated samples (4). In another study that had as a method of investigation the microscopic examination, animal tissues like blood vessels, adipose tissue, glandular tissue, and nerve were found in meat tortellini (12).

Another element pursued in some studies is the analysis, with precision, of quality parameters by detecting and measuring the content of specific animal tissues (muscular, connective, and adipose tissue). In this regard, several studies have been published which also included histopathological examination, namely: content evaluation of eight hotdog brands (29) and hamburger (30) in the United States of America.

In these products, the use of HE staining showed the presence of bone tissue, collagen, blood vessels, peripheral nerves, cartilage, and epithelial tissue, as well as plant materials (20). In addition to detecting unauthorized tissues, Prayson et al. (2008) noticed that the products examined contained the amount of meat less than 10% of their weight (as opposed to those mentioned on the label) (29).

A categorical detection of lung, ruminant stomach, large blood vessels, cardiac muscle, cartilage (hyaline fibrocartilage), spongy bone, and lymphatic tissue (spleen) in meat sandwiches was done using the histological method by (2). The same team detected onions in the samples examined using histological examination (in Kofta, Hawawshi, and Shawarma). Plant material has also been observed in sandwiches in some of the studies cited above (21, 29, 30).

Histological analysis can also be used to evaluate the quality of meat products obtained from mechanically separated meat, the properties of which depend on the nature and quantity of raw material used, as well as on the adjustment of the machine. Detection of mechanically separated meat (MSM) in meat products is a major challenge for the meat industry. Most meat producers illegally replace meat, partially or totally, with mechanically separated meat in meat products in order to reduce the economic cost. Thus, emulsion sausages (preserved meat) were prepared, with mechanically separated meat 0%, 10%, 30%, 50%, 70%, and 90% as a substitute to meat per se, and then examined. Histological sections showed: the presence of skin and cartilage, typical elements of MSM, the addition of 10% MSM could not be detected after cooking, the use of CSM 30% is easy to detect; significantly changed the technological properties of the product (23). The effectiveness of the histological examination for the quantitative determination of the unauthorized animal and vegetable tissue content of the minced meat was evaluated and used by Sadeghinezhad et al. (2015) (17). Similar results have been reported for chicken skin as not allowed content in minced meat. However, the histological method is not suitable for assessing the amount of adipose tissue in minced meat, probably because the fat is dissolved in xylene or other organic solvents used in the processing of permanent products (17, 38).

IDENTIFICATION OF PARASITES THROUGH MICROSCOPIC EXAMINATION IN MEAT PRODUCTS

Sarcocystis spp. is an intracellular parasite in mammals that presents a considerable infection rate especially in sheep and cattle. Human infection with *Sarcocystis* may be related to consumption of raw meat, or meat products containing the enclosed parasite (9).

The results of the Prayson et al. (2008) study showed that more than 80% of the tested samples were infected with *Sarcocystis*. The infection rate in sausages and hamburgers was 83.33%, respectively 87.5%, the sections were stained with the Giemsa method and examined with the optical microscope.

Histopathological examination allowed the identification of parasites in fast food burgers, whose characteristics were the localization of the protozoan in the cytoplasm of muscle fibres (29, 30).

In a study by Ghisleni et al. (2010) regarding the identification and prevalence of sarcocystosis in canned beef, the prevalence was up to 25% infected samples, and the histopathological impairment of the muscle tissue variable from discrete dystrophies up to inflammatory processes (12).

Literature data on parasitism with *Sarcocystis* spp. generally refer to meat samples collected in the slaughterhouse, directly from the carcasses (26). Only a few studies refer to the prevalence of the parasite in meat preparations or products. In Belgium, *S. hominis* was detected at 97.4% in the samples of minced beef (42). In the same context, histology was recommended by Delphine (2010) as an accessible method for detecting parasites in fresh fish samples, processed foods (fish paste), and in finished products (smoked salmon). The method allowed the identification of two important parasites, due to the frequency of their presence in fish, namely *Anisakis simplex* and *Kudoa* spp (8).

HISTOLOGICAL AND CHEMICAL TECHNIQUES USED TO ESTIMATE THE PERCENTAGE OF MEAT AND UNAUTHORIZED TISSUES

A comparison between the histological and chemical analyses was also studied by Botka-Petrak et al. (2011) to evaluate the sustainability of mechanical boning of chickens (MBC) used in foodstuff. The basic principle of meat separation varies depending on the machine, which can affect the functional properties of the MBC produced. The MBC used in this study was produced from the broiler chicken carcass, back, wings and neck, after commercial cutting. The separation was performed mechanically, on a «Beehive» separator, which separates the muscular tissues, the adipose tissues, and the skin from the cartilage, the bones, and the lymphatic tissues (7).

Histological examination was performed on permanent products, HE stained. In the histological analysis performed by Botka-Petrak et al. (2011) on the mechanically recovered poultry meat, the results showed a longitudinal and transversal reduction of the muscle tissue, but also of the organic products, of the cartilage and the bone tissue in a quite large number, with the presence of the connective, lipid, and lymphatic tissue characteristic elements of the mechanically separated

meat (because it indicates their quality).

Also, the content of water, lipids, proteins, ash, and calcium was analysed. The average water content was highest in the boneless meat carcasses (69.14%). The total lipids had the highest value (20.85%) in the boned back and the value of the proteins was the highest (15.57%) in the boned carcass. In the boned wings, the ash content was very high (1.65%), as well as the calcium content (0.29%). In the secondary products, the highest average water content was in the whole carcass (59.02%). Total lipids had the highest value in the wings (11.56%) and total protein (32.88%), ash (20.19%), and calcium (7.41%) had the highest value in the back. The results of the chemical analysis confirmed the result of the histological examination (7). This comparative analysis of the histological and chemical examination results could provide relevant information on the content of the raw material, which directly influences the quality of the final products (7).

The approach of the combined histological and chemical analysis has been studied and applied in some researches, especially to evaluate the quality of mechanically separated meat (MSM) obtained through different separation methods. The results of the histological analysis performed by Komrska et al. were consistent with the results of the chemical analysis. The morphometric examination included the quantification of muscle, fat, connective tissue (collagen) and bone fragments. The chemical analysis included connective tissue, calcium, and fat (15). Quality parameters for chemistry (hydroxyproline content (collagen) and calcium (bone)) and histology (determination of connective tissue and bone particles) were evaluated by Nagy et al. (2007). The results showed that MSM contained twice as much collagen and bone content (27).

To compare the histological method and the chemical analysis as the most used techniques in detecting unauthorized tissues from meat products, Sezer et al. (2013) examined from the microbiological, physico-chemical and histological point of view different types of fermented sausages, produced by traditional methods by local butchers from Kars province (Turkey). Following the examination, none of the samples conformed to the standard. The level of nitrates and nitrites in sausages was between 14.88-943.71 mg / kg and 0.46-378.16 mg/kg respectively, made by chemical analysis. The histological method detected in 13 of the examined samples epithelial tissue (32.5%), in 11 samples (27.5%) epithelial glandular tissue, and in 5 samples (12.5%) cartilage and bone tissue with smooth muscle tissue (37).

In another study, the histological method was applied and compared with chemical analysis to identify unauthorized tissues in 44 hamburgers. Histopathological examination (HE) stain detected chicken epithelial tissue, chicken gizzard, hyaline cartilage, bone and

mammal tissue in meat samples, while chemical analysis (determination of collagen and hydroxyproline) did not confirm anything (3). Sharedeh et al. (2015) applied the histological method to determine chemical changes such as pH and mineral content of the meat by evaluating the structural characteristics of the muscle fibre. Histological changes were examined, interpreting the diameter of the muscle fibres on the cross-sections and the areas with extracellular space in the meat samples with different pH values. The results showed an increase in extracellular space and a decrease in the cross-sectional diameter of the fibre when the pH decreased (40).

Tremlova and Štarha (2003) studied the procedure of quantitative evaluation of the bone content of meat products by histological examination. The results were confirmed by comparison with chemical analyses, determining calcium levels. The correlation between the two methods, histological and chemical, was expressed by a coefficient of 0.78 (41). However, the histological analysis may indicate a more complete picture of the composition of meat and meat products than that obtained only by chemical analysis (14, 24, 41).

IDENTIFICATION OF FRESH MEAT AND THAWED MEAT USING HISTOLOGICAL AND CHEMICAL METHOD

Preservation by freezing has been frequently used as a method of preserving raw materials, for many meat products or directly to guarantee the quality of the meat until it reaches the consumer, although it causes structural changes directly related to frost speed. When freezing is fast (0.5 °C / minute), it causes the formation of many small ice crystals, evenly distributed inside and outside the muscle cells.

When freezing is slow (0.05 °C / minute), it allows the formation of large ice crystals, at lower levels and in the extracellular region, causing less cell damage. Also, freezing usually results in a decrease in muscle fibre diameter and the length of sarcomeres (6, 28, 44).

The histological method is mainly applied to detect fresh meat and previously frozen meat by evaluating changes in ice crystals and muscle fibres.

Zhu et al. (2004) examined the microstructures of ice crystals (size, shape, location) in pork muscle tissue during freezing with pressure change (PSE - pressure-shift freezing) using the histological method and comparing them with freezing with air blaster freezing (ABF - air blaster freezing) and freezing with liquid immersion freezing (LIF - liquid immersion freezing). The results show that the size of ice crystals in pork samples treated with PSE (pressure-shift freezing) was smaller than those treated with ABF and LIF (45). Rahelic et al. (1980) analysed the microscopic changes in beef which appeared at different freezing temperatures, noticing a

link between the degree of deterioration of the meat and the position of the ice crystals. It has been proven that the greatest fibre damage was caused by the formation of inter- and intra-cellular ice crystals, when the ice crystals formed between the fibres generate pressure that separates the fibres, while the ice crystals formed in the fibre generate pressure in the opposite direction (33).

Less deterioration was observed when only intracellular ice crystals formed, when pressure is generated in one direction only (33). The modification of ice crystals from frozen and stored beef caused by the fluctuation of storage temperatures (-5 °C, -10 °C, -15 °C, and -20 °C) was analysed by Martino et al. (1998) by histological examination over a 5 months to investigate the recrystallization effect of frozen meat tissue (25).

In a similar study, frozen and non-frozen sections of pork tissues in 3 freezing methods (assisted high-pressure freezing, frozen air currents freezing and cryogenic fluid freezing) were stained using HE method. The results revealed that the smallest destruction of the structure was caused by the assisted high-pressure freezing due to the small size of the ice crystals formed during freezing (25). Also, the histological changes of thawed beef samples at -18 to + 10 °C, at the frequency of 50 Hz and different voltage levels (10, 20 or 30 V/cm) were compared with the conventional thawing method also by Icier F et al (2010). The optical microscopy of the tissue sections revealed the destruction of the myofibrillar elements in both defrosting methods, at the same time, the structural changes of the defrosted samples, according to the conventional method were significantly higher than those defrosted using the ohmic method (16).

The optical microscopy of the tissue sections revealed the destruction of the myofibrillary elements in both defrosting methods, at the same time, the structural changes of the defrosted samples according to the conventional method were significantly higher than in the case of the defrosted ones using the ohmic method (16). No significant differences were observed in the structural changes between the non-frozen and frozen samples based on the RF-assisted cryogenic freezing method (1).

DISCUSSION

In some cases, products from the meat processing industry are not exclusively made from animal materials, and some producers use unauthorized methods in the preparation of these products. Thus, it is important to use the accurate and reliable methods of quantitative and qualitative analysis of ingredients and constituents of vegetable origin in meat products, their detection in meat products makes it possible to monitor the quality of these products (43).

Examination by histological and chemical techniques in parallel could give a relevant idea about the content of raw materials, which directly affects the quality of the final products (7). Both methods offer an objective analysis of the quality and quantity of the essential components, of the meat products, but at the same time, the two methods also involve certain errors, which is why they must be used together and not independently. Nevertheless, the histological analysis may indicate or provide a more complete picture of the quality of meat and meat product composition than that obtained only by chemical analysis. At the same time, the use of microscopy has been sufficiently justified as an instrument for assessing the degree of cellular damage caused by frost, as severe deteriorations with ruptures in muscle fibre groups. The diameters of the ice crystals were between 60 µm and 95 µm, depending on the freezing speed. Occasionally, endomysium ruptures have been identified. Histological analysis of the samples after thawing showed that: these methods affected the structural characteristics of the meat, destroyed the myofibrillar elements, and favoured the appearance of intracellular oedema and even degeneration of the muscle cells. (32).

However, more research is needed to establish the exact relationship (in addition to the major impacts) between the freezing rate and the consequent cellular damage. The general idea that fast freezing damages meat to a lesser extent than slow freezing should be verified (6).

Histological analysis can also be used to evaluate the quality of meat products obtained from mechanically separated meat, the properties of which depend on the nature and quantity of raw material used, as well as on the adjustment of the machine (31). The concept is not recent, several researchers claim that the optical microscope has always been used to evaluate such meat (7, 13). In general, the histological method is used to a large extent to detect the unauthorized content of different plants, animal tissues and to evaluate and highlight structural nonconformities.

CONCLUSIONS

The physico-chemical methods alone cannot control the quality of meat products. The histological approach is a reliable tool to evaluate the quality of meat products. Based on this information, histological techniques can be a simple and conclusive tool for controlling the quality of foodstuff.

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