THE USE OF HISTOLOGICAL METHODS IN MEAT AND MEAT PRODUCTS FOR FRESHNESS DETERMINATION - A POSSIBLE FUTURE TREND?

Raluca-Aniela GHEORGHE-IRIMIA, Manuella MILITARU

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăşti Blvd, District 1, Bucharest, Romania

Corresponding author email: irimiaral@gmail.com

Abstract

Food products preservation was and will be of general interest, especially due to the current challenges faced by the industry. In this regard, data collection methods diversity represents an opportunity for extending the current knowledge in meat preservation. Meat and meat products microscopical imaging leads to obtaining some extremely useful information that cannot be substituted by any numerical equivalents of other comparative methods. This fact has been noted in many countries worldwide and was adopted as a complementary method for assessing the integrity, quality and shelf life of food products. In this regard, the aim of this literature review was to assess the benefits of using the histological method for freshness determination in meat and meat products, along with the documented procedure and interpretation.

***Key words**: meat, meat products, histological assessment, meat freshness.*

INTRODUCTION

The histological method is a valuable tool for assessing the meat and meat products freshness (Avinee et al., 2010). This method involves the examination of tissue samples under a microscope to identify changes in tissue structure and composition that occur as the meat ages. These changes can provide important insights into the quality and safety of meat, and can be used to optimize processing and packaging techniques to ensure the production of fresh, high-quality meat products (Abbasy-Fasarani et al., 2012; Abdel Hafeez et al., 2016).

One of the key advantages of the histological method is its ability to detect changes in muscle tissue that occur at the cellular level. As meat ages, the structure of muscle fibres begins to break down, resulting in changes in the appearance and texture of the meat. These changes can be observed and quantified using histological techniques, allowing researchers and food producers to determine the optimal time frame for processing and packaging meat products (Kalyuzhnaya, 2020).

Histological analysis can also provide important information about the meat tissue composition. For example, the presence of connective tissue or fat can affect the tenderness and flavour of meat, and changes in the distribution of these tissues can indicate changes in the quality of the meat.

Histological techniques can be used to identify the types of bacteria (bacilli or cocci) present in meat samples, allowing food producers to respond appropriately in order to prevent contamination and ensure the safety of their products. In this regard, the aim of this paper is to identify the histological assessment main advantages and disadvantages along with the current perspectives of use in meat and meat products freshness determination.

HISTORICAL PERSPECTIVE AND CURRENT TRENDS

The invention of the optical microscope at the end of the 16th century represented a significant opportunity for food researchers, as it was the first instrument that could magnify the images of samples 20 to 30 times their original size. The resolution of contemporary optical microscopes is 103 times that of the human eye, resulting in a 4 to 1500 times magnification. In addition, they are adaptable and can be used in a variety of configurations, including bright field, phase contrast, differential interference contrast, polarised light, and fluorescence (Toldrá et al., 2009).

Since 1850, it has been acknowledged that microscopic food evaluation technologies provide a comprehensive, inexpensive, and rapid alternative for getting essential data regarding the control and quality of food products. Since 1920, meat and meat products have been evaluated using histological techniques. Currently, 75% of European studies on food analysis are based on meat and meat products, with 25% of these researches focusing on histological evaluation (Guelmamene et al., 2018).

Acquiring a microscopic image of the meat and meat products structure results in the import of incredibly relevant data that cannot be replaced by the numerical equivalents of other comparative methods for assessing the food quality or safety (Řezáčová et al., 2011). This fact has been recognised in numerous countries, where it has been used as a complementary method for evaluating the food safety, quality, and preservation (Isaconi et al., 2020).

In addition, food products microscopic examination enables the determination of content and types of unauthorised animal or vegetable tissues, the labelling accuracy by histomorphometric analysis, raw materials specificity (origin), tenderness analysis, the detection and evaluation of mechanically separated meat, and meat quality prediction under freezing and thawing conditions, ante-mortem animal handling effects evaluation, parasites or microbial cells detection. Under these circumstances, the histological evaluation for meat and meat products quality and preservability control can be rapid, sensible, and accurate (Guelmamene et al., 2018).

From the evaluation methodologies point of view, current microscopic technologies have surpassed the spatial resolution limitations of conventional optical microscopy, which is based on a light beam and objectives.

Nonetheless, conventional optical microscopy is still utilised for numerous reasons. One of these is the chromatic capability of distinguishing different structures or substances by staining, which facilitates interpretation and imaging visualisation. In addition to optical microscopy, other types of microscopy (e.g., electron and X-ray microscopy) use stains, albeit in considerably less numbers than in optical microscopy (Toldrá et al., 2009).

In terms of costs, optical microscopy is far less expensive than other methods of microscopy. Moreover, no particular environmental conditions are necessary (Toldrá et al., 2009).

ASSOCIATED CHALLENGES

One of the primary challenges associated with the histological method is the need for specialized equipment and expertise (GOST 23392-2016, 2016). The method requires the use of a microscope and specialized staining techniques to prepare tissue samples for examination. Additionally, interpreting the results of the histological analysis requires specific expertise, which can be challenging to acquire. As a result, many small-scale meat producers may not have access to the necessary equipment or expertise, limiting their ability to utilize this method for freshness determination.

Another challenge associated with the histological method is the time and cost required for analysis. Preparing tissue samples for examination can be time-consuming, and the process requires specialized staining techniques, which can be costly.

Accuracy is another challenge associated with the histological method. While the method can provide valuable information about the quality and freshness of meat and meat products, the accuracy of the results can be influenced by several factors, including the quality of the tissue samples, the expertise of the technician performing the analysis, and the staining techniques used (GOST 23392-2016, 2016). Any inaccuracies in the analysis can affect the reliability of the results and potentially lead to incorrect conclusions about the freshness of the product.

The histological method is also limited in its ability to assess other important attributes of meat quality, such as flavour, aroma, and texture. While the method can provide information about the structure and composition of the tissue, it does not provide information about other sensory attributes that can influence the overall quality and freshness of the product. As a result, the histological

method may need to be used in conjunction with other methods for assessing meat quality and freshness.
Moreover, the optical microscopy has a limited depth of focus, difficulties in excluding optical defects, and limited magnification possibilities (Toldrá et al., 2009).
In contrast, considering the fact that none of the freshness assessment methods used in the food industry is perfect in terms of execution, costs or precision, optical microscopy is still a practical, simple, and cost-effective approach compared with others.

WORLDWIDE PERSPECTIVE

The histological method has been adopted in the meat industry by several countries worldwide, including the European Union (EU), United States, Canada (Canadian Food Inspection Agency, 2018), Russian Federation and Australia. In the EU, histological assessment is used as part of the post-mortem inspection of meat from some species. In some particular situations, the EU has strict regulations in place to ensure that the meat from these animals is subject to histological examination to determine its fitness for human consumption. For example, according to Regulation (EC) no. 999/2001, the histological method can be used to diagnose Bovine Spongiform Encephalopathy in bovines. The EU also requires that any imported meat must meet the same standards (European Commission, 2005; European Commission, 2004, European Commission, 2001).
Outside of the EU, other countries have also adopted histological assessment for meat and meat products. In the United States, for example, the USDA Food Safety and Inspection Service has approved the use of histological analysis as a means of evaluating the quality and freshness of meat. The method is used primarily for detecting and preventing cases of meat adulteration, particularly in ground beef products (USDA Food Safety and Inspection Service, 2021).
Other countries that have adopted histological assessment methods for meat and meat products include Japan, South Korea, and Australia. In the Russian Federations' constituent republics, the GOST standards describe the histological method of meat and meat products assessment by optical microscopy (GOST, 2013; GOST, 2016).

THE MEAT AND MEAT PRODUCTS HISTOLOGICAL ASSESSMENT METHOD

General steps

The following are the general steps involved in the meat and meat products histological assessment (Figure 1):

1. Tissue sampling: A meat or meat product sample of approx. 1 cm^3 is collected for analysis.
2. Tissue fixation: The tissue is fixed using a solution such as formalin. This helps to preserve the tissue structure and prevent degradation.
3. Dehydration: The tissue is dehydrated using a series of alcohol washes, which remove water from the tissue.
4. Embedding: The tissue is embedded in a medium such as paraffin wax. This helps to support the tissue and make it easier to cut thin sections.
5. Sectioning: The embedded tissue is cut into thin sections using a microtome. The thickness of the sections depends on the type of analysis required.
6. Staining: Depending on the coordinates of the research or examination, the routine staining is haematoxylin eosin, in tissues of animal origin, the nuclei of the cells having a dark blue colour, and the cytoplasm red tones varying in intensity and shade. Other stains such as toluidine blue can also be used to identify the origin and evaluate the quality (vegetable and animal tissues). Additionally, PAS (Periodic Acid Schiff) Calleja with Trichromic blue or green (collagen evaluation) or Alizarin Red can be used for the detection of bone fragments (Petrášová et al., 2016).
7. Microscopic examination: The stained sections are examined under a microscope, and images are captured for analysis. The images can be analysed using various software programs to quantify features such as muscle fibre size, fat content, and connective tissue content (Lukášková et al., 2011).

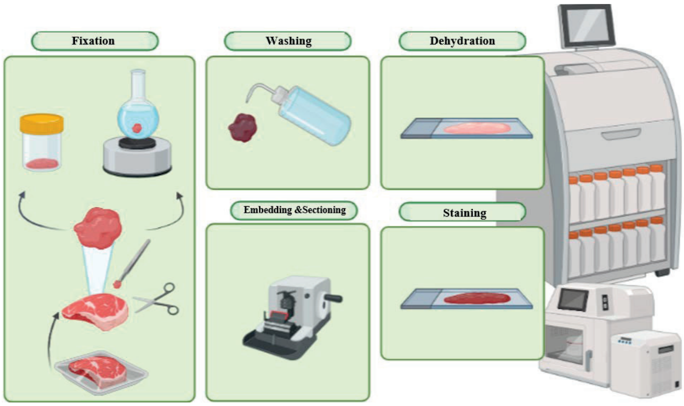

Figure 1. Histological method procedure (illustration made via www.BioRender.com)

Meat and meat products freshness evaluation by using the histological method

The main components of fresh meat are muscle fibers, connective tissue, and fat cells. Muscle fibers make up the bulk of the meat, and are organized into bundles called muscle fascicles.

Each muscle fascicle is surrounded by connective tissue, which helps to hold it together and provides support. The fat cells are distributed throughout the muscle tissue, and their distribution varies depending on the cut of meat (Kalyuzhnaya, 2020).

Under a microscope, fresh meat appears as a highly organized structure of muscle fibers surrounded by connective tissue. The muscle fibers are long and cylindrical, with distinct striations that reflect their contractile properties. The connective tissue appears as a network of thin, white fibers that criss-cross the muscle tissue. The fat cells are typically small and round, and are located in between the muscle fibers (Figure 2 and Table 1) (GOST 19496-2013, 2013).

Hematoxylin stains nuclei and other acidic structures blue-purple, while eosin stains basic structures such as collagen and muscle fibers pink-red. In fresh meat samples, the histological aspect may show well-preserved muscle fibers, with a clear striated appearance and an abundance of nuclei. The fat content may also appear well-preserved, with a distinct pink staining and minimal eosin staining. The connective tissue may be visible as thin collagen fibers, which appear as pinkish strands (Petrášová et al., 2016). Moreover, fresh meat samples may show few or no signs of microbial activity, with a minimal presence of bacteria and fungi (GOST 19496-2013, 2013).

In spoiled meat samples, the histological aspect may show signs of muscle fiber degradation, with disintegration and separation of the fibers, a reduction in the number and size of nuclei, and an increase in eosin staining. The fat content may also show signs of degradation, with a loss of the normal pink staining and an increase in eosin staining. The connective tissue may appear disrupted, with a loss of elasticity and a reduction in collagen content. Additionally, microbial changes may be visible under the microscope, with an increase in the number of bacteria and fungi, which may appear as clusters or filaments. These changes can result in unpleasant flavors and odors, as well as potential health risks (GOST 19496-2013, 2013).

In ralative spoiled meat samples the general appearance has both fresh and spoiled characteristics, as it’s an intermediary stage (GOST 19496-2013, 2013).

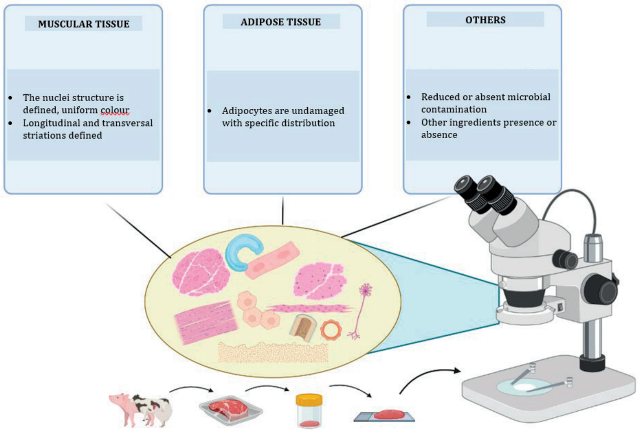

Figure 2. Main tissue characteristics in fresh meat and meat products (illustration made via www.BioRender.com)

As for cured artisan meat products, the maturation stages are determined by the muscle fibres autocatalytic destruction intensity, subsequent fragmentation into myofibrils and disintegration into sarcomeres in the form of a granular conglomerate located in the endomysium, with the preservation of the primary elements colour.

For the histological evaluation of meat products such as sausages, the muscle tissue evaluation is the first step (Khvyla et al., 2016). Fresh sausages should have a uniform and smooth appearance, with well-defined muscle fibers (Chen et al., 2019). As it changes, the muscle tissue begins to disintegrate and become more fragmented, with less distinct fibers (Khvyla et al., 2013).

Another aspect underlying the sausages freshness histological evaluation is the presence of adipocytes (Khvyla et al., 2013; Khvyla et al., 2012). Fresh sausages should have a high percentage of adipocytes, which is transposed into a moist texture (Donkova et al, 2018). Along with the spoilage progression, the adipocytes begin to disintegrate and become more fragmented, the sausage being drier and less tasty (Alshejari et al., 2017). Additionally, the presence of other types of muscle tissue (cardiac or smooth) is evaluated, depending on the nuclei location.

Table 1. Histological aspects of meat and meat products in different freshness stages (GOST 19496-2013, 2013; GOST 23392-2016, 2016; Kalyuzhnaya, 2020)

	Fresh	Relatively spoiled	Spoiled
Meat	The muscle fibre nuclei structure is clearly defined, the colour is uniform. Clearly defined and expressed muscle striations, with a uniform colour. Focal areas of cocci can be detected in fresh meats, at the section surface or in the loose connective tissue of the superficial fascia.	Nuclei in different stages of karyolysis, the colour being uneven, slightly intense and discoloured. Complete disappearance of the striations, the changes being identified up to about 15 mm deep, there are areas with a mucous appearance that have a basophilic color at the surface of the meat. Both focal areas with cocci and bacilli flora can be identified in the loose connective tissue of the superficial fascia, in the perimysium and endomysium. These aspects can be identified up to 5 mm deep.	Nuclei are almost undetectable; the colour is absent or slightly visible. Complete disappearance of the striations, the changes being identified up to more than 30 mm deep in relation to the cross-sectional surface of the sample, the color being absent or barely perceptible. Diffuse areas of bacillary flora extending up to 10 mm deep on the entire surface of the section and in the loose connective tissue of the superficial fascia, perimysium and endomysium.
Meat products – Cured meat products	Microflora is absent or up to 10 bacterial cells (cocci or bacilli) are present, without signs of muscle destruction.	Intermediate aspects between fresh and spoiled	More than 30 cocci/bacilli along with a significant degradation of muscle tissue
Meat products - Sausages	Uniform and smooth appearance, with well-defined muscle fibres. High percentage of adipocytes.	Intermediate aspects between fresh and spoiled.	Muscle tissue begins to disintegrate and become more fragmented, with less distinct fibres. Adipocytes begin to disintegrate and become more fragmented

Subsequently, the presence of integumentary epithelial structures, as well as dense connective tissue and organs, is determined. In the individual sections, the presence of starch and tissues of plant origin can also be identified.
Moreover, the presence of bacteria or other contaminants can also be evaluated (Khvyla et al., 2016).
In general, histological examination is a useful tool for determining the freshness and safety of meat and meat products (Lukášková et al., 2011; Khvyla et al., 2013).

CONCLUSIONS

In conclusion, the histological method is a powerful tool for assessing the freshness of meat and meat products. This method involves the examination of tissue samples under a microscope to detect changes in tissue structure and composition that occur as meat ages. The histological method can provide valuable information about the quality and safety of meat products, including its tenderness, juiciness, flavour, and overall palatability.
However, the use of the histological method for freshness assessment of meat and meat products is not without its challenges. These challenges include the need for specialized equipment and expertise, time and cost constraints, accuracy limitations, and a limited ability to assess other important attributes of meat quality.
Despite these challenges, the histological method remains an important tool for assessing the freshness of meat and meat products. By providing detailed information about the structure and composition of meat tissue, this method can help food producers ensure the quality and safety of their products and provide consumers with fresh, flavourful, and nutritious meat products. Further research is necessary to overcome the limitations of the histological method and to develop improved techniques for assessing meat and meat product freshness.
Additionally, the histological examination is internationally accepted for assessing the freshness of meat and meat products. The method is regulated and standardized in several countries worldwide, including EU, United States, Canada, and Australia.

REFERENCES

Abbasy-Fasarani M., Hosseini H., Jahed-Khaniki G.R., Adibmoradi M., Eskandari S. (2012). Histological study of industrial hamburgers containing 30 and 60 percent meat for presence of unpermitted edible tissues and correlation of this factor to meat connective tissue chemical indices (Persian). *Iranian Journal of Nutrition Sciences & Food Technology,* 7(5):311-318.

Abdel Hafeez H., Zaki R., Abd El-Magiud D. (2016). Applying light, histochemical and scanning histological methods for the detection of unauthorized animal and herbal content in street meat sandwich: What is in the sandwich we eat? *Food Process Technol*., 7(12):11.

Alshejari, A., Kodogiannis, V.S., (2017). An intelligent decision support system for the detection of meat spoilage using multispectral images. *Neural Computing and Applications*, 28(12), 3903-3920.

Avinee G., Charfi S., Stocker N., Gyde E., Hebert A., Mabille M.P., Sevestre H., Chatelain D., (2010), Pathological analysis, an original way to study foodstuffs. *Ann Pathol.,* 30(5):344-349.

Canadian Food Inspection Agency (2018). Meat hygiene manual of procedures - Chapter 4 - Inspection procedures.

Chen, J., Gu, J., Zhang, R., Mao, Y., Tian, S. (2019). Freshness evaluation of three kinds of meats based on the electronic nose. Sensors (Switzerland), 19(3).

Donkova, N.V. (2018). Safety assessment of broiler chicken meat based on microstructural analysis. *Bulletin of the Krasnoyarsk State Agrarian University*, 2(137), 32-40.

European Commission (2004). Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin.

European Commission (2005). Commission Regulation (EC) No 2075/2005 of 5 December 2005 laying down specific rules on official controls for *Trichinella* in meat.

GOST 19496-2013. Meat and meat products. The method of histological examination. Retrieved from http://docs.cntd.ru/document/1200107317

GOST 23392-2016. Meat. Methods of chemical and microscopic analysis of freshness. Retrieved from http://docs.cntd.ru/document/1200144232

Guelmamene , R., Bennoune , O., and Elgroud , R. (2018). Histological Techniques for Quality Control of Meat and Meat Products. *Scholar Journal of Applied Sciences and Research,* 1(7), 26–32.

Isaconi, I. V., Militaru, M. (2020). Applied Histological And Chemical Analysis For Quality Assessment Of Meat And Meat Products: Review. Rev. *Rom. Med. Vet.*, 30(2), 79–85.

Kalyuzhnaya, T., Karpenko, L., Orlova, D., Drozd, A., Urban, V. (2020). An Express Assessment Method For Meat Quality And Safety. *International Transaction Journal of Engineering, Management, & Applied Sciences & Technologies*, 11(1), 1–9. http://doi.org/ 10.14456/ITJEMAST.2020.8

Khvyla, S.I. (2016). The histological method of assessing the effect of freezing and storage on the microstructure of meat. *Refrigeration equipment*. 11, 44-47.

Khvylya, S. I., Pchelkina, V. A. (2013). Standardized histological methods for the study of meat and meat products. *Meat industry*. 7, 28-31.

Khvylya, S.I., Pchelkina, V.A., Burlakova, S.S. (2012). The use of histological analysis in the study of raw meat and finished products. *Technique and technology of food production.* 3(26), 132-138.

Nollet, L. M., Toldrá, F. (2009). *Handbook of Muscle Foods Analysis.* Boca Raton, FL: CRC Press.

Petrášová, M., Bartlová, M., Pospiech, M., Luňáková, L., and Tremlová, B. (2016). The use of special PAS Calleja staining for the microscopic detection of selected hydrocolloids in model meat products. *MASO International*, 2, 124–130.

Řezáčová Lukášková, Z., Tremlová, B., Randulová, Z., & Bartl, P. (2011). Microscopic methods in food analysis. *Maso International,* 1, 27-34. https://doi.org/10.2754/avb201101010027

Regulation (CE) No 999/2001 of the European Parliament and of the Council of 22 May 2001 laying down rules for the prevention, control, and eradication of certain transmissible spongiform encephalopathies. *Official Journal of the European Communities,* L 147/1-40.

USDA Food Safety and Inspection Service (2021). Meat and poultry inspection manual.