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COMPARATIVE HISTOLOGICAL STUDY BETWEEN FILLET MUSCLE TRADITIONAL PREPARATION AND INDUSTRIAL PREPARATION

Andrei CĂLINOIU, Mihai-Romeo DINICĂ, Laura-Cătălina DUMITRA, Ștefania Mariana RAITA

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

Corresponding author email: andreicalinoiu95@gmail.com

Abstract

The aim of this paper is to highlight the invaluable contribution of histological techniques used to assess the quality of meat products deemed for human consumption. Additionally, histological analyses assist with the study of what effect natural vegetarian solution extracts have on the products' quality, effect reflected by the impact on preservation properties of meat products. One hundred industrially and traditionally processed and preserved fragments of tenderloin samples were prepared for histological analysis. After prelevation tissue samples were fixed with formalin solution. Samples were individually rinsed with distilled water, stained with hematoxylin-eosin and with Mallory technique. Results: muscle fibres rarely displayed their biological integrity and numerous vacuole were identified as a result of the use of brine in the industrially processed samples. Traditionally processed tenderloin displayed an unaltered muscular fibre structure. The quality of the traditionally processed product was assessed as superior when compared to the industrially processed product. Conclusion: traditional methods of preservation were assessed as giving a superior quality to the end meat product.

Key words*: histological analyses, industrial, muscle fillet, traditional.*

INTRODUCTION

Health protection is the primary objective of all EU rules and standards for agriculture, animal husbandry and the food industry (European Commission, Department - General for Agriculture and Rural Development, Department -General for Trade, *Ensuring food is safe: the veterinary and phytosanitary system of the European Union explained*, Publications Office, 2018, from https://europa.eu/european-union/topics/food-safety_ro).

The concept of food means food with nutritional and sensory properties which has a twofold role: 1) to maintain health and psycho-physical well-being, but also 2) to ensure disease prevention.

Food hides many traps for human health. Food production processes carry many critical points along the "farm to fork" journey. Primary material either gets exposed to contamination risks or, thereafter escapes various safety rules and procedures at any point in the processing chain: from the start of processing at production platforms up to the point they end up at the market stalls (Gallo et al., 2020; Dobrinas et al., 2013; Isaconi (Bulai) et al., 2019).

Our human way of life has changed profoundly over time and consequently our eating behaviors.

Modern man prefers to eat cooked food or pre-cooked food, wich is calories-rich as well as complemented by food additives. Salt is frequently used for preservation, but it has turned from a nutrient into a risk factor, being a real threat to health due to its excessive use.

The National Academy of Sciences states that "...salt improves the perception of the product's density, accentuates the sweet taste, hides metallic flavors or the taste of chemicals, having the overall effect of intensifying flavors and improving them" (Durack et al., 2008).

The World Health Organization (WHO) proposes a reduction in salt consumption to 5 g per day to reduce stroke by 23% and cardiovascular disease by 17%, which can prevent 4 million deaths globally. Recommendation goes even further for children's health with: no salt added up to the age of 14 years and 2 g per day after that until adult life. In order to reduce premature

mortality (deaths occurring before the age defined by life-expectancy at birth), WHO relaunched its international appeal to further reduce salt consumption from its current levels by 30% by 2025 (The WHO European Food and Nutrition Action Plan 2015–2020 provides a framework for action to progress towards healthy diets for all in the WHO European Region from https://www.euro.who.int/__data/ assets/pdf_file /0006/ 457611/Accelerating-salt-reduction-in-Europe.pdf).

When foodstuff composition is assessed and evaluated along with nutritional composition as well as customs and eating habits of the modern man, such evaluations can identify or confirm risks associated with nutritional imbalances. Such evaluations have the aim to draw evidence informed nutritional policies and recommend appropriate prophylactic measures.

The aim of this research is to place an emphasis on the utility of microscopic morphology for the analysis of industrially prepared fillet muscle and traditionally prepared fillet muscle; and to compare the additive content used with each of these methods of preservation.

A good quality histological analysis of any meat product can validate and confirm nutritional qualities of that product. It provides, with an in-depth insight, information on the effect and impact which some plant extracts have on the preservability as well as on the nutritional and market quality of the final product.

Interdisciplinary research remains the way forward to increasing the use of complex yet efficient and sensitive methods for the monitoring of food's quality and safety with impact on human health. This research may open new avenues in quality and safety procedures for the meat processing industry.

MATERIALS AND METHODS

The harvesting of the materials consisted of the sampling of fragments of pork and beef tenderloin prepared traditionally as well as industrially - we obtained fragments with flat faces and parallel to the side of approximately 1 cm. The tissues were processed by usual techniques, for the purpose of preservation and histological fixation, using 10% formalin solution, for a period of 48-72 hours, depending on the size of the harvested tissue. The fixative was removed by washing with distilled water. Later I included the fragments in paraffin, going through the following steps: dehydration (by passing the pieces through 6 baths of ethyl alcohol 96° for a duration of 4-6 hours for each bath), clarification (through 3 baths with benzene for a total duration of 8-9 hours), liquid paraffin impregnation (through 3 paraffin baths for 6-8 hours, in an oven at 56°C), block casting (embedding in a block of solidified paraffin). We sectioned the fragments obtained with the help of the paraffin microtome, at a size of 6 μm under a ribbon aspect. To remove the existing creases, I put the obtained sections in a heated water bath. I used the paraffin microtome in Laboratory of the discipline of Histology and Embryology of the Faculty of Veterinary Medicine of Bucharest.

We glued the sections to the slides by applying a thin layer of Mayer's ovalbumin, then they were placed in the thermostat for a very short time to achieve proper adhesion. In order to create a contrast between the tissue and cellular elements, we stained the sections using the HE (Hematoxylin Eosin) and Mallory staining methods. The staining process was preceded by dewaxing (by passing the slides in 3 successive baths of benzene for 2-5 minutes) and hydration (in 3 baths of ethyl alcohol in decreasing concentration from 90°, 80° and 70° for 5-6 minutes).

We obtained over 100 permanent histological preparations that we observed under the Motic Panthera microscope with video camera. The examination of the microscopic preparations was carried out in the laboratory of the discipline of Histology and Embryology within the Faculty of Veterinary Medicine of Bucharest. We developed and used Kiernan method (Kiernan, 2015).

RESULTS AND DISCUSSIONS

Following the microscopic examination (with the objectives of 10X, 40X and 100X) of the sections made from traditionally prepared smoked pork and beef muscle, compared to the industrially prepared one, we observed major preservation differences.

In the traditional preparation, both in pork and beef muscle, the integrity of the skeletal

striated muscle fibers has been preserved, the sarcolemma being intact, the arrangement of the nuclei is preserved. Skeletal striated muscle fibers are cylindrical in shape with nuclei located at the periphery on either side of the sarcolemma.

In the traditionally prepared pork tenderloin, the presented characteristics are supported by the sections observed under the microscope. In hematoxylin eosin staining, the cytoplasm apears pink-red, the nuclei blue-violet, the perimysium and endomysium are discolored (Figure 1).

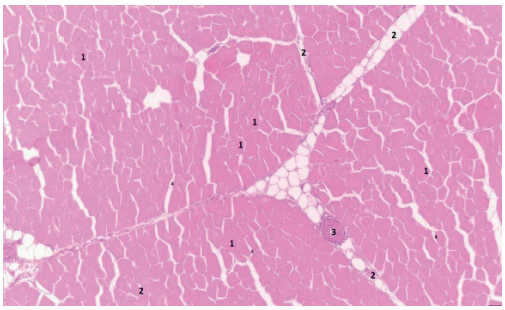

Figure 1. Traditional pork tenderloin, staining HE, ob.10 (original): 1 - transversely sectioned skeletal striated muscle fibers, 2 - perimysium, 3 - artery at the level of perimysium, 4 - endomysium

At the level of the sarcoplasm, the arrangement of the striations was preserved, which means that no noticeable changes took place at the cellular level. There are no major changes in the connective tissue. At the level of the perimysium, we observed numerous blood vessels - arteries, veins, but also nerves, arranged morphologically as in living tissues (Figure 2).

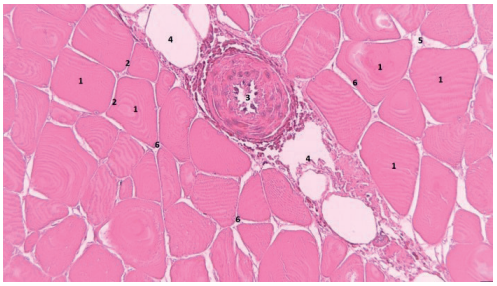

Figure 2. Traditional pork tenderloin, staining HE, ob.40 (original): 1 - transversely sectioned skeletal striated muscle fibers, 2 - sarcolemma, 3 - artery at the level of the perimysium, 4 - perimysium - numerous white fat cells, 5 - endomysium, 6 - nuclei of muscle fibers located at their periphery

Adipocytes have a typical shape with the nucleus located eccentrically, and the cytoplasm is discolored due to lipids that have been washed away by organic solvents (Figure 3).

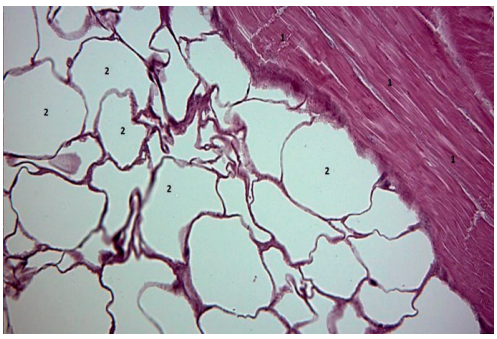

Figure 3. Traditional pork tenderloin, staining HE, ob.40 (original): 1 - longitudinally sectioned skeletal striated muscle fibers - longitudinal and transverse striations are observed, 2 - numerous white fat cells

The endomysium can be seen very well, enveloping each individual fiber, where capillaries and connective fibers are present (Figure 4).

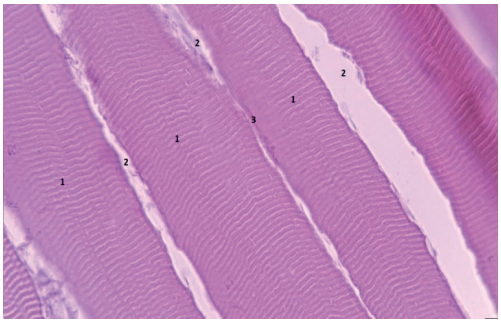

Figure 4. Traditional pork tenderloin, staining HE, ob.100 (original): 1 - longitudinally sectioned skeletal striated muscle fibers - the longitudinal and transverse striations are clearly visible, 2 - endomysium

Compared to pork muscle in cross-sectioned traditionally prepared beef muscle, the perimysium is more colorful, suggesting a richer vascularity preserved by the preparation technique, as well as muscle fibers more clearly differentiated from each other by the endomysium (Figure 5).

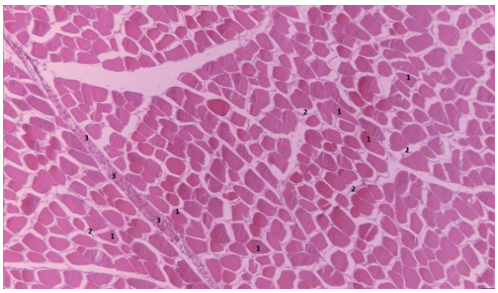

Figure 5. Traditional beef tenderloin, staining HE, ob.40 (original): 1 - transversely sectioned skeletal striated muscle fibers, 2 - endomysium, 3 - perimysium

Adipocytes show typical shape but with discolored cytoplasm because lipids have been removed by organic solvents (Figure 6).

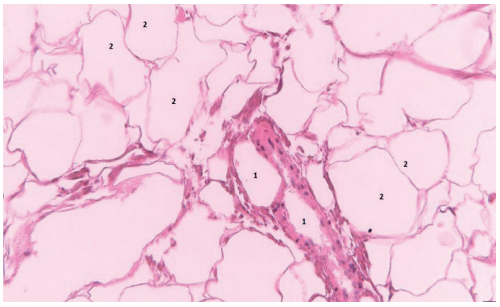

Figure 6. Traditional beef tenderloin, staining HE, ob.40 (original): 1 - blood vessels, 2 - fat cells

In Mallory staining nucleus, cytoplasm and elastic fibers are in red, red blood cells and myelin sheaths in yellow, collagen, mucus and connective tissue in blue (Figure 7).

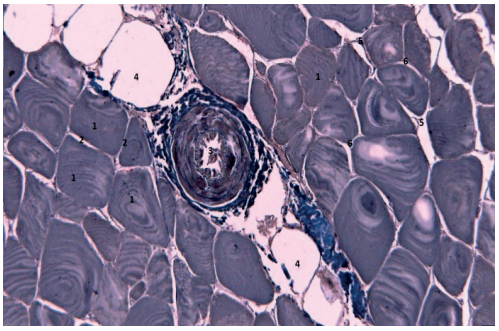

Figure 7. Traditional pork tenderloin, Mallory staining, ob.40 (original): 1 - transversely sectioned skeletal striated muscle fibers, 2 - sarcolemma, 3 - artery at the level of the perimysium, 4 - perimysium - numerous white adipose cells, 5 - endomysium, 6 - nuclei of muscle fibers located at their periphery

In the industrial preparation, the pork and beef muscle fibers show numerous vacuoles, which indicates that these muscle fillets have been injected with brine, and some fat cells are fragmented.

In the HE staining of the sections, the spacing of the spaces between the muscle fibers can be observed, the endomysium and sarcoplasm being flooded with brine (figure 8).

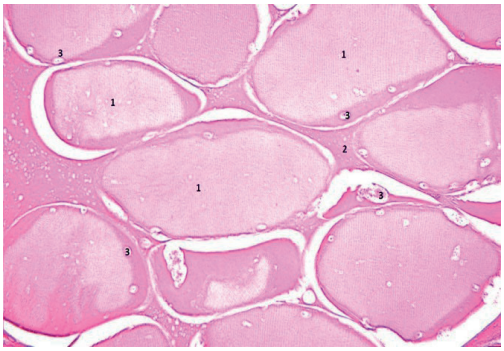

Figure 8. Industrial pork tenderloin, staining HE, ob.100 (original): 1 - transversely sectioned skeletal striated muscle fibers, 2 - endomysium, 3 - brine - both at the level of the endomysium and at the level of the sarcoplasm

In the case of industrial prepared beef muscle, in the transversal sections stained with hematoxylin-eosin, the striations of the muscle fibers are better preserved compared to industrial pork muscle, the edomysium and perimysium with preserved vasculature, as well as brine vacuoles, but in a much smaller amount compared to pork tenderloin (Figure 9).

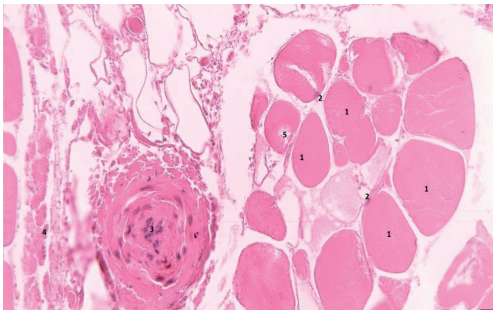

Figure 9. Industrial beef tenderloin, staining HE, ob.40 (original): 1 - transversely sectioned skeletal striated muscle fibers, 2 - endomysium, 3 - artery at the level of the perimysium, 4 - connective collagen fibers, 5 - brine vacuoles at the level of the sarcoplasm of muscle fibers

When comparing pork tenderloin with beef, we noticed that pork muscle fibers have many more vacuoles in the sarcoplasm. A brief conclusion was in the case of the beef tenderloin samples, these were injected with a smaller amount of brine (Figure 10).

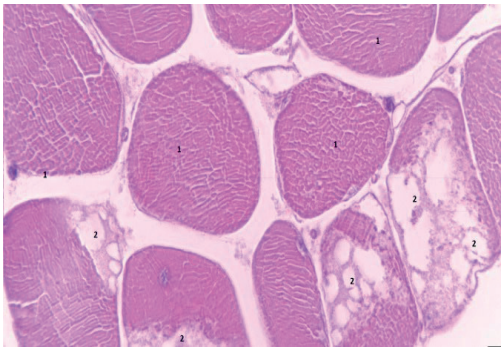

Figure 10. Industrial beef tenderloin, staining HE, ob.100 (original): 1 - transversely sectioned skeletal striated muscle fibers, 2 - brine vacuoles at the level of the sarcoplasm of muscle fibers

In Mallory's staining, collagen and connective tissue are found to be colored blue, white, discolored adipocytes, brine vacuoles at the level of the sarcoplasm are highlighted, as well as the lack of muscle fibers striations (Figure 11).

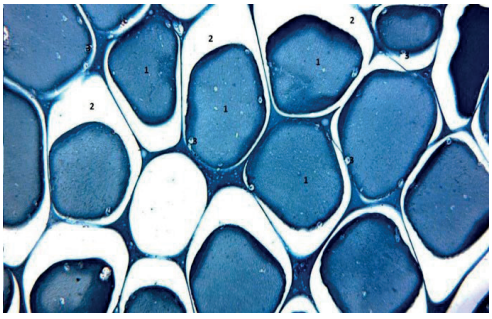

Figure 11. Industrial pork tenderloin, Mallory staining, ob.40 (original): 1 - transversely sectioned skeletal striated muscle fibers, 2 - intercellular fluid, 3 - brine vacuoles at the level of the sarcoplasm of muscle fibers

When the microscope's objective was enlarged to 100X we noticed the abundance of vacuoles of brine in industrially prepared muscle fibers, both at the level of the sarcoplasm and at the level of the endomysium (Figure 12).
In all similar samples the brine preservation method destroyed the normal histological appearance.

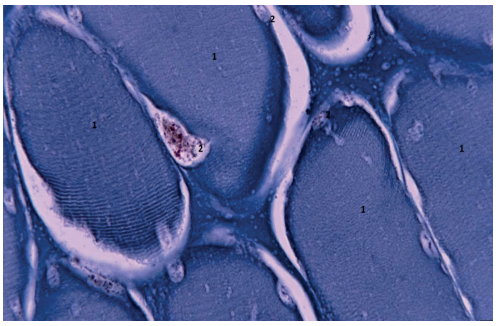

Figure 12. Industrial pork tenderloin, Mallory staining, ob.100 (original): 1 - transversely sectioned skeletal striated muscle fibers, 2 - brine vacuoles at the level of the sarcoplasm of muscle fibers

We expected to find other categories of tissues, in the observed sections, but we did not find parasites or other tissues - glandular, cartilaginous.

RESULTS AND DISCUSSIONS

With the help of optical microscopy, we were able to identify all the structures and morphological characteristics of striated muscle tissue prepared for food consumption similar to those described by Bell and Morris (2010). The special colors used allowed us to highlight the "selected" structures, with colors different from those of the other parts of the examined product, according to methods of Afanasiev (1993) and Cornilă (2011). Upon microscopic examination of pork and beef muscle sections with 10X, 40X and 100X objectives, in line with Șincai recommandations (2003), we observed that:
In the traditional preparation, the integrity of skeletal striated muscle fibers has been preserved, the sarcolemma being intact, the perimysium appears clearly around the muscle fibers, the arrangement of the nuclei is preserved, at the periphery of the fibers, the blood vessels are present (Bacha and Wood, 2011; Pavelka, 2005). Adipocytes show the typical shape with the nucleus located eccentrically, with discolored cytoplasm because the lipids have been washed away by organic solvents. These results correlate closely with the findings of Ross and Pawlina (2011) and Young and Heath (2001).
In the industrial preparation few muscle fibers kept their normal histological appearance, predominating the vacuoles which indicated

that these muscle fillets were injected with brine, the preparation method destroyed the normal histological appearance. Similar characteristics of the tissue is described by Dănacu (2015), Georgescu and Raita (2014) and Cui (2011). Adipose tissue contains fragmented fat cells (Mirancea, 2010; Yonkova et al., 2012). Comparing beef and pork tenderloins, we found a higher number of vacuoles in pork tenderloin, so it can be concluded that beef tenderloin muscle is injected with a smaller amount of brine.

CONCLUSIONS

The histological assessment and evaluation of the traditional fillet muscle structure showed 1) integrity and therefore 2) a superior quality of the end meat product in the traditionally preserved samples compared with the industrially preserved samples. Industrially preserved samples use brine. This adds to an already high salt consumption which is detrimental to human health.

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