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Histological Techniques for Quality Control of Meat and Meat Products

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Abstract

Histological examination of meat and meat products allows for direct identification and differentiation of individual components. The purpose of this article is to highlight the importance of the results of the qualitative and quantitative evaluation of meat and meat products on the basis of histological analysis. Such an analysis allows quality control of meat and meat products through content determination, detection of unauthorized (animal or plant) tissue, verification of labeling (histomorphometric analysis), the specificity of the animal source of the raw material, the assessment of tenderness, the detection and evaluation of mechanically separated meat, the detection of parasite, the prediction of the quality of the meat subjected to freezing and the defrosting, evaluation of the impact of post-slaughter manipulations on quality, detection of muscle degeneration, evaluation of certain methods of treatment or preservation of meat and meat products. On the basis of this evidence, histological techniques can be a simple, fast, economical, decisive and conclusive tool for quality control of such foodstuffs.

Keywords: Histological techniques, Quality, Meat, Control, Meat products.

Introduction

Microscopy methods, alongside those of chemistry, immunochemistry and molecular biology, represent yet another, and in some cases less expensive, alternative for the examination and control of foods. The history of food microscopy dates back to 1850 (the study focused on coffee) [1].

In parallel, histological techniques belong to the oldest methods used for the analysis of foodstuffs in order to detect their intentional contamination or falsification, in particular the meat products in which they have been used since 1910 [2]. In Europe, for example, Clinquart [3] gave an overview of research in the same field, food analysis. 75% of the studies were done on meat products, of which 25% were made using histology as a discipline [3].

Histological techniques are widely used for assessing the quality of meat and meat products as well as highlighting unauthorized tissues (to include subtitles in this paragraph).

Evaluation of content and quality

Today, different bioimaging techniques are available for the microscopic determination of food components. Commonly, the most used method is optical microscopy. This one makes it possible to identify all the structures, present, by their morphological characteristics. In addition, the special stains allow to highlight "selected" structures, with different colors from those of the other parts of the examined product [1].

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The topographic histology of meat products allows:

- The evaluation of the quality of the raw material used as well as the effect of the transformation steps [4].
- The analysis, with precision, quality parameters by detecting and measuring the content of tissues, animals, specific (muscle, connective and adipose ...). In this regard, several histological studies have been published, namely: the evaluation of the contents of eight brands of hotdog [5], hamburger [6], and other meat products [4,7-12].
- Authentication and identification of the composition. In recent years, interest in authenticity has increased and many consumers are concerned about meat and the meat products they eat [8].

The objective is usually a qualitative review; that is, detecting the presence of individual tissues and evaluating their acceptability or suitability for a given product [2]. In other words, make a definite diagnosis of the composition when all the characteristic elements are highlighted, especially since very low levels can be detected in the histological sections [13].

Detection of unauthorized animal tissue

This is the major advantage of histological techniques. Published studies have shown:

- The addition of certain unwanted tissues of a slaughterhouse, namely viscera, gizzard, gonads, ovary, lymph nodes, hyaline cartilage, bone, skin and peripheral nerves [11].
- The presence of striated muscle, adipose and connective tissue, and which is quite normal, but also blood vessels, glandular tissue and nerves. Cartilage and bone were not seen in the sausage rooms, examined by Malakauskiene et al. [4].
- A clear observation of lung, ruminant stomach, large elastic blood vessels, cardiac muscle, cartilage (hyaline fibrocartilage), cancellous bone and lymphatic tissue (the spleen), in meat sandwiches [14].
- Avinee et al. [7] examined six samples of sausages, merguez and chipolatas. All samples contained fragments of fibrotendinous tissue and some of the bone and cartilage. While salivary glands have been present in sausages and fragments of lymphoid tissue in merguez. However, nerve tissue was not found in the evaluated samples.
- Apart from skeletal muscle, a variety of tissues was observed by Prayson et al. [6] in both the hot dog and hamburger studies, namely: bone and cartilage, blood vessels, peripheral nerve and skin [6].

Plant tissue detection

The products of the meat processing industries are not composed solely of materials of animal origin.

A simple microscopic observation, with H & E stained slides, makes it easy to identify constituents of plant origin in their traditional form [1]. Their detection in meat products, combined with an estimate of the actual quantity, makes it possible to monitor the quality of such products [15].

Moreover, many methods, such as immunochemistry, molecular biology and histology, have been developed to detect plant materials in meat products. This is the consequence of the fact that the fraudulent addition of plant tissue in such foods is important, not only for the quality of the product (considered an adulteration), but also for food safety: it is allergens for some consumers [16]. The same team used histology to identify soy in minced meat. Hafeez et al. [13] detected onions in the examined samples (Kofta, Hawawshi, and shawerma sandwich). Plant material has also been observed in some of the studies cited above [5,6,10].

Pest detection

Sarcocystis spp., is an obligate intracellular parasite in mammals, which expresses a considerable infection rate especially in sheep and cattle.

Human infection with Sarcocystis may be related to consumption of raw, undercooked meat or meat products containing the encysted parasite [16]. The results, from the same team study, showed that more than 80% of the samples tested were infected with Sarcocystis. The infection rate in sausage and hamburger was 83.33% and 87.5% respectively. Note that all samples were treated with Giemsa staining and observed under an optical microscope.

Histological examination allowed the identification of parasites, in fast food burgers, whose characteristics were “Sarcocystis spp.”: parasite located in the cytoplasm of muscle fibers [6].

In the same context, histology has been described as a simple and inexpensive method for the detection of parasites in fresh fish samples, food for processing (fish pulp) and in finished products (smoked salmon). The method allowed the diagnosis of two important parasites, because of their frequency in fish and their cosmopolitan distribution, which are: Anisakis simplex and Kudoa spp. [17].

Verification of labeling

In fact, the evaluation of the content and the detection of unauthorized tissues (animal or plant) intertwine to constitute key elements to verify the labeling.

For meat products, although the labels indicate meat, most often as the first ingredient, histomorphometric techniques have demonstrated other things. For example, Prayson et al. [5] found that meat comprised less than 10% of the weight of the products examined (contrary to what was mentioned on the labeling).

Assessment of tenderness

The content and spatial distribution of the connective tissue between the muscle bundles determines the tenderness of the meat. Dubost et al. [18] used histology to evaluate the impact of structural features of intramuscular connective tissue on muscle quality. The results showed that the content in proteoglycans, that is to say in collagen, contributes negatively to succulence. Intramuscular lipids are positively related to tenderness and flavor. The method was also studied, with the Warner-Bratzel test, to predict the tenderness of beef [19].

However, the molecular architecture of the sarcomere, as defined by the interaction of actin-myosin filaments and the boundaries between Z-discs, influences not only tenderness but also water retention capacity [20].

The specificity of the animal species source of the raw material

The identification of the animal species af the origin of the raw material in meat product samples is relevant to consumers and producers.

This is because of the possible economic loss due to fraudulent falsifications, the medical requirements of individuals, who may have specific allergies, and for religious reasons [21].

Several modern techniques make it possible to identify the animal source species of meat, which is used in the meat products industry, among other things, histology and image analysis [22]. For example: Singh and Sachan [23] were successful in identifying the species in mixtures of cow and buffalo meat, with a view to combating the illegal killing of the latter. The measurement of muscle fiber length, diameter and density in a microscopic visual field allowed the distinction between the two species [23].

The same team mentioned that the area needs specialized attention. Species specificity is an important management area for quality control in the meat industry and meat product technology.

The prediction of the quality of meat subjected to freezing and thawing

Freezing is a very important mean of preserving meat. The latter is frequently used, after thawing, as a raw material for many meat products or directly for the preparation of different dishes [24].

Freezing

Frozen storage has always been used to guarantee the quality of meat until it reaches the consumer [25], while it causes structural changes directly related to the freezing speed. When it is fast (0.5°C/minute), it causes the formation of many small ice crystals, evenly distributed inside and outside the muscle cells. When it is slow (0.05°C/minute), it promotes the formation of large ice crystals, at lower levels and in the extracellular region, causing less damage to the cells. In addition to this, freezing usually results in a decrease in the diameter of the muscle fiber and in the length of the sarcomeres [26-28].

At the same time, the use of microscopy has been sufficiently justified as a tool for assessing the degree of cellular damage caused by freezing. This depends on the size and location of the crystals:

The optical microscope showed:

- Serious deformities with ruptured bundles of muscle fibers. Diameters of ice crystals were between 60 μm and 95 μm, depending on the freezing rate [25].
- Altered myocytes and intramuscular connective tissue.
- Ruptures of endomysia [27].
- The electron microscope confirmed this damage by highlighting:
- Degrees of deterioration of the microstructure, depending on the freezing rate [26].
- Alterations of intracellular myofibrils.
- Breaks of sarcomeres [27].

However, more research is needed to establish the exact relationship (in addition to the major impacts) between the rate of freezing and the consequent cellular damage. The general idea that fast freezing damages meat to a lesser extent than slow freezing is to be verified [26].

Thawing

Regarding thawing, the notion that the quality of meat is not only related to freezing methods, but also to the conditions and methods used for thawing were confirmed in the study by Oliveira et al. [28]. The objective was to evaluate the structural characteristics of chicken halves subjected to rapid freezing (at -36°C for two hours) and then thawed by five different methods: under refrigeration, in the microwave, in an oven with air circulation, placed in cold water and at room temperature. Histological analysis of the samples, under the light microscope, after thawing revealed that:

All these methods affected the structural characteristics of the meat:

- Increased intercellular spaces, and decreased cell diameters.
- Disintegration of the characteristic striation of the muscle cell.
- Intracellular edema and even degeneration.
- Thawing in cold water has shown the best results and appears to cause less damage; slight damage to the cell structure allowing it to maintain its properties [28].

The impact of post-slaughter handling on the quality of meat

In the study by Bayraktaroglu and Kahraman [29] on the effect of the "stretching" method on meat quality and ultrastructure (biceps femoris of beef), histological analysis conclude that muscle stretching is a very useful way to improve the tenderness of meat.

After slaughter, the right side of the carcasses was suspended from the Achilles tendon, while the left side was hooked from the pelvic bone and then suspended:

- Under the light microscope, the length of the sarcomeres increased from 0.13 μm to 0.14 μm for the hooked muscle of the pelvic bone, and from 0.12 μm to 2.7 μm for the suspended muscle of the pelvic tendon. Achilles, in 10 days post mortem.
- The electron microscope showed that the ultrastructure of the femoral biceps had the longest sarcomeres and the smallest fiber diameters after stretching (elements directly related to tenderness) [29].

Muscular degeneration

Muscle degeneration, cytoplasmic vacuoles, and disorganization of muscle cytoplasmic striation (a precursor to edema) were observed under the light microscope in the study by Oliveira et al. [28]; this was carried on frozen-thawed chicken halves. Latorre et al. [10] applied the histological method for the detection of unauthorized tissue in meat products, the results showed, in addition, skeletal muscle with signs of degenerative changes, on sausage samples examined. The electron microscope, too, has illustrated degenerate myocytes in the study done on the hot dog by Prayson et al. [5].

Histological examination as an alternative to chemical analysis

On the one hand, chemical analysis does not offer a true approach to detect mechanically separated meat in meat products, an alternative method has to be established [30]. On the other hand, image analysis has been described in the literature as a method that gives objective, precise results comparable to those of chemical tests [2].

The objective of the study by Tremlova and Štarha [2] was to establish a quantitative evaluation procedure by histological examination, to control the bone content in meat products. The results were confirmed by comparison with chemical analyzes, determining calcium levels, and by atomic absorption spectrometry. The correlation of the two methods, histological and chemical, was expressed by a coefficient of 0.78 [2].

For Durand (2005), histology expresses global and complementary results to chemical assays, not only for the detection of bone. For example, the low levels of starch titrated by chemistry may “come from spices”, while starch was not “added”, neither as a binder nor fraudulently. The histological analysis “knows how to differentiate” the two origins of starch [12].

The analytical approach to the use of histological and chemical techniques in parallel could give a relevant idea about the content of raw materials, which directly affects the quality of end products [31]. Both methods were of equal value and had limits; the concept of the used together has, in addition to the advantages, some disadvantages. Nevertheless, histological analysis may indicate or provide a more complete picture of the composition and/or quality of meat and meat products than that obtained through chemical analysis alone [2,4,32].

The histomorphometry

The histological quantitative test, histometry, is one of the reliable scientific means for determining the quality and composition of meat products [32].

The development of “food processing histology” allowed considering its possible use for evaluation, individual components, of these on the quantitative basis, it was a qualitative examination.

For meat products, the quantities were initially described. Later, the content was expressed in percentages; this fact initiated the actual start of the so-called “histometry”, thanks to in-depth analyzes and the proposal of the objective procedures for the evaluation of the results [2].

Studies on the feasibility of using histological methods for the quantitative determination of the components of meat products have been published in a number of documents since the eighties of the last century. Koolmees and Bijker [32] discussed the advantages and disadvantages of using histometric methods for determining the percentage of fibrous tissue and collagen in meat products.

Recently, the quantitative and qualitative accuracy of histomorphometric methods has been largely re-evaluated. For example, for the determination of tissues, animal and plant, not authorized in minced meat previously prepared with contents of 10, 15 and 20% of soy and gizzard, the histometric analysis has proved that it There was no significant difference between the estimated percentages of the added tissue and the true relative percentages, and suggests the technique as an effective method for qualitative and quantitative evaluations of such products [15].

Moreover, histomorphometry is a revolutionary approach and more and more applicable. Several studies have focused on this, to evaluate meat and fat contents, especially in different brands of meat products, and to compare their results with label data [5-7].

Combined histomorphometric and chemical analysis

As meat products are subjected to histometric examination, each tissue is determined directly by microscopic identification, its surface is proportional to its volume in the initial sample. While in chemical analysis, it is evaluated indirectly.

To quantify collagen or muscle proteins, for example, their components which are hydroxyproline and nitrogen respectively, are detected (chemically), then the total protein and collagen contents of/~~in~~ the starting material are calculated by converting: quantify hydroxyproline for collagen and nitrogen for total protein. The muscle protein content can be determined indirectly by subtracting the amount of collagen from the total protein and vice versa [32].

The combined histomorphometric and chemical analysis approach has been studied and applied in some research, especially to evaluate the quality of mechanically separated meat (MSM) obtained by different separation methods:

- The results of the histological analysis, made by Komrska et al. [33], were in agreement with the results of the chemical analysis. Morphometric examination included quantification of muscle, fat, connective tissue (collagen) and bone fragments. Chemical analysis included connective tissue, calcium and fat.
- The quality parameters for chemistry (hydroxyproline (collagen) and calcium (bone) content) and histology (determination of connective tissue and bone particles) were evaluated by Nagy et al. [34]. The results showed that MSM contained twice as much collagen and bone contents.

- The advantages and disadvantages of using combined histometric and chemical methods to detect and quantify collagen in meat products have been studied by the same team 34; the results obtained showed a strong correlation, r=88.

These combined techniques, histometric and chemical, are valid and both provide an objective judgment of the quality and quantity of essential components, meat products. Nevertheless, both methods have certain disadvantages and involve certain errors, they should be used together and not independently [4,32].

Immunohistochemistry

The first use of the immunohistochemical method in the meat products sector, was made in 1999. The study revealed, using specific antibodies "anti-ESN" (anti-enolase specific to neurons), the presence of central nervous tissue; it was a fraudulent addition of bovine brain, in cooked sausages made from meat of the same species. The results have led to the conclusion that "ESN immunohistochemistry" is an appropriate and selective method for detecting central nervous tissue in meat products, which is the main infectivity in bovine spongiform encephalopathy [35].

Immunohistochemical methods were further tested to determine whether brain tissue could be detected in meat products. These were prepared with known levels of central nervous tissue; the results of the application of this technique were variable:

- The heat treatment applied to the products influenced the extent of the immunohistochemical reactions (pasteurization or sterilization).
- Immunoreaction and intensity of staining varied with the type of antibody used, even when the samples contained the same amount of brain tissue.
- The technique has proven its ability to detect brain tissue in finely ground-heated products [36].
- In the two studies done by Prayson et al. [5,6], to detect central nervous tissue, immunohitochemia was also used. While there was no evidence of brain tissue either with microscopic examination or with the use of specific antibodies (glial fibrillary acid protein).

Another study has proven the ability of the immunohitochemical technique, in combination with image analysis, to detect and quantify, this time, soy protein, when it is added fraudulently in meat products [37].

Detection and evaluation of mechanically separated meat

The detection of mechanically separated meat (MSM) in meat products is a major challenge for the meat industry. Most meat processors illegally replace meat partly or wholly with mechanically separated meat in meat products to reduce the economic cost. Thus, emulsion sausages (luncheon) experimentally formulated, with 0, 10, 30, 50, 70, 90% mechanically separated meat instead of meat, were cooked and then examined. Histological sections showed:

- The presence of skin and cartilage, typical elements of MSM.
- The addition of 10% VSM could not be detected after cooking.
- The use of 30% VSM is easily detected; it has significantly changed the technological properties of the product [9].
- As regards the evaluation of mechanically separated meat used in food products, the functional properties of these products must be taken into account; in all cases, it is relatively non-standardized raw materials [31]:
- The notion is not recent, the optical microscope has always been used to evaluate such meat [30].
- In the histological analysis carried out by Botka-petrak et al. [31] on mechanically recovered poultry meat, the results showed longitudinal and transversal cuts of muscle tissue, but also offal products, cartilage and bone tissue in fairly large numbers, with the presence of connective tissue, lipid and lymphatic, which are typical elements of mechanically separated meat (as they indicate the quality of these).

Histological analysis can also be used to evaluate the quality of meat products derived from mechanically separated meat, the properties of which depend on the nature and supply of the raw material used, as well as the adjustment of the machine [1].

Evaluation of certain methods of processing or preserving meat and meat products

One of the most important devices for meat and meat products influencing their qualities, to be accepted by the consumer, is texture; it is understood as the result of the treatments that these undergo [38].

The dynamics of the changes of the histological parameters of the muscle under the influence of the manipulations which the meat undergoes, to be conserved or transformed into a meat product, can be estimated using the Bio imaging:

- The injection of the brine to the muscles has resulted in loosening the muscle fibers, with rounding to the cut.
- Mixing did not cause changes in muscle fiber structure, but it decreased their percentage in a visual field examined.
- Pasteurization increased myocyte circumference and percentage in a visual field examined, compared with the kneaded muscle [39].
- The use of proteolytic enzymes to improve the tenderness of the meat led to the disappearance of the Z disks, following a degradation of the actin myofilaments. Nevertheless, the technique was effective in increasing the tenderness of meat [40].
- The preservation of fresh meat by the addition of salt has also been studied. Salt was added at varying percentages (0.8 and 1.6%). Histological examination mentioned that:
- Perimysium is a barrier to the diffusion of salt in the muscle, unlike the endomysium.

- Cell damage, sarcomere length, and sarcomere attachment to sarcolemite depend on the percentage of salt added [39].

Conclusion

The present study showed the ability of histological techniques in quality control of meat and meat products. Consequently, such an analysis, if properly carried out and interpreted, can provide objective and valid information not only to verify the compliance of these products with the regulations in force but also to ensure the composition, both qualitative and quantitative, of such foods and to detect any malfunction in processing units.

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