Review Paper: Application of Histomorphological Method to Assess Meat Products

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A B S T R A C T

Meat is rich in protein, minerals, and vitamins and therefore a main source of the human diet. Meat products are usually made from processed red meat and preservatives such as nitrate, to improve the overall quality of the food. Histologically, meat is mainly composed of skeletal muscle fibers, water, adipose and connective tissues. Growing bodies of evidence suggests that unauthorized ingredients or tissues in meat products are quite common, and this alters the product quality and safety.

Histological methods make it possible to examine the composition of meat products directly; hence these methodologies are largely used to detect unpermitted herbal and animal contents. Histological and histometric methods are also used to test the percentage and quality of meat in meat products. Freezing and thawing meat cause formation of ice-crystals and muscle fiber modification. This can be identified using histological methodology. Moreover, the changes in pH and mineral content in meat products can be determined using the aforementioned approach. Overall, the histomorphological approach represents a reliable method to investigate the meat product contents and make customers and markets more certain about meat quality. This brief review is focused on the use of the histological method in the assessment of meat products.

1. Introduction

Meat is a food rich in a variety of vitamins, protein, and minerals such as iron and zinc. It is known as a popular source of a variety of essential nutrients that improve the overall diet quality [1, 2].

Processed meat is usually made from red meat, which is cured, salted, or smoked to increase its durability, color and taste [3]. Meat processing technology comprises the steps and procedures of manufacturing processed meat products [4].

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Histology is the study of the microscopic structure of tissues. Tissue is a group of similar, identical, or dissimilar cells, and their extracellular products, performing a specific function [5]. From a histological aspect, meat is composed of skeletal muscle fibers, adipose tissue, connective tissues and other components. Histological study provides the possibility of detecting tissues in meat products directly [6]. In this study, the various histological methods used to assess the quality of processed meat will be discussed based on the available data.

2. Histological Method

Detection of unauthorized tissues

The use of unauthorized animal tissue in meat products is possible due to the economic value of meat itself. Producing meat products with ingredients which are not consistent with the label is considered fraud [7]. Authentication problems in meat products could include replacing meat with other meat species and tissues, vegetable proteins, organic compounds, and also substitute vegetable fats with animal fats [7].

The unauthorized animal and plant tissues can be identified by their histological structure [8]. Histological methods could provide fraudulent and quality control of meat products for the government [9]. The histological methods can also be applied to identify non-meat proteins such as plant proteins (wheat, soya, and flour) [9]. The various types of tissues detected in meat products by the histological method are summarized in Table 1.

Latorre et al. [10] applied haematoxylin and eosin (H&E) to detect the meat content in processed meat products (Kabab loghmeh, sausage, handmade hamburger, and Kabab Koobideh). The histological evaluation of meat products markedly showed that the formulation used to prepare these products does not respect the standard and hygiene food regulation. The unpermitted tissues identified in this study included soya, cartilage, gizzard, ovary, lymph node, and glandular tissues. Another histological examination was carried out on 8 different hamburger [11] and hotdog [12] brands in the United States of America. In these products, hematoxylin-eosin staining showed the presence of bone, collagen, blood vessels, peripheral nerves, adipose, cartilage, and skin tissues as well as plant materials.

Jahed-Khaniki and Rokni [13] showed a 10% presence of the following unpermitted tissues: chicken gizzard, mammary glands, lungs, head soft tissues, and cattle visceral organs in prepared sausage, using Masson's tri-

chrome (MT) histological staining. Sadeghinezhad et al. [14] verified the efficacy of MT and toluidine blue staining for detecting animal and herbal additive tissues in minced meat besides the common H&E staining. Jahed-Khaniki and Rokni [15] showed fine details of soya histology, i.e. palisade, extrudate tissue, and cotyledon cells in 80 samples of frozen raw hamburger using H&E staining. Izadi et al. [16] detected residuals of visceral organs in 20 samples of trade frozen minced meat using a histological approach.

In a study of 60 heated sausage samples from 10 different factories in Iran, researchers revealed the presence of salivary gland tissue and nuchal ligament using histology. This shows that meat from the head region of the slaughtered animals was used [17]. Histological analysis was also used by Botka-Petrak et al. [18] in the evaluation of mechanically deboned poultry used in food products. In this study Botka-Petrak recognized adipose, skin, cartilage, bone, muscular, and lymphatic tissues. In another study, also using a histological approach, animal tissues such as nerves, blood vessels, adipose tissue, mucosa of upper digestive tract, cartilage and glandular tissue were found in "tortellini" (filled pasta) from Italian commercial brands [19].

Sadeghi et al. [20] examined 720 samples of sausages and revealed unauthorized tissues, including adipose tissue (30.8%), heart muscles (19.2%), cartilage and bone (96.2%), immature bone (57.6%), spleen, aorta, esophagus, salivary glands, alimentary glands, lymphatic node, hair, lung and tongue tissues (3.8%), breast, skin and nerve tissues (7.7%), connective tissue and smooth muscles (27%), and blood vessels (46.1%). In addition, herbal tissue was recognized in 100% of the examined samples. Moreover, this research showed that even though skeletal muscle is one of three components of meat products, it did not exist in 3.8% of the samples.

To compare histological method and chemical analysis, as the most usable techniques in detecting unauthorized tissues in meat products, Abbasy-Fasarani et al. [21] examined 44 industrial hamburgers containing 30% and 60% meat. The histological method (H&E staining) detected unauthorized tissues in hamburgers, including chicken skin, chicken gizzard, hyaline cartilage, lymph nodes, bone and breast tissues in meat samples, whereas the chemical analysis (collagen and hydroxyproline determination) did not confirm any. This finding agreed with the observations made by Fekri et al. [22] in samples of heated sausages with 40%, 55%, and 70% meat content.

The immunohistochemical method (IHC) has been mostly used to detect the central nervous tissue (CNT)

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Table 1. Summary of various unauthorized tissues in meat products detected by the histological method.

Product Name	Unauthorized Tissues	Reference
Minced meat	Chicken gizzard, soya, visceral organs, chicken skin, adipose	[14, 16, 26]
Sausage	Soya, cartilage, ovary, lymph node, glandular, chicken gizzard, mammary glands, lungs, head soft tissues, visceral organs, salivary gland tissue, nuchal ligament, chicken skin, hyaline cartilage, adipose, kidney, heart muscles, bone, immature bone, spleen, aorta, esophagus, salivary glands, alimentary glands, hair, tongue, breast, skin, nerve, connective tissue, smooth muscles, blood vessel, herbal tissues, brain tissue, central nervous tissue	[10, 13, 17, 20, 22, 23, 24, 25]
Hamburger	Gizzard, soya, cartilage, ovary, lymph node, glandular tissues, collagen, blood vessels, peripheral nerve, adipose, plant materials, chicken skin tissues, chicken gizzard, hyaline cartilage, bone and breast tissues	[10, 11, 12, 15, 21]
Kabab logh-meh	Gizzard, soya, cartilage, ovary, lymph node and glandular tissues	[10]
Kabab Koo-bideh	Gizzard, soya, cartilage, ovary, lymph node and glandular tissues	[10]
Deboned meat of broiler chicken	Adipose, skin, cartilage, bone and lymphatic tissue	[18]
Tortellini	Nerves, blood vessels, adipose tissue, mucosa of upper digestive tract, cartilage and glandular tissue	[19]

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in heated meat products due to its high specificity [23]. Aside from the adulteration aspect, some animal tissues, like the brain and the spinal cord, can bear infective agents, transmissible to humans [24]. The neuron-specific enolase (NSE) immunoreaction was suggested as a reliable marker of CNT in meat products as a consequence of the extraordinary resistance of the enolase enzyme [25].

The immunohistochemical method using antibodies against NSE was able to detect the brain tissue in cooked sausages by adding different percentages of bovine brain (i.e. 0%, 7.4%, 33.3%); whereas the histological method was not able to detect it [25]. Additionally, Tersteeg et al. [23] used 4 different antibodies i.e. anti-neurofilament (NF), anti-myelin basic protein (MBP), anti-glial fibrillary acidic protein (GFAP), and NSE to detect bovine (in 0%, 1%, 5%, 10%, and 20%) and porcine brain (5%) tissues in raw, pasteurized, and sterilized meat products. Overall, the anti-MBP was suggested as the most useful antibody for detecting brain tissue in heated meat products.

Estimating the percentage of meat and unauthorized tissues

The histological method using image analysis has been used in different studies to estimate the percentage of

meat and unauthorized tissue in meat products. There are some experimental studies that emphasized the accuracy of this method. The efficacy of the histological method for the quantitative determination of unauthorized animal and herbal content in minced meat has been evaluated by Sadeghinezhad and associates [14].

In the mentioned study, the estimated percentages of prepared minced meat with 5%, 10%, 15%, and 20% soya and chicken gizzard showed that the content closely resembled those real percentages. Thus, the histological method is suggested as an acceptable method for the quantitative evaluation of meat products. Furthermore, similar results have been reported for chicken skin as an unpermitted content in minced meat. However, the histological method is not adequate in assessing the quantity of adipose tissue in minced meat, probably due to the fact that the fat is dissolved in xylene during the sample preparation process [26].

Tremlova and Starha [27] applied the histologic method to determine the size and the number of bone fragments in 26 samples of poultry meat products, including sausage, salami, and minced meat. In another study, the histological method was applied and compared with chemical analysis to identify bone fragments and de-

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termine the amounts of these fragments in 29 different poultry products [28].

The percentages of herbal and animal content in meat products have been estimated. In this respect, the samples of muscle tissue with 0.5%, 1%, 2.5%, 5%, and 10% plant material were examined for the detection of additive tissues [29]. In addition, the immunohistochemical method was used to detect soya protein. The study by Pospiech et al. [30] proposed this method as a functional technique in detecting additive soya with different percentages (0.5%, 1%, 2.5%, 5%, and 10%).

Image analysis of histological sections was used to determine the percentage of skeletal muscle in 8 different brands of hamburgers [11] and 8 different brands of hot-dogs [12] in the USA. The results showed a small percentage of skeletal muscle tissue in the mentioned meat products. The percentage of skeletal muscle in the 80 samples of tortellini was determined using histological study. Thus, it was concluded that using histology was an effective method to estimate the amount of skeletal muscle in meat products [19].

Detecting fresh and thawed meat

The histological method mostly applied for detecting fresh and thawed meat by evaluating the ice crystal and muscle fiber modification. Zhu et al. [31] examined ice crystal microstructures (size, formation, and location) in pork muscle tissues during pressure-shift freezing (PSE) using the histological method and compared them with air blaster freezing (ABF) and liquid immersion freezing (LIF). The results showed that the ice crystal size in the PSE-treated pork samples were smaller than the ABF and LIF samples.

In addition, the ice crystal modification in frozen stored bovine meat due to oscillating storage temperatures (-5°C, -10°C, -15°C, and -20°C) were analyzed by histological examination over a 5-month period to investigate the effect of recrystallization on frozen meat tissue [32]. In a similar study, the sections from frozen and unfrozen pork tissues in 3 freezing methods (high-pressure assisted freezing, air blaster freezing, and cryogenic fluid freezing) were stained with H&E. The results revealed that the high-pressure assisted freezing method caused the least structural destruction due to the small size of ice crystals formed during freezing [33].

The histological changes in the thawed beef cut samples in -18 to +10°C at 50 Hz frequency and different voltage gradients (10, 20, or 30 V/cm) were compared with the conventional thawing method. The light microscopy of

tissue sections showed the destruction of myofibrillar elements in both thawing methods, meanwhile the amount of structural changes in conventionally thawed samples, were observed to be much higher than the ohmic thawed samples [34]. Also, Anese et al. [35] carried out a histological study on 3 freezing methods in meat: radio-frequency (RF) to assist cryogenic freezing, cryogenic freezing, and air freezing. The results showed a larger number of smaller ice crystals at intracellular level in RF to assist cryogenic freezing. Also no significant difference was seen between structural changes in the unfrozen samples and RF to assist cryogenic freezing.

Detecting meat structure

The histological method has been used to determine the chemical changes like pH and mineral content of meat products by evaluating the structural features in muscle fibers. Sharedeh et al. [36] examined histological modifications like the fiber cross-section area and the extracellular space in meat samples with different pH range. The histological modifications in fiber cross-section area and the extracellular space in meat samples with the pH range of 6.5, 5.4 or 4.3 and 0.9% or 2% NaCl content were evaluated. The image analysis showed an increase in extracellular space and a decrease in fiber cross-section area when pH decreased from 6.5 to 4.3.

The histological analysis in prepared sausage using sodium chloride (NaCl) and potassium lactate (K-lactate) showed effects on the meat structure. These structures were more sensitive in sheep meat than bovine and pork meat [37]. In another study; pale, soft, exudative (PSE) zones in normal ham samples were analyzed by histochemistry. Light microscopy of PSE zones in the meat showed disruption and disorganization of fiber alongside inter fiber spacing increment [38].

4. Discussion

In some cases the meat production does not fulfill the standards and some producers use unauthorized methods in preparing these products. Thus, it is important to use accurate methods for the quantitative and qualitative analysis of the ingredients in meat products with regard to authentication problems in this field. Chemical and microbiological methods alone cannot control the quality of meat products. Based on histological features, the histological method can be applied to identify non-meat tissues and morphological changes in the meat structure. Thus this approach is a reliable tool to assess the quality of meat products.

In addition, the histological method is able to estimate the percentage of meat and unauthorized animal and herbal tissues in meat products using image analysis. However, the histological technique is not able to reveal all combinations of meat products and their percentages with high sensitivity.

Currently other methods such as immunoassays, genetic techniques, and spectrometry analysis have been used as complementary techniques for quality assessment in meat products. In general, histological analysis of meat products is useful to reduce the factors which threaten the consumer's health and above all, it can be used to detect and control unauthorized agents.

Acknowledgments

Conflicts of Interests

The authors declared no conflict of interest.

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