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Detection of adulteration in locally marketed retail meat products: Combined histological and chemical analyses

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ABSTRACT

Species authentication in food of animal origin is an urgent necessity for food control since food adulteration is relevant to religious, economic, and public health concerns. This study was conducted to apply histological and chemical means of identification to detect commercial fraud in different meat products. To achieve this aim, about 60 samples including, 20 samples each of minced meat, luncheon meat, and sausage were collected from various markets in Beni-Suef, Egypt. All samples were examined histologically using hematoxylin and eosin stains for the detection of foreign tissue. The examination was confirmed with acid-chiffon blue and Masson's trichrome stain. Additionally, meat product samples were chemically examined for determination of fat, protein, starch, and nitrite. The results revealed that the examined minced meat, luncheon, and sausage samples were adulterated by different tissues including elastic artery, spongy bone, skin, fibrous connective tissue, visceral muscles, visceral organs, cartilage, tendon, secretory gland, and plant tissue. The percentages of fat accounted for 17.75±0.9, 13.97±0.40, and 11.23±0.5 %, while the percentages of protein were 19.47±0.6, 15.99±0.3 and 16.67±0.7 % in minced meat, luncheon meat, and sausage samples, respectively. Sodium nitrite was below the detection limit in all minced meat samples and 40% of luncheon samples, nonetheless, 60% of luncheon samples and all sausage samples contained detectable levels of sodium nitrite. Furthermore, starch was undetectable in all minced meat samples and 40% of sausage samples, on the other hand, all examined luncheon samples and 60% of examined sausage samples contained detectable starch levels. In conclusion, the histological and chemical examination could be valuable methods to detect adulteration in meat products.

Introduction

Meat is an abundant source of different essential nutrients, such as proteins, long-chain polyunsaturated fatty acids, vitamins, and minerals (Binnie *et al*., 2014). A combination of meat items with fat, water, and other ingredients can be used to make processed meat products. Meat products are highly popular and widely consumed all over the world. This is regarding their taste, flavor, juiciness, palatability, and ability to provide high biological values, in addition to their low price (Decker and Park, 2010). Accordingly, meat products should be produced under hygienic conditions following standard rules. Therefore, the authentication of food has been one of the most worrisome issues in the global food industry, especially for consumers with unique nutritional, lifestyle, cultural, and religious needs (Haider *et al.*, 2024).

The adulteration of food is a current socioeconomic worldwide problem affecting economic investment, and public health, among others. Accurate labeling is vital to support and promote fair trade. The regrettable mismatches between the ingredients used and those declared on the meat product labels are becoming increasingly common, leading to spikes in consumer concerns (Ballin, 2010; Hrbek *et al*., 2020). There are several types of meat adulteration. One relatively common fraudulent type that poses ethical, religious, and dietary issues includes the replacing of highly commercial-valuable meats with cheaper or unattractive ones (Chuah *et al*., 2016). Undeclared meat species can cause foodborne or zoonotic infections and allergic reactions (Di Pinto *et al*., 2015). Moreover, substituted species may be unsanitary and not pass meat inspection (Alarcon *et al*., 2017). Another sort of meat product adulteration is the addition of various flavors to mask the presence of media needed by microorganisms to perform their actions; however, this addition increases the toughness of meat. Furthermore, many undesired ingredients may be added to meat products such as sausage and luncheon meat to reduce their price or processing cost (Lumley, 1996).

Nowadays, over 2500 additives are used around the world to create the desired taste and smell (Mega and Tu, 1995). Even though chemical additives are necessary for meat product processing, higher levels than permissible may pose public health hazards and/or technological problems. Nitrates and nitrites are the two most commonly used curing agents in meat products. Nitrites, with antioxidant and antibacterial properties, are responsible for the red color and taste of cured meat (Honikel, 2008). On the other hand, the excessive addition of nitrites beyond the permissible limits has a potential carcinogenic risk due to the formation of N-nitrosamine compounds that react with the secondary amines in the acidic environment of the stomach. This reaction may cause death due to increasing the risk of colorectal, stomach, and pancreatic cancer (Larsson and Wolk, 2012; Rohrmann *et al*., 2013). As a concern in this field, the WHO (2002) established an acceptable daily intake (ADI) of 0-3.7 mg nitrates /Kg body weight.

Starch has traditionally been used in meat products to improve quality and occasionally to extend the more expensive meat fraction of various products. The impact of adding starch relies on its capacity to undergo gelatinization when exposed to heat in a medium containing water, resulting in the binding of significant quantities of water. The addition of a high starch content (< 5%) is considered a sign of adulteration and deteriorates the slicing capacity, resulting in dry products (Sison *et al*., 1975).

Currently, various methods are used for food authentication such as protein-based methods including high-performance liquid chromatography (HPLC), electrophoretic techniques, enzyme-linked immunosorbent assay (ELISA); and DNA-based assays. These methods are complex, time-consuming, and expensive. The best methods for quality control are histological tests, which in many studies have been shown to detect the fraud of food products (Latorre *et al*., 2015). Therefore, the present study aimed to investigate the efficiency of histological and chemical analyses of meat products to detect adulteration by other animal tissues and chemical ingredients above permissible limits.

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Materials and methods

Sample collection and preparation

A total of sixty meat product samples, 20 each of luncheon, sausage, and minced meat were collected from retailers and markets in Beni-Suef governorate. The samples were identified and immediately transported in an isolated ice box to the Faculty of Veterinary Medicine, Beni-Suef University. The collected samples were kept frozen at -18oC and analyzed within 5 days of collection. All chemicals used in the current study were chemical grade and purchased from Sigma Aldrich (Saint Louis, MO, United States) and El-Gomhouria Company (Egypt).

Histological examination

The samples collected were processed according to the techniques recommended by Bancroft & Stevens (1996) and Bancroft & Gamble (2008). The samples were first dehydrated by ascending grades of ethyl alcohols, cleared in xylene, impregnated in soft paraffin, embedded, and blocked in hard paraffin. The blocks were cut by microtome 4-6 µm thickness and mounted on clean and dry glass slides. The obtained slides were stained by H&E as a general stain, PAS technique for demonstration of glycogen and Masson's Trichrome stain for demonstration of collagen fibers for histopathological examination using LEICA (DFC290 HD system digital camera, Heerbrugg, Switzerland) connected to the light microscope using 10, 20, 40 objective lens.

Chemical examination of samples

At the Laboratory of Food Hygiene and Control, Animal Health Research Institute, Dokki, Egypt, the chemical profiles (protein %, fat %, nitrite %, and starch) of the collected samples were determined as follows:

Determination of protein percentage

The AOAC Official Method 981.10 (1982) was applied, briefly 2 g well-ground and thoroughly mixed sample was digested using 15 mL H_2SO_4, 3 mL 30–35% H_2O_2 at 410°C for 45 min. The digest was distilled and received in a flask containing 25 mL H_3BO_3 solution with a mixed indicator. The absorbing solution was titrated with 0.2M HCl to neutral gray end point and the required volume of acid was recorded to 0.01 mL.

N, % = (VA − VB) ×1.4007 ×N/g test portion

Protein %= (VA − VB) ×1.4007 ×N ×6.25/g test portion

Where VA and VB stand for volume standard acid required for test portion and blank, respectively; 1.4007 = mile equivalent weight N ×100 (%); N = normality of standardized acid; and 6.25 = protein factor for meat products (16% N).

Determination of Fat percentage

Three grams of sample were weighed into a thimble. The thimble was dried in the oven (Heraeus, Germany) for 1 h at 125°C, then removed and left to cool. The thimble was transferred to the extraction unit and extracted with 40 mL petroleum ether in boiling position for 25 min and in a rinsing position for 30 min. The temperature of the extraction unit was adjusted to ensure a condensation rate of ≥5 drops/s. The cup and contents were dried for 30 min at 125°C, cooled and weighed (AOAC Official Method 991.36, 1992).

Estimation of nitrite percentage

The residual nitrite level was determined using the spectrophotometric method prescribed by AOAC (2002). Briefly, ten grams of thoroughly mixed, finely comminuted sample were de-proteinized using saturated borax solution. A series of nitrites standard solutions were prepared. For color development, sulphanilamide ($NH_2C_6H_4SO_2NH_2$) and N-1-naphthylethylenediaminedihydrochloride ($C_{10}HNHCH_2.2HCl$) solutions were added to tested, standard nitrite solution and blank samples. The absorbance of the solutions of both standard and tested samples was measured spectrophotometrically (Unico-UV-2100 spectrophotometer, USA) at 538 nm against the blank. The nitrite content of the sample was expressed as milligrams of sodium nitrite per kilogram (ppm).

Estimation of starch content

Five grams of the samples were treated with boiled water, then cooled and the superior liquid portion was treated with Lugol solution (obtained by solving 0.5 g iodine and 1.5 g potassium iodide in water and then completed to 25 ml volume with water). The appearance of blue color indicates positive results. If the color is very strong, this may be a clue that there is a high starch content or other cereal products supplement (with a fraud intention) (AOAC, 2005).

Statistical analysis

Means were compared at a significant level of 0.05 by Analysis of variance (ANOVA) using SPSS 17.0 for Windows (SPSS Inc, Chicago, IL, USA).

Results

In minced meat various non-meat tissues were recorded including a large artery or elastic artery, spongy bone [recognized by hematopoietic spaces, and bone trabeculae (arrow) that contained osteocytes located in the lacunae (arrowhead)], skin (identified by epidermal membrane), degenerated muscle fibers (recognized by the loss of cross-striations), fibrous connective tissue rich with collagen bundles, visceral muscles (smooth muscle fibers with an elongated flat nucleus), and white fat cells (Figs. 1 and 2).

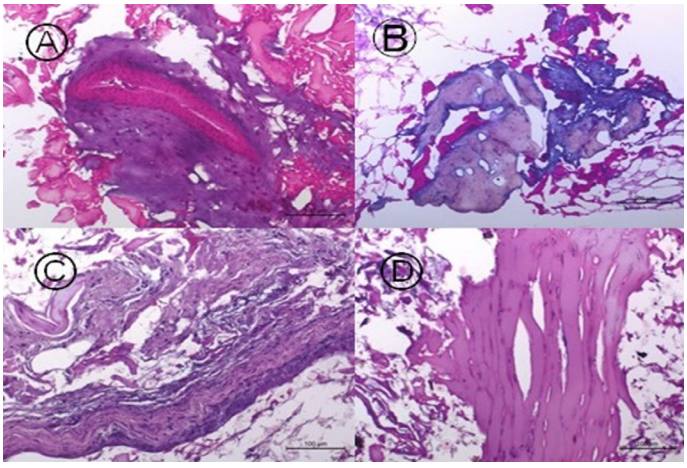

Fig. 1. Paraffin sections showing adulteration of the minced meat. (A) Minced meat sample was adulterated by a large artery or elastic artery. (B) Minced meat sample containing spongy bone, which was recognized by haematopoietic spaces and bone trabeculae (arrow) that contained osteocytes located in the lacunae (arrowhead). (C) Minced meat samples were adulterated by skin, which was identified by epidermal membrane. (D) Minced meat sample containing degenerated muscle fibers that were recognized by the loss of cross-striations.

Luncheon samples were adulterated with different kinds of other animal tissues including visceral organs which were identified by the characteristic spindle-shaped muscle cells that had centrally located nuclei, avian gizzard, proventriculus, cartilage, skin with epidermal layer, and compact bone (Figs. 3 and 4).

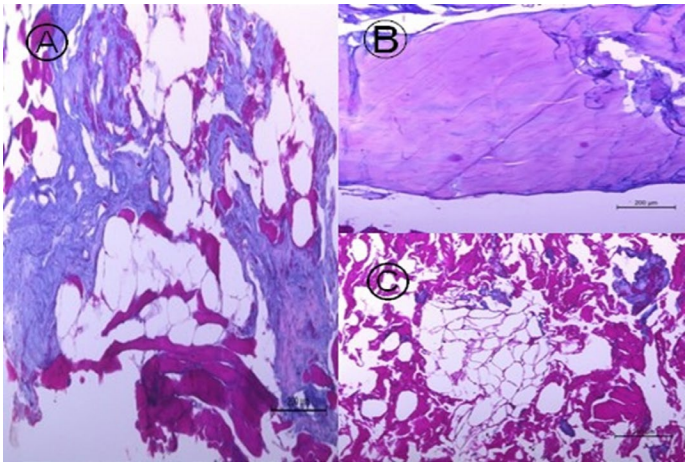

Fig. 2. Paraffin sections showing adulteration of the minced meat. (A). Minced meat sample containing fibrous connective tissue rich with collagen bundles. (B). Minced meat sample containing visceral muscles (smooth muscle fibers) with elongated flat nuclei. (C). The Minced meat sample containing white fat cells.

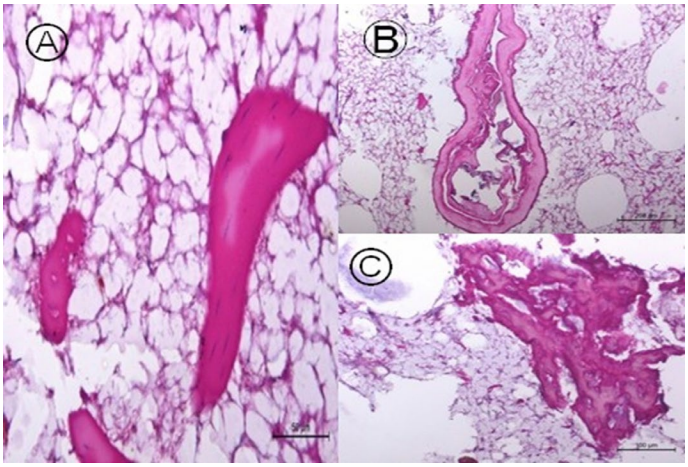

Fig. 3. Paraffin sections showing adulteration of the luncheon. (A) luncheon sample containing smooth muscle fibres of the visceral organs were identified by the characteristic spindle-shaped muscle cells that had centrally located nuclei. (B) luncheon sample containing avian gizzard and proventriculus. (C) luncheon sample containing cartilage with chondrocytes inside the lacuna.

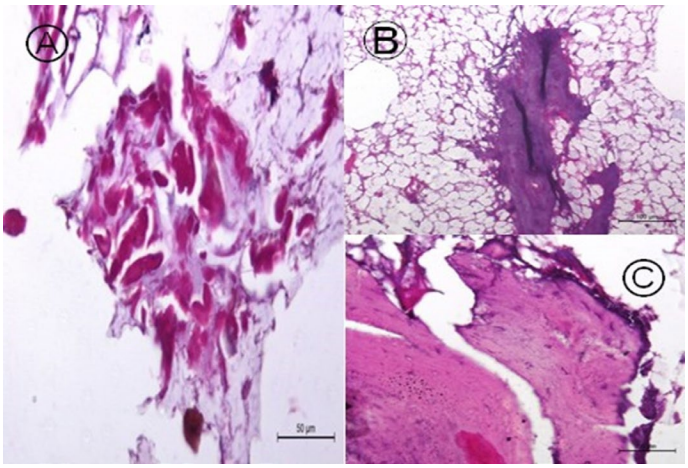

Fig.4. Paraffin sections showing adulteration of the luncheon. (A) luncheon sample containing degenerated muscle fibers. (B) luncheon sample containing skin with epidermal layer. (C) luncheon sample containing compact bone with characteristic Haversion system with central canal, concentric bone lamellae, and osteocytes inside lacuna.

In sausage samples, several unauthorized tissues were noticed in sections observed by light microscope including part of a tendon that was distinguished by the regularly arranged collagen fibers, part of hyaline cartilage, part of a secretory gland, part of compact bone, part of lung tissue with bronchus, bronchioles and alveoli, part of degenerated muscles and fibrous tissue with collagen fibers and part of plant tissue (Figs. 5 and 6).

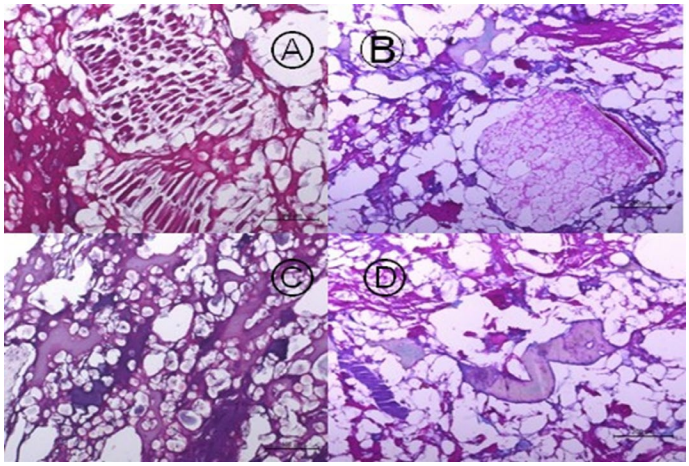

Fig. 5. Paraffin sections showing adulteration of sausage. (A) Sausage sample adulterated by part of a tendon was distinguished by the regularly arranged collagen fibers. (B) Sausage sample adulterated by part of a secretory gland. (C) Sausage sample adulterated by part of hyaline cartilage, which was identified by chondrocytes located in the lacunae (arrowhead) and embedded in the cartilage matrix (arrow). (D) Sausage sample adulterated by part of compact bone with characteristic Haversion system and osteocytes inside lacuna.

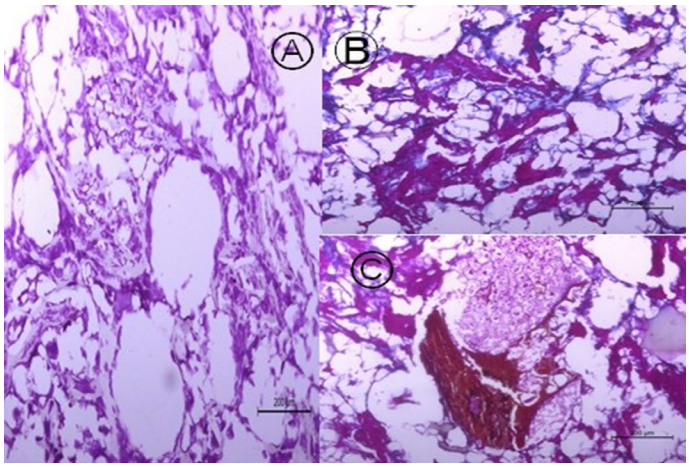

Fig. 6. Paraffin sections showing adulteration of sausage. (A) Sausage sample adulterated by part of lung tissue with bronchus, bronchioles and alveoli. (B) Sausage sample adulterated by part of degenerated muscles and fibrous tissue with collagen fibers. (C) Sausage sample adulterated by part of plant tissue.

In the present study, protein content in minced meat samples ranged from 14.16 to 24.45 % with a mean value of 19.47±0. 6%, while it was 13.83 to 19.59% with a mean value of 15.99±0.3% for luncheon samples, and 10.77 to 21.94 % with a mean value of 16.67±0.7 % for sausage samples. The fat content in the examined minced meat samples ranged from 11.07 to 25.47 % with a mean value of 17.75±0.9%, 10.02 to 16.88% with a mean value of 13.97±0.4 % for luncheon samples, and 7.01 to 15.84 % with a mean value of 11.23±0.5 % for sausage samples.

Sodium nitrite was under the detection limit (11.59 PPM) in all examined minced meat samples and 40% of luncheon samples. While it was above the detection limit in 60% of luncheon samples and all sausage samples. Starch was undetectable in all examined minced meat samples and 40% of sausage samples while all examined luncheon samples and 60% of examined sausage samples were positive for starch.

Discussion

The diversity of the finished meat products in the consumer market in Egypt is quite great, but many products vary in quality. As such, quality control of meat and meat products is very important (Lyubchyk *et al.*, 2016). Manufacturers should ensure that the meat product quality and ingredients agree with the applied local and international food regulations and standards (Malakauskienė *et al.*, 2016).

Recently, the single most troublesome issue that has become visible worldwide is meat adulteration (Sadeghinezhad *et al.*, 2015), which violates food safety, health regulations, and religious beliefs. Accordingly,

precise methods can be utilized to quantitatively and qualitatively analyze the ingredients in processed meat products to solve the authentication problem in the meat industry. Chemical methods alone could not be used to assess the properties of meat products. Histological techniques could be used to directly identify other tissues in addition to meat and changes in the structure of meat (Sadeghinezhad *et al*., 2016).

In the current study, different authorized and unauthorized tissues were detected in the examined samples including skeletal muscles, cartilage, connective tissue, bone, nerve fibers, adipose tissue, growing bone, secretory glands, smooth muscles, alveolar elastic tissue, and fibrous tissue, gizzard, in addition to the presence of plant tissues in sausage samples. These results were similar to those of Mokhtar *et al.* (2018); Malak *et al.* (2020) and Shaltout *et al.* (2023).

In this regard, Dayyani *et al.* (1998) detected histologically smooth muscles in meat products. Prayson *et al*. (2008) detected adipose tissues, connective tissue, cartilage, blood vessels, and bone in meat products. Furthermore, Sepehri Eraei (2008) detected unauthorized tissues such as nerves, cartilage, blood vessels, and adipose tissues. Additionally, Latorre *et al.* (2015) showed the presence of different ratios of unpermitted tissues, such as cartilage, heart muscles, bone, spleen, lymph node, and esophagus, in different sausage samples. The results of the present study reported the presence of secretory glands in sausage samples in agreement with Dayyani *et al.* (1998) who also reported the presence of the salivary gland in Iranian sausage; however, it could not be detected by Abdel-Maguid *et al.* (2019). This difference may be attributed to the fact that sections were taken randomly from the used organ.

The examined samples were also adulterated with hollow organs, an observation that agrees with the studies of Cetin *et al.* (2010) and Abdel Hafeez *et al*. (2016). In this concern, İnce and Özfiliz (2018) detected alveolar tissues in Turkish-type sausage samples. However, Ayaz *et al.* (2006) detected bronchi with no alveolar structures in the examined samples.

The smooth muscle fibers of visceral organs were detected in the luncheon meat samples, these findings follow those of İnal (1992), who reported that the muscle cells of the intestinal mucosa and heart muscles were detected in salami and sausage samples.

The results obtained showed that immature or growing long bone was present in minced meat, sausage, and luncheon samples. The appearance of bone tissue in samples was frequent, as mentioned by Tremlová and Štarha (2003), who quantified bone tissues in meat products by image analysis. Since bone fragments do not ordinarily exist in meat products, their presence can highlight the trouble of minimally processed meat material (Pospiech *et al*., 2011), which has a real negative impact on the quality of these meat products.

The results indicated that luncheon samples were adulterated by skin, which is in accordance with Izadi *et al.* (2016), who reported the presence of avian skin and adipose tissues in minced meat samples. Additionally, the results of the current study revealed the presence of gizzard tissues in the luncheon samples examined. The presence of gizzard in meat products is considered a fraud and confirms the mixing of avian organs in minced meat. These results agreed with Shaltout *et al.* (2023).

As revealed by the results obtained, the sausage samples were adulterated by part of plant tissue. Sadeghinezhad *et al.* (2015) focused on the qualitative and quantitative accuracy of histological investigation as 5, 10, 15, and 20% of soya and chicken gizzard were constructed in order to determine the amount of herbal content and unapproved animal in minced beef meat. The use of plant-based additives in meat products lowers meat quality and may have an allergic effect on some consumers (Pospiech *et al*., 2009).

The current study revealed the existence of degenerative muscle in all types of samples in agreement with the results obtained by Abdel-Maguid *et al.* (2019). Consumers need to know about processed food and the differences between the 1st type of skeletal muscle (slow contracting, dark fiber) and the 2nd type (fast contracting, light fiber). This knowledge is important because the detection of meat softness depends on the percentage of various fiber types according to the proportion of light and dark fibers (Picard *et al*., 1998). Additionally, the proportion of light and dark fibers is a fitting metric for adult and fetal meat detection. The skeletal muscle of a bovine fetus contains primarily more dark fibers than light fibers (Crosier *et al*., 2002). In this regard, Buche and Manron (1997) evaluated meat quality through image analysis to determine the marbling of meat or to measure the different muscle fiber parameters.

All the unauthorized tissues, whether edible or inedible, could cause a risk to humans as a major cause of food poisoning in humans (Scallan *et al*., 2011). For these reasons, the criteria established by the International Commission on Microbiological Specifications for Foods (ICMSF) have been extensively used for estimating the hygienic quality of edible offals (Roberts *et al*., 1996).

The present study revealed that the presence of authorized and unauthorized tissues in minced meat, luncheon, and sausage samples is detectable by the histological method. Thus, the histological technique may be a simple and affordable tool to assess meat adulteration and enhance the quality and hygiene of meat. Different kinds of staining methods were used to detect authorized and unauthorized tissues in food products, which concludes that histological methods are practical techniques for routine assessment of the authenticity and quality of food products to protect consumers from fraudulent practices.

The protein content in minced meat and sausage in the current study was nearly similar to the results obtained by Mokhtar *et al*. (2018) who examined minced meat and sausage and found that protein content was 20.56 and 19.78 %, respectively. On the other hand, they found lower fat percent (9.92 and 8.06 %) in minced meat and sausage respectively.

The protein and fat contents in examined minced meat and sausage do not comply with the Egyptian standards (ES, 2005), which state that minced meat preparation must have a fat percentage of not above 20% and a protein percentage of not below 18 %, while the sausage preparation must be of no higher than 30% fat and no lower than 15% protein.

the obtained results revealed that sodium nitrite was under the detection limit (11.59 PPM) in all minced meat samples and 40% of luncheon samples. Yet it was above the detection limit in 60% of luncheon samples and 100% of sausage samples. Nitrite was not found in the examined minced meat samples in agreement with the results of El Bayoumi *et al*. (2023) and Shaltout *et al*. (2020). While 92.5% of the sausage samples analyzed were within the permitted limits regarding their nitrite level. The obtained nitrite levels (ppm) for sausage samples in the current study were higher than those recorded by Nayel (2013); EL-Zahaby (2013); Maky *et al.* (2020); Saad *et al*. (2018a); Abdel-Atty *et al.* (2022) and Shaltout *et al.* (2020). The results of nitrite levels in luncheon were similar to those obtained by Abdel-Atty *et al*. (2022) and Shaltout *et al*. (2020). Higher values of nitrite levels than the values of the current study in luncheon samples were recorded by Nayel (2013); Sorour *et al*. (2022); EL-Khawas, (1996), and Saad *et al*. (2018a). In this respect, higher results for luncheon were also obtained AOAC Tolba *et al.* (1994) and Aiedia (1995) as they recorded 137.7±8.05, 118.9 and 134.7±2.4 ppm, while lower values were recorded by Viuda-Martos *et al*. (2009).

When added to meat, nitrite, and nitrate serve the purpose of giving the meat three desired qualities. First, it stabilizes the reddish-pink hue in nitrite-cured meat. Second, nitrite improves flavor by delaying its breakdown. The third role is to prevent Clostridium botulinum from producing toxins. Thus, meat processors insist on using nitrite for these purposes because there is now no other options (Gray *et al*., 1981).

In this study, starch was undetectable in minced meat samples while was detectable in all examined luncheon samples and 60% of examined sausage samples. Lower percentages of positive samples were recorded by Saad *et al.* (2018b) who found that about 63.3% of examined sausage samples and 83.3% of examined luncheon samples gave positive results to starch detection. In this respect, EL-Sayed (1995) said that the mean values of starch % in luncheon were 5.17±0.35. While lower results were recorded by El-Zahaby (2013) for luncheon samples. The composition of

the products examined in this study showed that all the suppliers of these meat products do not completely comply with the standards of meat product ingredients and specifications.

Conclusion

Results of the current study demonstrated that minced meat, luncheon, and sausage samples were adulterated by different tissues including elastic artery, spongy bone, skin, fibrous connective tissue, visceral muscles, visceral organs, cartilage, tendon, secretory gland, and plant tissue. The results of protein and fat analysis in minced meat and sausage are not harmonized with the Egyptian standards specification. Sodium nitrite was detected in 60% of luncheon samples and all sausage samples Furthermore, all the luncheon samples examined and 60% of examined sausage samples were positive for starch.

Conflict of interest

The authors declare that they have no conflict of interest.

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