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Histological assessment of the quality, safety, and fraudulence risk of commercially frozen processed beef and chicken meat products

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ABSTRACT

Ensuring the quality and safety of frozen beef and chicken products is crucial for protecting human health, especially when food adulteration poses a major challenge. To fulfill this target, 120 samples of commercial frozen processed beef meat (beef burger, cocktail beef sausage, beef hotdog, beef salami) and chicken products (chicken burger, chicken cocktail sausage, chicken nuggets, chicken kofta), 15 samples each, were histologically examined to identify various unfavorable tissue types. Samples were subjected to tissue sectioning, then they were stained using both hematoxylin & eosin (H&E) and Crossmon's trichrome stain to detect unauthorized plant or undesirable animal tissues. The findings revealed highly significant ($P \leq 0.001$) adulteration in the majority of examined commercial frozen beef and chicken product samples, with the presence of unauthorized animal tissues such as lung, heart, smooth muscle, blood vessels, brain, intestine, uterus of a bitch, and excessive plant tissues with low skeletal muscle content. This study demonstrated the effectiveness of histological examination in detecting adulteration and evaluating the quality and safety of frozen beef and chicken products. The results highlight the need for strict control over formulation processes and continuous monitoring and inspection to ensure the quality and safety of frozen meat products.

Introduction

Food adulteration, particularly in the meat industry, significantly challenges food safety and consumer trust, one of the most common forms of fraud in this sector is the substitution of high-quality meat with cheaper alternatives, which undermines product integrity and consumer rights (Sohrabi *et al*., 2020), especially in developing countries. Thus, the diversity of meat products in the market necessitates rigorous monitoring to ensure both safety and quality (Tokarev *et al*., 2019). Consumers in these countries increasingly demand meat products that meet their expectations in terms of quality, safety, and affordability, making the detection of adulterants crucial for maintaining market integrity (Agamy and Hegazy, 2011; Potter, 2001).

Food adulteration of meat products often involves the addition of non-meat ingredients or the substitution of valuable meat species with cheaper ones (Maghami *et al*., 2022) as unauthorized animal tissues, such as organs, cartilage, and bones (Frank and Habn, 2003; Trienekens and Zuurbier, 2008) or with plant-based proteins, such as soybean and grain derivatives (Ballin, 2010; Parchami Nejad *et al*., 2014). Additionally, the use of unauthorized animal tissues, such as organs and cartilage, in processed meats like hamburgers and sausages violates food hygiene regulations and deceives consumers.

Processed chicken products are widely accepted for their sensory appeal, including color, odor, taste, and texture (Agamy and Hegazy, 2011; Potter, 2001), the issue necessitates more restricted monitoring to avoid fraudulent and adulteration.

Since grinding and heating alter meat's sensory characteristics, complicating the identification of fraud (Ayaz *et al*., 2006), the traditional methods like serological tests are often limited in sensitivity and are less commonly used today (Amaral *et al*., 2014; Terrell and Hernandez-Jover, 2023), the conventional laboratory techniques for detecting meat fraud are complex and time-consuming, there is a growing demand for faster forensic methods (Edwards *et al*., 2021). Histological examination has proven to be an effective tool for identifying fraud in meat products (Latorre *et al*., 2015), enables precise differentiation of animal and plant components in meat products, and is highly effective in identifying bone fragments and assessing tissue suitability (Ghisleni *et al*., 2010).

The aim of this study was to evaluate histological techniques foe the assessment of quality and safety and to detect risky ingredients in commercial frozen processed meat and chicken products from various Egyptian brands. This study also sought to verify the accuracy of product labeling, ensure compliance with food safety standards, and identify any undeclared or unauthorized components in these products.

Materials and methods

Samples collection

A total of 120 specimens were obtained from retailed frozen processed meat with different trademark qualities, which included 4 beef meat products (beef burger, cocktail beef sausage, beef hotdog, and beef salami), as well as 4 chicken meat products' samples (chicken burger, chicken cocktail sausage, chicken nuggets, and chicken Kofta). 15 samples for each were randomly collected from different hypermarkets and local shops in various localities in Assiut City, Egypt, spanning the period from October 2023 to July 2024.

Histological examination technique

Three 1×1×0.5 cm pieces were dissected from each sample area. These specimens were immediately fixed in Bouin's solution for 22 h. The following fixation, the tissues underwent dehydration through a series of graded concentrations of ethanol, were cleared with methyl benzoate, and embedded in paraffin wax. Three paraffin blocks were prepared

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from each sample, and serial sections of 5–7 µm thickness were cut using a Richert Leica RM 2125 microtome (Germany). The sections were then mounted onto glass slides and incubated at 40°C for drying. Then, they were stained with Hematoxylin & Eosin (H&E) (Mokhtar *et al.*, 2018) as well as Crossmon's trichrome stain, following the methodology outlined by Bancroft *et al.* (2013). Images of the stained sections were captured using an OLYMPUS DP72 camera mounted on an OLYMPUS BX51 microscope to facilitate the detection and identification of various tissue types.

Morphometric Measurements

Morphometric measurements were conducted using Image-J software to estimate the percentage of skeletal muscle (SKM) against undesirable contents (animal tissues and plant tissues) present in the beef and chicken products.

Statistical analysis

Data were analyzed using Statistics Package for Social Sciences (SPSS) version 27.

Results

The results in Figure (1A) showed that frozen beef meat products were adulterated and non-conforming to their labeling. The adulteration rate was higher in beef salami (26.7%), than beef burgers (20.0%), beef hotdogs (130.3%) and beef cocktail sausage (6.7%).

The data in Figure (1B) showed that the highest adulteration percentage was in chicken burgers (86.7%), followed by chicken nuggets (80.0%), and cocktail chicken sausage (73.3%). Chicken kofta had the lowest adulteration rate (60.0%) among the examined products.

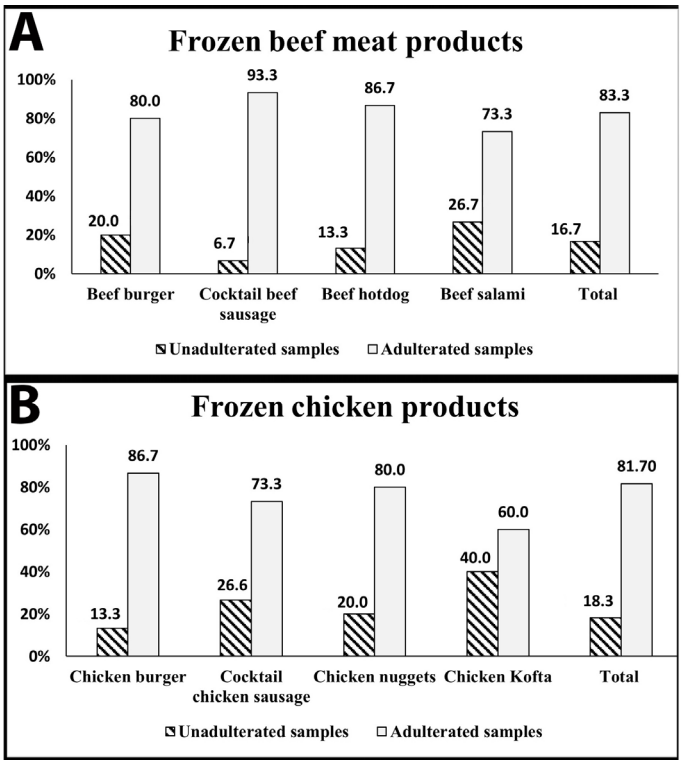

Figure 1. Percentage of unadulterated and adulterated frozen beef (A) and chicken (B) meat product samples based on histological examination.

Figure (2A) provides that the percentage of SKM content was lower than the percentages of animal and plant tissues (unauthorized adulterated components) of all examined frozen beef products. The lowest percentage of plant tissues (21.89%) was observed in beef burgers.

Based on histological examination, Figure (2B) shows a high percentage of plant tissues in all chicken products and animal tissues in chicken nuggets and chicken kofta compared with a notably low SKM content, reflecting a greater reliance on non-muscle components.

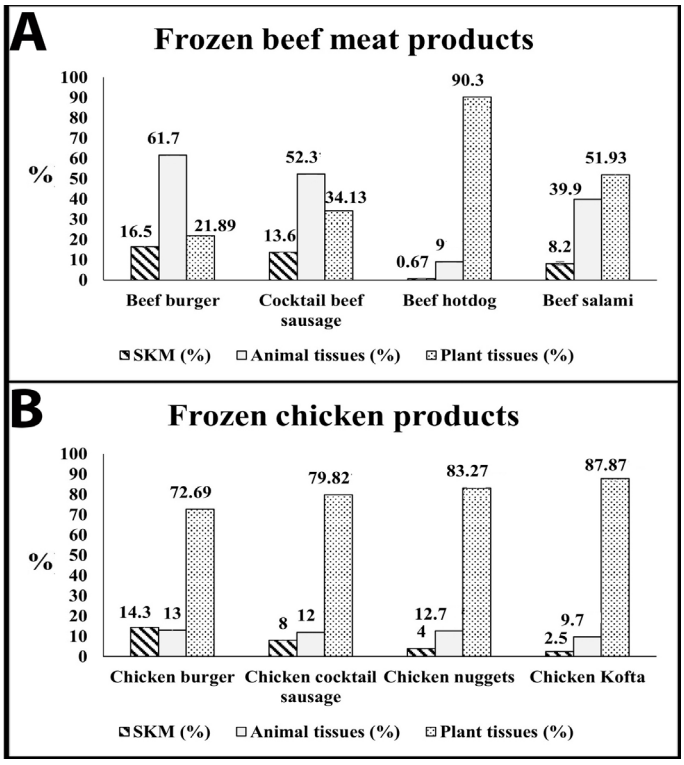

Figure 2. Percentage of SKM, animal, and plant tissues determined in different frozen beef (A) and chicken (B) meat product samples.

The findings presented Figures 3, 4 & 5, revealed the presence of unauthorized animal tissues in frozen beef meat products (of brain tissue, collagen fibers, spongy bones, blood vessels, intestinal tissue, gastrointestinal (GIT) wall and inflammatory cells, lung, intestine, smooth muscle, artery heart, tendon, and smooth muscle fibers). Additionally, a lot of plant materials, particularly soya was identified.

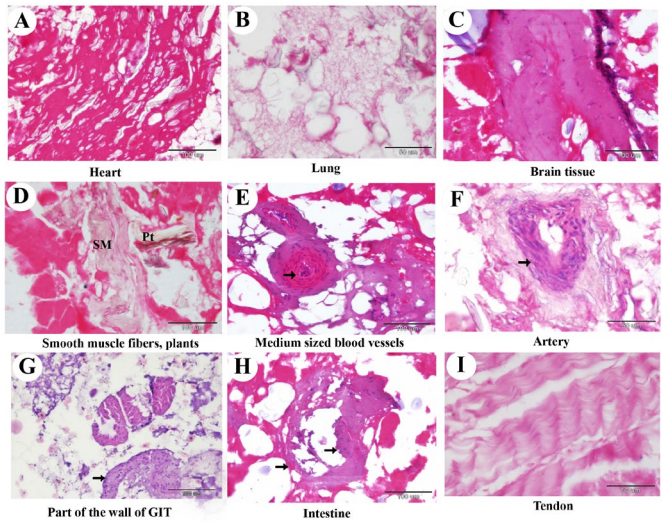

Figure 3. Paraffin sections of frozen raw beef meat products stained with H&E, revealing various unauthorized animal tissues. (A) Heart tissue found in beef salami, (B) Lung tissue detected in beef hotdogs, (C) Brain tissue identified in beef burgers and cocktail beef sausages, (D) Smooth muscle fibers and plant material observed in beef hotdogs, (E) Medium-sized blood vessels (arrow) present in cocktail beef sausages, (F) Artery (arrow) found in beef hotdogs, (G) Section of the gastrointestinal tract wall (arrow) detected in cocktail beef sausages, (H) Intestinal tissue (arrows) identified in beef hotdogs, and (I) Tendon present in beef salami.

Also, the histological analysis of frozen chicken products, as shown in Figures 6, 7 & 8, revealed the presence of various unauthorized tissues and cells. Frozen chicken products contained skeletal muscle with freezing vacuoles, smooth muscles, nerve fibers, intestinal villi, spongy

bone, and even a uterus of bitch, lung tissue, smooth muscle fibers, blood vessels, liver of chicken, cartilage, gastrointestinal tract wall, lymphocytes, intestinal tissue. In addition, high levels of plant materials, inflammatory cells, and excessive fat were detected.

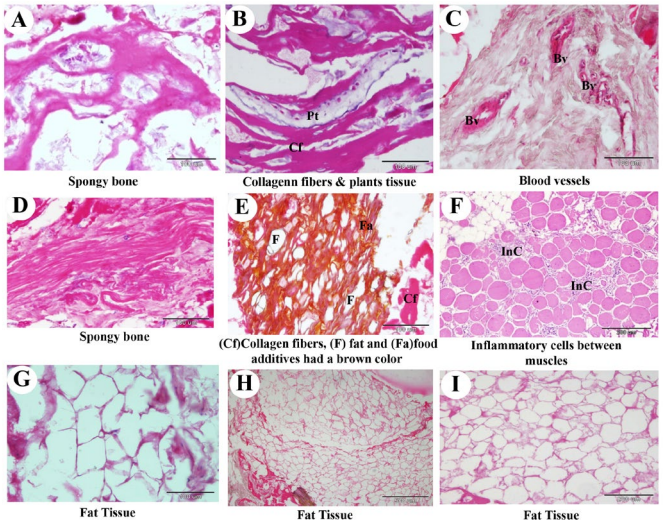

Figure 4. Paraffin sections of frozen raw beef meat products stained with H&E, revealing various unauthorized animal tissues. (A) Spongy bone detected in beef burgers and cocktail beef sausages, (B) Collagenous fibers (cf) and plant tissues (pt) identified in beef burgers, (C) Blood vessels (Bv) present in beef burgers, (D) Spongy bone found in beef hotdogs, (E) Collagenous fibers (cf), fat (F), and food additives (Fa) observed in cocktail beef sausages, (F) Inflammatory cells (InC) detected between muscle fibers in beef burgers, (G) Fat tissue present in cocktail beef sausages, (H) Fat tissue identified in beef hotdogs and beef salami, and (I) Fat tissue found in beef burgers.

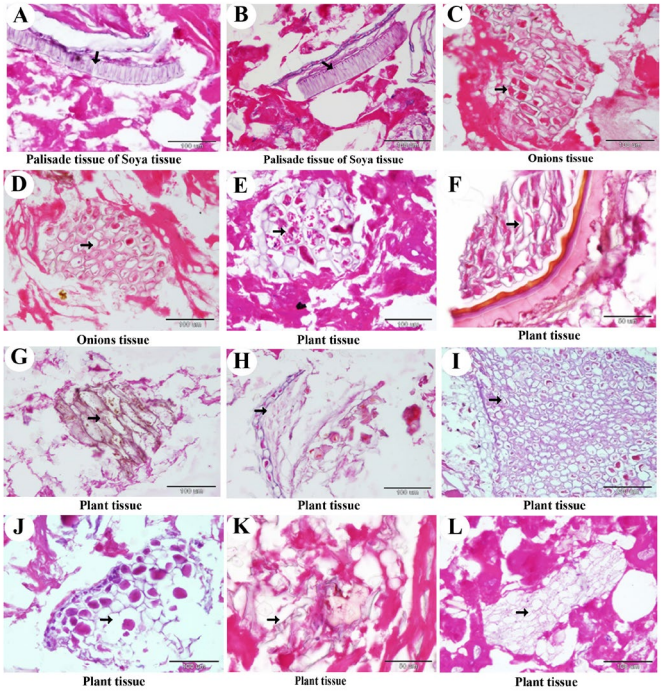

Figure 5. Paraffin sections of frozen raw beef meat products stained with H&E, highlighting various plant tissues (indicated by arrows). Plant tissues were identified in (A, C, E) beef burgers, (B, D, F) cocktail beef sausages, (G, H, I) beef hotdogs, and (J, K, L) beef salami.

Discussion

Processed beef & chicken are preferable for kids, easily prepared junk food and convenient especially, for working women. Adulteration with unauthorized animal tissues or plants may reduce the feeding value. Therefore, this investigation evaluated the most favorite forms based on histological analysis, which is crucial for analyzing the sample's composition, quality, and different tissue types (Agamy and Hegazy, 2011; Sadeghinezhad *et al.*, 2015) histometry's unique perspective on tissue

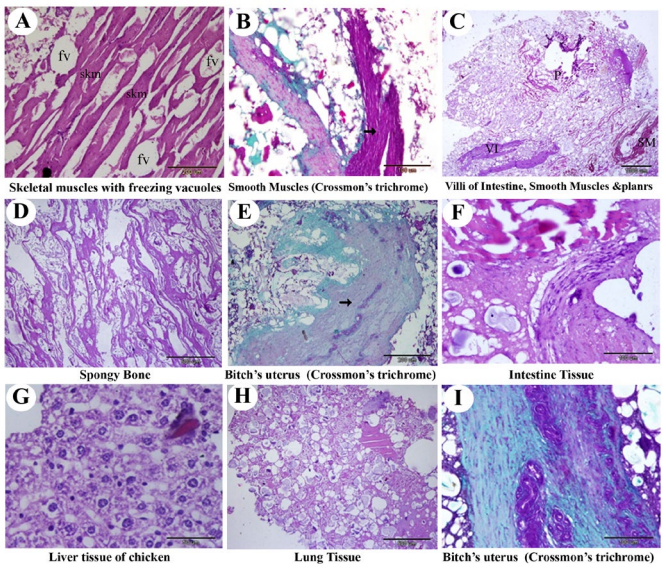

Figure 6. Paraffin sections of frozen raw chicken meat products stained with H&E and Crossmon's trichrome, revealing various unauthorized animal tissues. (A) Skeletal muscle fibers (skm) and freezing vacuoles (fv) detected in chicken burgers and chicken kofta, (B) Smooth muscle fibers (arrowhead) identified in chicken kofta, (C) Intestinal villi (VI), smooth muscle (SM), and plant material (P) present in chicken burgers, (D) Spongy bone found in chicken burgers and chicken nuggets, (E) Uterine tissue from a bitch (arrowhead) detected in chicken burgers, (F) Intestinal tissue present in chicken cocktail sausages and chicken kofta, (G) Chicken liver tissue found in chicken cocktail sausages, (H) Lung tissue detected in chicken cocktail sausages, and (I) Uterine tissue from a bitch identified in chicken nuggets.

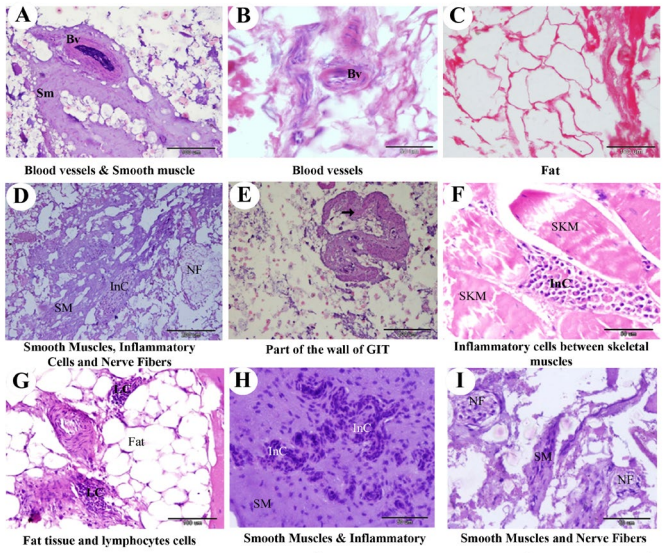

Figure 7. Paraffin sections of frozen raw chicken meat products stained with H&E, revealing various unauthorized animal tissues. (A) Blood vessels (Bv) and smooth muscle (Sm) detected in chicken cocktail sausages and chicken nuggets, (B) Blood vessels (Bv) identified in chicken kofta, (C) Fat tissue present in chicken cocktail sausages, (D) Smooth muscle (SM), inflammatory cells (InC), and nerve fibers (NF) observed in chicken burgers, (E) Section of the gastrointestinal tract wall (arrow) detected in chicken kofta and chicken nuggets, (F) Inflammatory cells (InC) located between skeletal muscle fibers (SKM) in chicken burgers, (G) Fat tissue and lymphocytes (LC) found in chicken nuggets, (H) Smooth muscle (SM) and inflammatory cells (InC) identified in chicken nuggets and chicken kofta, and (I) Smooth muscle (SM) and nerve fibers (NF) detected in chicken kofta.

structure quantitatively (Tremlová and Štarha, 2003). Those histological analysis techniques detected frozen beef and chicken meat products fraud worldwide (Ghisleni *et al.*, 2010; Sezer *et al.*, 2013; Abdel Hafeez *et al.*, 2016; Malakauskienė *et al.*, 2016; Abdel-Maguid *et al.*, 2019) containing different types of animal and plant tissues.

According to Egyptian standards specification (E.S.S., 2005) of beef meat and chicken products, skeletal muscle within meat tissues must be not less than 60% and must be free from any unauthorized tissues (internal organs). In the present study, the majority of the examined samples (beef or chicken products) fulfilled these requirements either with SKM content or the presence of other unauthorized components (Fig. 2 A&B). It is obvious that the level of adulteration in frozen beef products was

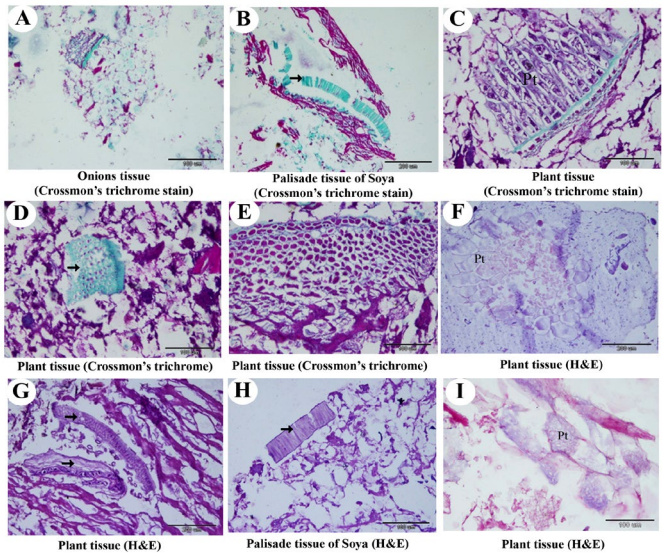

Figure 8. Paraffin sections of frozen raw chicken meat products stained with H&E and Crossmon's trichrome, highlighting various plant tissues. Plant tissues were identified in (A, B (arrow)) chicken burgers and chicken cocktail sausages, (C) chicken burgers, (D, arrow) chicken cocktail sausages, (E, F) chicken nuggets, (G, arrow) chicken kofta, (H, arrow) chicken nuggets and chicken kofta, (I) chicken cocktail sausages, and (I) chicken kofta.

83.3% (Fig. 1A) and chicken meat products were 81.7% (Fig. 1B) from all categories, which achieved a high decrease under 60%, suggesting that these products were more prone to tampering with unauthorized ingredients. The substitution of ingredients with meat from other animal species, internal organs, or excessive quantities of plant material to increase volume or bulk frequently falls victim to counterfeiting and other forms of deceptive labeling (Visciano and Schirone, 2021). Large amounts of plants adversely impact the quality and nutritional value of the products, as noted by Malak *et al.* (2020).

Identification of plant and animal tissues in meat products (beef and chicken) is crucial for ensuring quality and safety. Fraudulent plant and animal tissues additions can lower product quality and introduce allergens, posing risks to consumers (Sadeghinezhad *et al.*, 2015). The widespread incorporation of plant-based fillers and lower-quality animal tissues in processed meats (Visciano and Schirone, 2021), while the high level of plant material not only reduces the overall quality of the products but also significantly be adulterated, likely for cost-saving purposes (Tolba *et al.*, 2023).

The present examination of beef meat products as shown in Figs. 3 –5, different unauthorized animal tissues (brain tissue, collagen fibers, spongy bone, blood vessels, wall parts of GIT) and illegal plant tissues volume (practically soya bean), while in tested chicken products as shown in Figs. 6 –8, smooth muscles, nerve fibers, villi of the intestine, spongy bone, bitch's uterus, lung, intestine, blood vessels, smooth muscle fibers, cartilage, many of fats and plants as soya bean, also freezing vacuoles were detected. Soya beans adversely impact the quality and nutritional value of such products, as noted by (Malak *et al.*, 2020). Cocktail beef sausage and chicken nuggets were found to contain excessively high levels of soy tissue, exceeding the 10% limit established by the E.S.S. (2005). Our findings agree with (Abdel Hafeez *et al.*, 2016), who confirmed the presence of soy and onions in various meat products. Although soy proteins having certain health benefits, such as reducing plasma cholesterol, the risk of organ toxicity and hormonal imbalances associated with excessive soy consumption has been documented (Sukalingam *et al.*, 2015).

Several studies (Latorre *et al.*, 2015; Prayson *et al.*, 2008a; Prayson *et al.*, 2008b) have histometrically determined the fraudulence of processed meat products. These studies detected the unauthorized tissues in meat products such as hamburgers, hotdogs, and sausages, İnal (1992) reported the presence of intestinal mucosa and heart muscle cells in salami and sausage samples. Additionally, (Abdel-Maguid *et al.*, 2019) found lung tissue in 25% of beef burgers. The very low SKM content across all beef and chicken products lowers their nutritional value. Such deviations not only compromise consumer safety but also violate food regulations and undermine trust in product labeling. Through the present work, isometric processed meat examination proved its usefulness in identifying the unauthorized tissues (Prayson *et al.*, 2008b). Our findings align with those of Prayson *et al.* (2008b), who revealed that skeletal muscle constituted less than 10% of the cross-sectional surface area in most brands.

Other than fraudulence risks, the present findings indicated the presence of blood vessels, heart tissue, and arteries in some examined beef meat and chicken products, where they can serve as sources of blood contamination (Russell *et al.*, 2006) and increase the risk of bacterial proliferation, posing a significant threat to human health and adversely affecting the quality, safety, and shelf life of meat products. Also, brain and nervous tissue in meat products are known as carrier of infectious agents (Herde *et al.*, 2005). FDA (2004) has strict regulations to prevent bovine spongiform encephalopathy in the US since prohibiting the use of high-risk specified risk materials like the brain and spinal cord. Moreover, peripheral nerves can facilitate pathogen transmission from the alimentary tract to the central nervous system (Gallo *et al.*, 2020) contaminating the processed products. In the present investigation, unallowed tissues presented in examined beef and chicken products such (smooth muscles, lung tissue, uterus of bitch, intestine, GIT tissue, and chicken liver) might lead to contamination with bacteria and fungi, posing a risk to human health. The International Commission on Microbiological Specifications for Foods (ICMSF) criteria assess the hygienic quality of edible offals (Mavi *et al.*, 2020) as meat products must be evaluated for microbial and fungal contamination to ensure their suitability for human consumption (Hassanien *et al.*, 2018; Shaltout *et al.*, 2020). The same risks may be attributed to the presence of microcomponents as lymphocytes and inflammatory cells in beef burgers, cocktail beef sausage, chicken burgers, chicken nuggets, and chicken kofta, suggesting the possibility of antemortem (pre-slaughter) infections (Sohrabi *et al.*, 2020). So, consuming meat and chicken products derived from diseased animals can pose significant risks to human health and transmit infections.

On the other hand, freezing is a crucial method for preserving meat, which is used in various meat products or consumed post-thawing (Carballo *et al.*, 2000), ensuring quality until it reaches the consumer (Xia *et al.*, 2012). The presence of freezing vacuoles observed in chicken burger and chicken kofta samples revealed that these products had undergone multiple freezing and thawing cycles (Jiang *et al.*, 2019) that might lead to the presence of freezing vacuoles, ice crystal formation, deformation of muscle microstructure, bacterial contamination, and deterioration of the product (Martino and Zaritzky, 1988; El-Sayed, 2023), which could be identified only through histological techniques.

Generally, based on the findings of this study, the histological technique is a highly accurate and rapid method that has been successfully utilized to detect adulteration with both unauthorized animal and plant tissues and (histomorphometry) evaluate the composition and quality of frozen beef and chicken products (Emara and Nouman, 2002; Tremlová and Štarha, 2003). It helps verify product labels and ensure and control meat quality (Buche and Mauron, 1997).

Upon all the above findings, these tested products often did not meet food regulation standards (Latorre *et al.*, 2015) and Egyptian Standard Specifications (E.S.S., 2005) of meat and chicken products, underscoring the importance of rigorous quality control measures to ensure meat product authenticity and safety, protecting public health.

Conclusion

The investigation revealed that frozen processed meat products, including beef and chicken are adulterated, contain low skeletal muscle content, and exhibit high levels of unauthorized tissues. These findings violate Egyptian standards and food safety regulations and pose potential health risks and underscore the need for stricter regulatory oversight to protect public health and maintain consumer trust in these products.

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Conflict of interest

The authors have no conflict of interest to declare.

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