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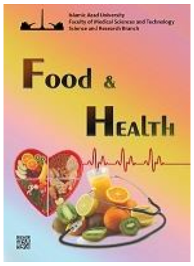

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Histological and microbial analysis of different types of raw meat products in Tehran, Iran

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ABSTRACT

Meat products are essential protein sources and play a significant role in human nutrition and overall health. However, unauthorized tissues in meat products can compromise their quality and safety. This study investigated the microbial and histological features of various minced meat products in Tehran province, Iran. A total of 200 samples were collected, including 50 samples of red meat Kabab Koobideh paste (RM), 50 of chicken meat Kabab Koobideh paste (CM), 40 of minced meat (MM), 30 of industrial hamburgers (IH), and 30 of homemade hamburgers (HH). The samples were analyzed for their histological composition and microbial counts (total bacterial count, *Staphylococcus aureus*, and mold and yeast counts). The most common unauthorized tissues found in the samples were adipose tissue (66.0%), smooth muscle tissue (46.5%), and herbal tissue (41.0%). The highest levels of unauthorized tissues were observed in the CM (N=198) and RM (N=129) groups, while the lowest levels were observed in the MM group (N=14). The total bacterial count (TBC) was highest in the RM group (7.69±0.46 log CFU/g), followed by the CM group (6.96±0.54 log CFU/g). The highest counts of *S. aureus* were observed in the CM group (4.84±0.23 log CFU/g), followed by the RM group (4.78±0.24 log CFU/g). The most elevated mold and yeast counts were observed in the HH group (4.58±0.34 log CFU/g), followed by the RM group (4.56±0.32 log CFU/g) and the CM group (4.50±0.45 log CFU/g). A significant correlation was observed between unauthorized tissues and bacterial contamination ($p<0.05$). This suggests that using unauthorized tissues in meat products can increase the risk of bacterial contamination. The findings of this study highlight the importance of avoiding the use of unauthorized tissues, especially those derived from the digestive tract, in meat products.

1. Introduction

Meat and its derivative products stand as paramount protein sources for human consumption. With the surge in population, ensuring the quality and health safety of foods, especially meat products, has gained notable importance (1, 2). Minced meat, utilized ubiquitously across the globe and specifically in Iran, features in numerous products. Economic incentives, however, sometimes drive the incorporation of unauthorized tissues into meat products (3-5). Research indicates a rising trend in substituting meat in meat products with unauthorized animal tissues such as connective tissue, gizzard, heart, skin, and vegetable proteins like soybean (6). To ensure quality and pinpoint unauthorized tissues in meat products, many methods like microbiological, chemical, and histological analyses are imperative (7, 8). Kabab Koobideh, a widely savored Iranian dish, is crafted from the minced meat of halal animals like beef, lamb, and chicken. The possibility of utilizing unauthorized tissues in these products depends on the type (6). The employment of unauthorized tissues and potential concomitant contamination may escalate microbial loads (9). For instance, Staphylococci are found on warm-blooded

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animals' skin, glands, and mucous membranes (10). In humans, Staphylococci are chiefly associated with the nasal cavity and can be isolated from feces and environmental sources like soil, water, and plants (11). Contamination in minced meats can transpire during processing and from tissue substitutions, such as viscera (12-14). Microbiological tests enable the detection of associations between organs in processed meats and contamination sources (15-17). Histological methods, pivotal for identifying unauthorized tissues, reveal that muscle strands appear scissor-like under microscopic examination. Smooth muscle nuclei are bar-shaped and centrally located in the esophagus, stomach, intestines, spleen, arteries, and skin (18). This study analyzed the microbial and histological characteristics of and discerning associations between unauthorized tissue usage and microbial pathogens in Kabab Koobideh, minced meat, and hamburger samples within Tehran province.

2. Materials and methods

2.1. Sampling

A total of 200 assorted raw meat product samples were collected, encompassing 50 chicken meat Kabab Koobideh paste (CM), 50 red meat Kabab Koobideh paste (RM), 40 minced meat (MM), 30 industrial hamburgers (IH), and 30 homemade hamburgers (HH). These were obtained from ten restaurants across the Tehran province, adhering to aseptic protocols, encased in ice bags, and conveyed to the Food Hygiene Laboratory of Islamic Azad University of Karaj, maintaining a refrigerator temperature and ensuring arrival within a 4-hour window.

2.2. Histological analysis

Each sample, weighing 100 grams, was subjected to meticulous histological procedures. Four spherical pieces, approximately 2 cm in diameter, were isolated from each sample, placed in containers filled with 10% formalin for 2 days, and prepared as paraffin molds. Four slices (each 6 µm thick) were stained with Hematoxylin-Eosin, and slides were examined under a light microscope (18).

2.3. Microbial analysis

For enumerating the total bacterial count (TBC), *Staphylococcus aureus*, and mold and yeast, respective media were utilized: Nutrient Agar (incubated for 48 hours at 37°C), Baird Parker Agar (48 hours at 37°C), and Sabouraud Dextrose Agar (5 days at 25°C) (19-21).

2.4. Statistical analysis

Data analysis was executed utilizing IBM SPSS Statistics 27 software for Windows. Histological analysis calculated the percentage frequency of unauthorized items (including gizzard, cartilage, adipose, bone, herbal, lymphatic, skin, smooth muscle, saliva, liver, and hair). The mean ± standard deviation of results was computed in the microbial analysis. A comparative assessment of results amongst various groups was facilitated by employing Friedman's test, while the Spearman-Kendall correlation test was deployed to analyze the relationship between different observations.

3. Results and discussion

3.1. Histological findings

The histological results, as presented in Table 1, indicate the presence of unauthorized tissues in various meat samples. Hair, salivary glands, and lymphatic vessels had the lowest occurrence (0.5%). In contrast, CM (Commercially Manufactured) and RM (Restaurant Manufactured) samples exhibited the highest counts of unauthorized tissues, with 198 and 129 occurrences, respectively. The MM (Minced Meat) group had the fewest unauthorized tissues, with only 14 instances. Furthermore, the highest percentage of gizzard tissue, indicating digestive tissue, was observed in the RM group (62.0%). Smooth muscle tissue was predominant in the CM (80.0%), HH (Homemade Hamburger, 60.0%), and IH (Industrial Hamburger, 43.3%) groups. In terms of specific tissue types, CM samples showed the highest numbers of herbal tissue (70.0%), skin tissue (50.0%), liver tissue (38.0%), and bone tissue (22.0%). Cartilage tissue was most prevalent in the RM group (62.0%), and RM also had one occurrence each of hair (2.0%) and lymphatic tissue (2.20%). Similar findings of unauthorized tissues in meat products have been reported in previous studies. For example, hamburgers contained cartilage, chicken gizzard, lungs, and smooth

Table 1. Numbers (percentage) of various unauthorized tissues in different meat products in Tehran province.

Unauthorized tissues	Hamburger		Koobideh Kabab Paste		Minced meat	Total
	Industrial	Homemade	Chicken meat	Red meat		
Gizzard Tissue	2 (6.7&%)^{c}	5 (16.7%)^{b}	7 (14%)^{b}	31 (62.0%)^{a}	7 (17.5%)^{b}	52 (26.0%)
Cartilage Tissue	1 (3.3%)^{b}	2 (6.7%)^{b}	2 (4.0)^{b}	3 (6.0%)^{a}	1 (2.5%)^{b}	9 (4.5%)
Adipose Tissue	21 (70.0%)^{b}	25 (83.3%)^{b}	34 (68.0)^{ab}	48 (96.0%)^{a}	4 (10.0%)^{c}	132 (66.0%)
Bone Tissue	1 (3.3%)^{c}	0 (0.0%)^{d}	11 (22.0%)^{a}	5 (10.0%)^{b}	1 (2.5%)^{c}	18 (9.0%)
Herbal Tissue	15 (50.0%)^{b}	14 (46.7%)^{b}	35 (70.0%)^{a}	18 (36.0%)^{b}	0 (0.0%)^{c}	82 (41.0%)
Lymphatic Tissue	0 (0.0%)^{b}	0 (0.0%)^{b}	0 (0.0%)^{b}	1 (2.0%)^{a}	0 (0.0%)^{b}	1 (0.5%)
Skin Tissue	0 (0.0%)^{c}	0 (0.0%)^{c}	50 (100%)^{a}	1 (2.0%)^{b}	0 (0.0%)^{c}	51 (25.5%)
Smooth Muscle Tissue	13 (43.3%)^{c}	18 (60.0%)^{b}	40 (80.0)^{a}	21 (42.0%)^{b}	1 (2.5%)^{a}	93 (46.5%)
Saliva Tissue	0 (0.0%)^{b}	1 (3.3%)^{a}	0 (0.0%)^{b}	0 (0.0%)^{b}	0 (0.0%)^{b}	1 (0.5%)
Liver	0 (0.0%)^{b}	0 (0.0%)^{b}	19 (38.0%)^{a}	0 (0.0%)^{b}	0 (0.0%)^{b}	19 (9.5%)
Hair	0 (0.0%)^{b}	0 (0.0%)^{b}	0 (0.0%)^{b}	1 (2.0%)^{a}	0 (0.0%)^{b}	1 (0.5%)

muscle tissue (22). Sausages made from chicken paste contained kidney tissues, fat, and skin (23). Sausages, in general, were found to contain stomach, intestine, heart muscle, lymph nodes, spleen, urinary tract, endocrine glands, salivary glands, liver, and heart tissues (2, 9, 24). Heated sausages contained salivary glands and post-serial ligand tissues (25). The presence of unauthorized tissues in food products can vary depending on the type of meat sample. In this study, minced meat samples exhibited the lowest occurrence of unauthorized tissues. In hamburger meat samples (both industrial and homemade), adipose tissue (21 out of 30 and 28 out of 30 samples, respectively), plant materials (15 out of 30 and 14 out of 30 samples, respectively), and smooth muscle tissue (13 out of 30 and 18 out of 30 samples, respectively) were the most frequently observed unauthorized tissues. It's worth noting that previous research conducted by Prayson et al. (26) on various fast-food hamburger brands in the USA found adipose tissue in seven samples and plant material in four samples. However, smooth muscle tissue was not observed in any of the samples. Additionally, all hamburger samples contained unauthorized tissues such as connective tissue, blood vessels, and peripheral nerves. In a study by Abbasy-Fasarani et al. (27) in Tehran, the most common unauthorized tissues in industrial hamburger samples were chicken skin (9 cases) and hyaline cartilage (7 cases). Julini et al. (2) also reported unauthorized sausage tissues, including the stomach, intestine, heart muscle, lymph nodes, spleen, urinary tract, and endocrine glands. The differences in results of unauthorized tissues in this study compared to other cases can be due to variations in sample type, sampling location, and sampling timing. Strict food safety regulations and oversight are important factors limiting the use of unauthorized ingredients in meat products. According to Iranian national standards, using tissues such as organs, breasts, liver, lungs, spleen, bladder, skin, gizzard, etc., in meat products is prohibited (28). In the current study, all tissues were identified using optical microscopy. Sample tissue images are shown in Fig. 1.

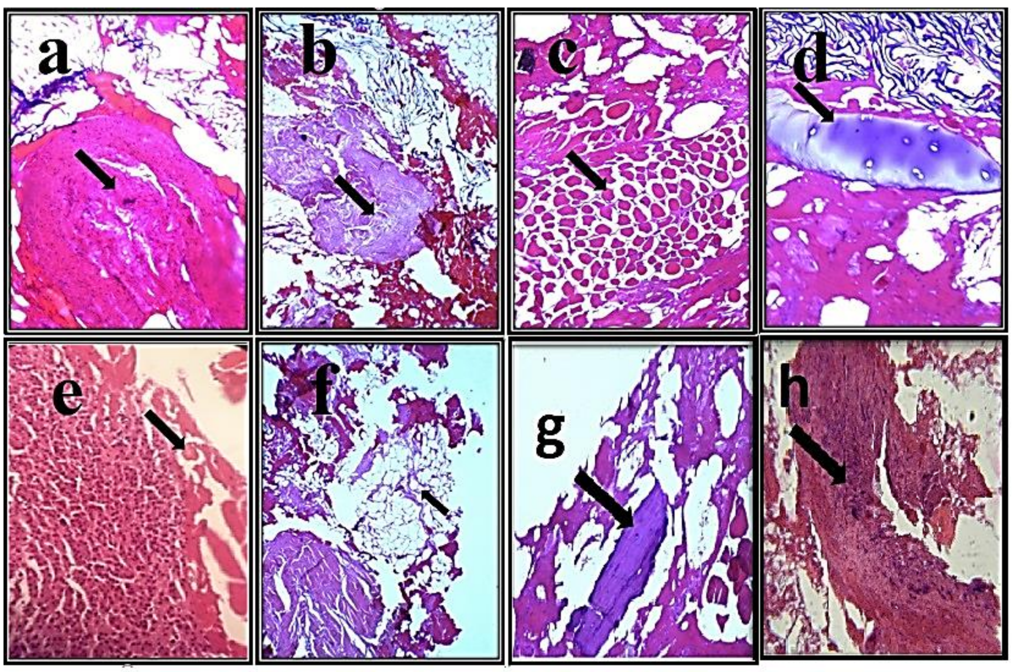

Fig. 1. Smooth muscle tissue (a), gizzard tissue (b), skeletal muscle tissue (c), cartilage tissue (d), liver tissue (e), adipose tissue accumulation (f), bone tissue (g), and skin tissue (h) in different raw meat products.

Histological analysis of meat product samples used H&E staining, following a methodology similar to several previous studies (2, 9, 25, 29). While microscopic examination is a standard method for histological analysis, Sohrabi et al. (30) also recommended using molecular techniques. The study collected 101 samples of ground meat (Group 1) and ready-to-eat meat products (Group 2) from supermarkets in Turin, Italy. Histological analysis revealed the presence of cartilage, bone, and glandular tissues. Ultimately, combining DNA microarrays and histology was proposed to enhance monitoring capacity in the bovine meat industry. This study showed the presence of bone, cartilage, smooth muscle, lymph nodes, vegetable protein (soy), hair, and skin tissues as unauthorized ingredients. The highest percentages of unauthorized tissues were adipose (66.0%), smooth muscle (46.5%), herbal (41.0%), and gizzard (26.0%) tissues related to ground meat and ready-made products. These are the cheapest and most popular meat products in Iran. Previous research has shown that organoleptic evaluation alone is insufficient for judging meat product quality, and histological outcomes provide the most effective assessment of meat product quality. In Chugunova's 2021 study, organoleptic and histological methods were used to examine the quality of ground pork and beef semi-finished products in Perm City, Russia. The organoleptic analysis found full compliance with regulatory requirements. However, unacceptably, some samples showed skeletal muscle and adipose tissues in a 1:1 ratio. Most samples (85%) contained connective and cartilaginous tissues (17).

3.2. Microbial results

The mean ± standard deviation of total bacterial count (TBC), *Staphylococcus aureus* count, and mold and yeast count in meat product samples are shown in Table 2. As shown in Fig. 2, TBC in RM was significantly higher than in other groups ($p<0.05$), and CM and HH groups did not differ significantly ($p<0.05$). *S. aureus* and mold and yeast counts in CM, HH, and RM groups were higher than others ($p<0.05$), and MM and HI differed significantly ($p<0.05$). Several studies in Iran and other countries have acknowledged a high prevalence of microbial contamination in foods (11, 31-33). Investigations of traditional Iranian foods like Rice, Gheymeh Stew, Eggplant Stew, Ghormeh Sabzi Stew, Plum Stew, Celery Stew, Koobideh Kabab, Chicken Kabab, Vegetable Rice, Chicken rice, Fish rice, Beans rice, Meat rice, Canned tuna, and Canned beans confirmed microbial contamination in these foods. The high microbial burden, especially bacterial pathogens, and the low nutritional value of unauthorized tissues render these meat products unsuitable for consumption. Additionally, some products may contain glands like the spleen, which are forbidden in Islam (11). The highest amount of gizzard tissue was found in RM samples. Considering these products' high gizzard, adipose, and smooth muscle tissue levels, a high microbial load could be predicted. The results of the microbial analysis in this study support this.

Table 2. Microbial analysis containing mean ± SD of total bacterial count, *Staphylococcus aureus*, and mold and yeast in different meat products in Tehran province.

Product		Mold and Yeast (Log_{10} CFU/g)	Total Bacterial Count (Log_{10} CFU/g)	*Staphylococcus aureus* (Log_{10} CFU/g)
Hamburger	Industrial	$3.32±0.30^{b}$	$6.08±1.04^{c}$	$3.24±0.35^{b}$
	Homemade	$4.58±0.24^{a}$	$6.62±0.82^{b}$	$4.81±0.28^{a}$
Kabab Koobideh paste	Chicken meat	$4.50±0.45^{a}$	$6.96±0.54^{b}$	$4.84±0.23^{a}$
	Red meat	$4.56±0.32^{a}$	$7.69±0.46^{a}$	$4.78±0.24^{a}$
Minced meat		$2.05±0.12^{c}$	$5.58±0.41^{d}$	$2.99±0.19^{c}$

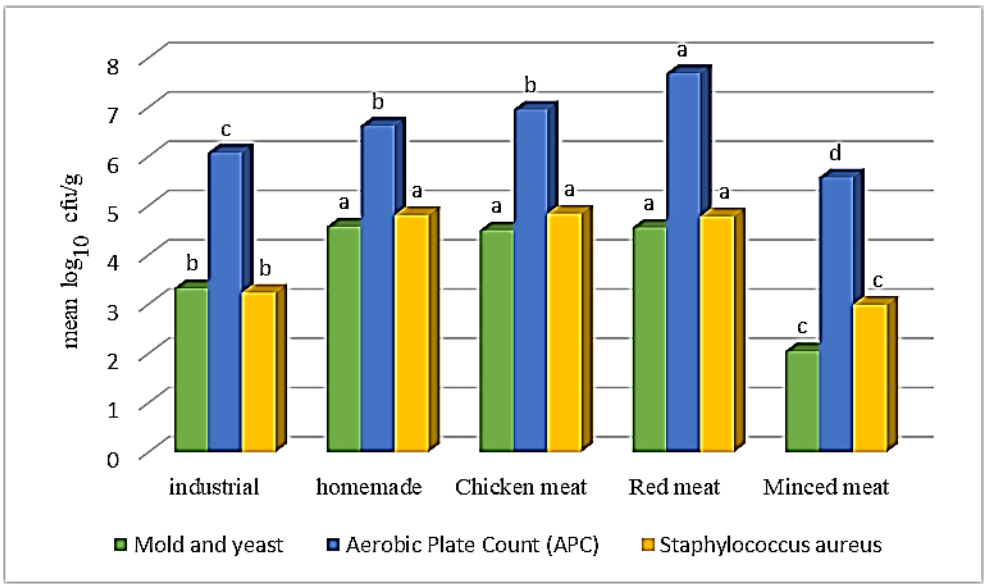

Fig 2. Mean microbial counts of total bacterial count (TBC), *Staphylococcus aureus*, and mold and yeast in raw meat (RM), chicken meat (CM), and homemade hamburgers (HH) kabab paste samples. Values within the same parameter with statistically significant differences ($p < 0.05$) between groups are indicated with different letters..

According to the WHO, foodborne diseases have increased substantially in countries like Australia (69.35% from 1985 to 2003) and Spain (74.13% from 1983 to 2004) (15). Foodborne diseases pose a major public health problem, as most countries have reported significant increases in food-related illnesses (13). The use of unauthorized tissues appears higher than microbial load, which can facilitate the transmission of infectious agents like *Salmonella* and *Escherichia coli*. Efforts have been made worldwide to identify unauthorized tissues in foods. Meat products provide a very nutritious environment for the growth of many microorganisms, some of which can be pathogenic to humans. Potential sources of microorganisms include raw materials of animal origin prohibited by Iranian standards, unhygienic handling by personnel, improper time-temperature storage, especially in summer, and use of contaminated additives like spices and flours during processing (34). The microbial results (TBC, *S. aureus*, mold, and yeast) exceeded Iranian national standards. TBC in RM and CM were significantly higher than other groups ($p<0.05$), which was predictable due to the use of visceral organs.

Furthermore, TBC is an appropriate indicator of spoilage and meat shelf life (32). A study on bacterial contamination of consumed foods in the cafeterias of Baqiyatallah University conducted by Tavakoli et al. (33) found that Kabab Koobideh had the highest levels of total bacterial counts (TBC) and coliforms. Additionally, 55.6% of samples tested positive for *S. aureus*. These results are consistent with the current study. *S. aureus* is an indicator of meat contamination, as Kabab Koobideh contains chicken and red meat, which can enter foods from the skin, mouth, or nose of food handlers (24). The increased prevalence of this bacterium in chicken meat can be due to poor hygiene practices among staff and potential missed evisceration during processing. Cattle and chicken carcasses can become contaminated by digestive tract contents, fecal matter, feathers, wool, and skin sheds during slaughter and butchering. In a previous study, *S. aureus* (5×10^5 CFU/g) and TBC (5×10^6 CFU/g) were isolated from 28 samples of Kabab Koobideh (28). Furthermore, high levels of microbial contamination have been attributed to inappropriate hygiene practices during food processing, particularly the presence of *S. aureus*, which likely results from cross-contamination between raw and cooked foods (34). This study's microbial results (TBC, *S. aureus*, mold, and yeast) exceeded the Iranian national standard. TBC in RM and CM was significantly higher than in the other groups ($p<0.05$). These elevated TBC levels were likely due to the use of visceral organs in RM and CM production, which can harbor higher bacterial loads. Additionally, TBC is a useful indicator of meat spoilage and shelf life. Hassan et al. (35) reported TBC levels between 10^6-10^7 CFU/g in red meats, which is consistent with the results. Production environment sanitation and hygiene maintenance are crucial factors influencing TBC counts. Therefore, the TBC level serves as an overall indicator of microbial contamination. Nemati et al. (11) reported a TBC of 3.22×10^3 CFU/g in a study of grilled Kabab quality in Tabriz. The presence of potential pathogens around premises, surfaces (walls and floors), and equipment can increase TBC. Conversely, contaminated meats can transmit pathogens to surfaces and equipment during production, allowing biofilms to form (36). Our findings showed higher *S. aureus* counts in RM, CM, and HH ($p<0.05$), possibly relating to poor worker hygiene and lack of sanitation during product handling and processing. Several studies have reported high levels of *S. aureus* and mesophilic bacterial contamination in chicken meats. Javadi and Safarmashaei (29) examined 80 fresh chicken meat samples. They found *S. aureus* contamination in 65% of samples, with TBC and *S. aureus* counts of 5×10^5 and 4×10^5 CFU/g, respectively, indicating poor slaughterhouse hygiene and handling practices. Another study compared the microbiological safety and quality of premium and economic grades of Egyptian beef luncheon sausages and burger patties collected from local markets (37). Histological examination revealed all products contained low muscle content and higher fat, heart, connective tissue, skin, bone, and cartilage. Total bacterial counts exceeded limits in 100%, 42%, 66%, and 84% of the premium and economic sausage and premium/economic patties, respectively. Premium patties also exceeded *S. aureus* (34%) and *E. coli* (36%) limits more frequently than economic patties (16 and 10%, respectively). While fecal coliform counts met limits for premium patties, 34% of economic products exceeded permissible levels, suggesting premium products had lower pathogen risks. These studies highlight poor hygiene and contamination control in raw meat production and retail. In another study, Teshome et al. (38) collected 60 raw beef samples from Dukem (N=20), Kara (N=20), and Burayu (N=20) shops around Addis Ababa, Ethiopia. The mean TBC, total coliform, mold and yeast, Staphylococcus spp., Bacillus spp., and psychrophilic bacterial counts were 8.34, 4.69, 6.01, 5.36, 5.45, 4.26 log10 CFU/g, respectively. Beef from Dukem had the lowest microbial counts compared to Kara and Burayu, which had the highest counts. This indicated poor sanitation conditions may have varied between sites. Cohen et al. (39) reported chicken meat TBC and *S. aureus* counts of 7.60 to 15.56 log10 CFU/g and 5.36 log10 CFU/g, respectively, with 10.4% *S. aureus* contamination. Therefore, contaminated meats used in kabab paste preparation can affect its microbial load. A previous study by Tavakoli et al. (33) found kabab koobideh was the most contaminated food in terms of TBC and coliforms, with 65.6% *S. aureus* contamination. Yuksek et al. (40) detected higher *S. aureus* in red versus chicken doner kabab, though coliform/*E. coli* levels were similar. The current study showed higher contamination percentages of red meat kabab paste samples. Mold/yeast counts were also higher in RM, CM, and HH ($p<0.05$), possibly due to spore transfer via additives like spices and flour.

4. Conclusion

This study revealed unauthorized tissues in some meat products, presumably for economic reasons. Histological and microbial analysis provides important safety assessments of meat products and protects consumer health. The results showed higher microbial contamination, specifically higher TBC, mold, and yeast counts, in RM and CM groups compared to other products. Additionally, the highest levels of digestive tract tissues like gizzard and smooth muscle were observed in RM products, which likely contributed to their higher microbial loads. *S. aureus* counts were also higher in RM, CM, and HH groups compared to other analyzed meat products. These findings emphasize the need for strict adherence to food safety standards and limiting the use of unauthorized ingredients during all stages of meat processing and product development. Education initiatives targeting production facilities could help reduce bacterial contamination issues. Overall, enhanced monitoring, avoiding non-compliant tissues, and ensuring hygienic practices throughout the supply chain are crucial to lowering health risks and improving the nutritional quality of meat products consumed by the public. Further research analyzing histological and microbiological parameters remains important for assurances of meat product safety.

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