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Comparative microbiological evaluation between fresh and frozen bovine liver

Samar. R. Mubarak[1], Nahla A. Abou EL-Roos[2], Mona N. Hussein[3], and Fahim A.E. Shaltout

[1]*Department of Food Hygiene and Control (Meat hygiene), Faculty of Veterinary Medicine ,Benha University, Benha 13736, Egypt.*
[2]*Food hygiene Department, Animal Health Research Institute, Agriculture Research Center of Shebin El Koom Branch, Egypt.*
[3]*Department of Histology and Cytology, Faculty of Veterinary Medicine, Benha University, Benha13736, Egypt*

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ABSTRACT

The consumption of fresh and frozen bovine liver is common in Egypt. Therefore, the purpose of the current study was to evaluate the hygienic indices of fresh compared to frozen bovine liver obtained from various supermarkets in Menofia governorate, Egypt. The aerobic plate count (APC), coliform count, *Escherichia coli* (*E. coli*), Staphylococcus aureus (Staph. aureus), and mold and yeast counts were evaluated in both fresh and frozen bovine liver. Our results indicated that APC, coliform count, and *E. coli* counts were significantly lower ($P \leq 0.05$) in frozen liver samples than in fresh liver samples. However, fresh liver samples were significantly lower ($P \leq 0.05$) than frozen liver samples in Staph. aureus count. Compared to the Egyptian Standard of chilled meat and frozen liver, all fresh liver samples exceeded acceptable APC and coliform counts of 6 log10 CFU/g and 2 log10 CFU/g, respectively. However, not all frozen livers exceeded the maximum acceptable limits of APC (5 log10 CFU/g) but surpassed the coliform count. These findings indicated that fresh liver and, to a lesser extent, frozen liver were produced, shipped, and handled unhygienically, and that best practices and legal requirements were not followed.

1. INTRODUCTION

Africa and Asia will account for the majority of the 8.6 billion people that will probably dominate the planet by 2030 (United Nations, 2015). This rapid human population growth will necessitate increased animal product production (Abebe et al., 2020). Therefore, there's a high tendency to obtain healthy, fresh, and natural alternatives for meat (Heard and Bogdan, 2021). Because meat and meat products are needed for good human nutrition, where they have high-quality protein and other necessary nutrients, which are difficult to obtain from plant-based protein, and there is an inadequate amount of animal protein for needed human consumption (Leroy et al., 2023; Pereira and Vicente, 2013). Therefore, offals would be the optimum substitute for meat consumption because they are more commercial and have a high nutritive value (Biel et al., 2019).

The liver is an edible organ which is highly nutritious and contains higher concentrations of some elements than muscle tissue, including iron, zinc, magnesium, folate, calcium, selenium, and B complex vitamins (Biel et al., 2019). Thus, offal typically spoils more quickly than meat due to easily accessible nutrients and unsanitary handling, collecting, and processing circumstances.

Large-scale food production, product processing, and strong demand for foods, including animal-derived food, all contribute considerably to noncompliance with best practices and legal requirements (Abebe et al., 2020). Inadequate processing and hygienic transportation and/or handling practices at any point in the farm-to-fork chain increase the risk of contamination and the spread of food- borne illnesses during this time (Elkholy et al., 2025). Various points in the food chain, such as during production, processing, distribution, preparation, and/or ultimate consumption, can result in the contamination of food, including the liver (Abebe et al., 2020; Heredia and García,2018).

Food contamination can include spoilage bacteria (the primary cause of food deterioration), food-borne diseases (bacteria, viruses, and fungi), and a variety of parasites (Majumdar et al., 2018; Shaltout et al., 2023a). Food-borne infections pose a considerable public health danger in both industrialized and developing countries, with developing nations carrying the majority of the burden (WHO, 2015). Thus, food contamination is a danger to food safety and can cause illnesses in humans by consuming contaminated animal products or their toxins or reducing keeping quality (Heredia and García, 2018; Lorenzo et al., 2018). APC, Salmonella, coliforms, Staph. aureus, *E. coli*, mold and yeastare considered the most common food contaminant microorganisms (Elkholy et al., 2024). Food management istherefore a top priority, and several strategies will be developed to lessen the effects of noncompliance with hygienic production, transportation, and storage of meat, especially offals (Heredia and García, 2018; Abebe et al., 2020; Shaltout et al., 2023b). Determining the kinds of foodthat lead to foodborne illnesses will not only improve food safety but also provide opportunities to fortify food safety regulations. Among many other things, regulatory bodies can employ source attribution estimates to drive agency goals, support the creation of legislation, performance requirements, and indicators, and carry out risk assessments (Gamil et al., 2025, 2024; IFSAC, 2022). Therefore, rather than keeping an eye on improper cooking habits, the most important first step in preventing the spread of contaminated food to the food chain and subsequent outbreaks is early detection of foodborne pathogens (Gamil et al., 2024). Consequently, the current study's goal was to ascertain the hygienic indices of bovine liver in Egyptian marketplaces.

* Correspondence to: mona.hussein@fvtm.bu.edu.eg

2.MATERIALS AND METHODS

Ethical of approval
This study follows the ethics guidelines of the Faculty of Veterinary Medicine, Benha University, Egypt with ethical approval number (BUFVTM 18-10-23).

2.1. Samples preparation and distribution
For this investigation, hundred samples of fresh and frozen (50 each) bovine liver were collected from different supermarkets located in Menofia governorate, Egypt, duringthe period (2023-2024). The samples were collected in a septic condition and transferred directly to the microbiology lab. Bovine liver samples were examined microbiologically (APC, coliforms, *E. coli*, and Staph. aureus and yeast and mold counts).

2.2 . Microbiological evaluation
The liver samples were prepared as 10% homogenate and serially diluted ten times (ISO, 2017). The microbiological examination methods were carried out following ICMSF (1996).

2.3 . Determination of total aerobic plate count (APC):

Using the deep seeding method on the Plate Count Agar (PCA) (Acumedia/UK) by ICMSF (1996), the total aerobic counts plate count was determined. It involved the aseptic transfer of 1 ml of the previously prepared solution into a sterile petri dish, followed by the addition of 15 ml of PCA to the inoculums. Following agar solidification, they were incubated for 24 to 48 hours at 37 °C. The total number of colonies per gram of the material was determined using Petri dishes holding between 30 and 300 colonies.

2.4 . Determination of coliform count:
After a 24-hour incubation period on Violet Red Bile Lactose Agar (VRBL) medium (HIMEDIA/India) at 37°C, the coliform count was determined using the same procedures used in the APC following ICMSF (1996). The pink-red colonies with a diameter greater than 0.5 mm were counted. The number of counted colonies was multiplied by the dilution factor to determine the number of coliforms per gram of sample.

2.5 . Determination of Staphylococcus aureus count:
After 48 hours of surface plating, Staphylococcus aureus level was assessed on Baird Parker agar plate, with round, glossy, smooth, convex, and black colonies enumerated. After 5-7 days of incubation at 25 °C.

2.6 . Determination of Escherichia coli count:
On Eosin Methylene Blue (EMB) plating medium (HIMEDIA/India), an *Escherichia coli* count was carried out. *E. coli* was identified by the colonies' metallic reflection and green color.

2.7 . Determination of yeast and mold count:
Yeast and mold were determined according to ICMSF (1996) guidelines. Onto Sabouraud dextrose agar (Biolife/Italy), one millilitre of the initial dilution was streaked, incubated at 25 °C, and monitored every day for seven days. The numbers of creamy white-yellow colonies were counted.

2.8 . Statistical analysis
Following the procedure outlined by Feldman et al. (2003), the gathered data were logarithmically converted and evaluated using a paired-samples t-test in SPSS. Bacterial log10 CFU/g) are expressed with standard error (SE). A difference was considered significant if $P \leq 0.05$.

3. RESULTS

The present investigation on microbiological evaluation of fresh and frozen liver showed that the aerobic plate count infresh liver samples had a significantly higher $P \leq 0.05$ mean(6.37 log10 CFU/g) and a larger range (6-6.75 log10 CFU/g)of APC than frozen samples (5.02 log10 CFU/g; 4.89-5.15 log10 CFU/g) (Table 1). Additionally, fresh liver samples had a substantially higher $P \leq 0.05$ mean of total coliform count (5.20 log10 CFU/g; 5.09-5.32 log10 CFU/g) than frozen liver samples (4.30 log10 CFU/g; 4.20-4.41 log10 CFU/g) (Table 2).

Table (1): Total aerobic plate count (APC) in fresh and frozen beef liver samples collected from Menofia Governorate (n=50).

	Total Aerobic plate count			EOS	P value
Samples	Mean ± SE	Min	Max.		0.05
Fresh liver	6.37±0.09a	6	6.75	< 6 log CFU/g	
Frozen liver	5.02±0.05b	4.89	5.15	< 5 log CFU/g	

Count expressed as a log10 CFU/g.
SE=Standard error; Min.: minimum; Max.: maximum.
[a,b] Mean within the same column of different samples are significantly different at ($P \leq 0.05$).
EOS 2007/1473 for frozen liver, EOS No. 3602 / 2013 for fresh liver.

Table (2): Statistical evaluation of total coliform count in fresh and frozen beef liver samples from Menofia Governorate (n=50).

	Total coliform count			EOS	P value
Samples	Mean ± SE	Min.	Max.		0.05
Fresh liver	5.20±0.10a	5.09	5.32	< 6 log CFU/g	
Frozen liver	4.30±0.09b	4.20	4.41	< 6 log CFU/g	

The mean *E. coli* count (4.44 log10 CFU/g; 4.13-4.75 log10 CFU/g) in fresh liver samples was significantly higher ($P \leq 0.05$) than in frozen liver samples (3.98 log10 CFU/g; 3.76- 4.2 log10 CFU/g) (Table 3). Also, the mean total mold and yeast count in fresh liver samples (3.22 log10 CFU/g; 3.09-3.35 log10 CFU/g) was not statistically significant in comparison with frozen liver samples (2.21 log10 CFU/g; 1.12-2.30 log10 CFU/g) (Table 4). However, the Staph. aureus count of fresh liver samples had a much lower $P \leq 0.05$ mean (2.79 log10 CFU/g; 2.45-3.14 log10 CFU/g) than frozen samples (3.27; 3.10-3.45 log10 CFU/g) (Table 5.

Table (3): Statistical evaluation of *Escherichia coli* count in fresh and frozen beef liversamples from Menofia Governorate (n=50).

	E. coli count			EOS	*P* value
Samples	Mean ± SE	Min.	Max.		0.05
Fresh liver	4.44 ± 0.10^{a}	4.13	4.75	free of pathogenic *E. coli*	
Frozen liver	3.98 ± 0.05^{b}	3.76	4.2		

Count expressed as a $\log^{10}$ CFU/g.
SE=Standard error; Min., minimum; Max, maximum.
[a,b] Mean within the same column of different samples are significantly different at ($P \leq 0.05$).
EOS 2007/1473 for frozen liver, EOS No. 3602 / 2013 for fresh liver.

Table (4): Statistical estimation of total mold and yeast count in fresh and frozen beef liver samples from Menofia governorate (n=50).

	Total mold and yeast count			*P* value
Samples	Mean ± SE	Min.	Max.	0.321
Fresh liver	3.22±0.05	3.09	3.35	
Frozen liver	2.21±0.09	1.12	2.30	

Count expressed as a $\log^{10}$ CFU/g.
SE=Standard error; Min., minimum; Max, maximum.

Table (5): Statistical estimation of *Staphylococcus* counts in fresh and frozen beef liversamples collected from Menofia governorate (n=50).

	Total *Staphylococcus* count			*P* value
Samples	Mean ± SE	Min.	Max.	0.001
Fresh liver	2.79 ± 0.05^{b}	2.45	3.14	
Frozen liver	3.27 ± 0.11^{a}	3.10	3.45	

Count expressed as a $\log^{10}$ CFU/g.
SE=Standard error; Min., minimum; Max, maximum.
[a,b] Mean within the same column of different samples are significantly different at ($P \leq 0.05$).

4. DISCUSSION

The current study aimed to assess the sanitary indices of fresh and frozen bovine liver sold in different supermarkets around Menofia governorate, Egypt. Similar to South Africa, offal dishes, especially liver, are popular among Egyptians of all backgrounds (van Heerden and Morey, 2014). One of the key determinants of customer attitude regarding the perception and purchase of meat and meat products is sensory attributes. Therefore, Egyptians still favor fresh liver meals over those that are frozen. However, consuming veal liver was linked to an increased risk of campylobacteriosis, as indicated by the presence of priority pathogens such as Salmonella, Campylobacter, and *Escherichia coli* O157:H7 in veal liver samples collected from slaughterhouses and retail outlets (Gaulin et al., 2018). Microbiological criteria, which are also called performance standards, are made to set control limits for the contamination of pathogens in food or environments where food is produced along the supply chain using a certain sampling plan and measurement method (Gamil et al., 2024) with the end goal of lowering the number of foodborne illnesses. The sanitary guidelines for chilled meats and coproducts from the Egyptian Standardization Organization (No. 3602/2013) say that the total number of coagulase-positive Staph. aureus and *E. coli* should not be higher than 2 logs CFU/gm and APC should not be higher than 6 logs CFU/gm. Moreover, neither Salmonella nor the typical enteropathogenic *E. coli* should be present in the examined samples. Additionally, the shelf life at 4°C cooling should not be longer than 6 days (EOS, 2008, 2004).

Current survey findings indicated that frozen livers had superior hygienic indices compared to fresh livers. Based on the Egyptian Standard of Chilled Meats No. 3602/2013, all of the fresh liver samples that were tested were higher than the maximum levels of APC and coliform, which are 6 log10 CFU/g and 2 log10 CFU/g, respectively. Although *E. coli* were not serotyped for pathogenicity identification, fresh liver samples showed significantly high *E. coli* counts (mean 4.44 log10 CFU/g; range 4.13-4.75 log10 CFU/g) that exceeded four logs. While all frozen samples were unsafe for the coliform count, the majority of them had APCs below the maximum allowable levels of 5 log10 CFU/g when compared to EOS 2007/1473 for frozen liver.

Butcheries typically dispatch non-refrigerated edible offals to the market separately in plastic containers, meat delivery containers, or carriers, and they are rarely packed. NFSA standards for food safety are only followed by modern abattoirs and stocks. Offals are packaged and shipped in refrigerated containers with clear instructions to hotels and restaurants. Of course, mixing offals with highly contaminated meat, as well as non-refrigerated transportation and display, may jeopardize their microbial safety and quality (Sirma et al., 2023). In agreement with the present findings, earlier studies found that the average pH of buffalo liver was 6.42 and that the average microbial numbers (log10 cfu/g) of the different organisms were as follows: APC levels were Enterobacteriaceae counts were 4.97, psychrotrophs were 4.30, and staphylococcal counts were 2.51 (Devatkal et al., 2004). In an earlier study, the fresh liver contained a lower average intrinsic microbial population than the current study: APC 5.72 log cfu/g, coliform count 5.30 log cfu/g, staphylococcal count 5.41 log cfu/g, and *E. coli* count 2.88 log (Selvan and Mendiratta, 2019).

Between October 2014 and March 2017, 339 animal livers—including 194 sheep and beef livers—were collected from 138 merchants and 16 slaughterhouses in Quebec, Canada. Salmonella, Campylobacter, and *E. coli* O157:H7 were evaluated in the livers. It was indicated that Campylobacter was present in 28.0% of the livers from vegetables, 22.2% of the livers from pork, 36.8% of the livers from chicken, and 19.1% of the livers from beef. The prevalence of Salmonella was higher in pig and chicken livers (19.1% and 22.1%, respectively) than in veal livers (3.1%); it was not detected in beef livers. *E. coli* O157:H7 was rarely detected in all liver types. The proportion of infected livers varied according to animal type and sample site. Compared to veal livers taken from slaughterhouses (16.2%), a larger percentage of veal livers (35.7%) obtained from retailers were contaminated with Campylobacter. The contrary tendency was observed with chicken and hog livers. The explanation for these differences was unknown at this time, but these findings could be due to the small number of samples collected at each location (Gaulin et al., 2018).

One of the control techniques for poor fresh liver hygiene quality and to avoid poor storage quality is that liver should be processed and cooked as soon as possible after slaughter or made into a product. Offals require the same attention as carcass meat in order to prevent contamination or an overabundance of microbial development (Sirma et al., 2023). The findings imply that retailers, food outlets, slaughterhouses, and the public should adhere to NFSA-approved food handling practices for veal liver and other offal.

5. CONCLUSIONS

The results showed that frozen liver samples had significantly lower mean APC, coliform count, *E. coli*, and mold and yeast counts than fresh liver samples. However, compared to frozen samples, the mean load of Staph. aureus was much lower in fresh liver samples. In comparison to the Egyptian Standard of Chilled Meats No. 3602/2013, all collected fresh liver samples surpassed permissible APC (6 log10 CFU/g); however, all frozen samples met acceptable APC. Both fresh and frozen liver samples exceeded the permissible limits of coliforms, *E. coli*, and Staph. aureus (2 log10 CFU/g). These findings indicate unsanitary fresh liver production, shipping, and/or handling processes, as well as a failure to follow best practices and legal norms.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

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