Assessment of hygienic and nutritive quality of retailed minced meat in Egypt

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A B S T R A C T

The study was conducted on ninety fresh, chilled and frozen minced meat to determine their bacterial and nutritional quality and to be screened for meat-borne pathogens in the Menofiya governorate. Statistically, the findings of Aerobic mesophilic, coliform, Staphylococci, and *Staphylococcus aureus* counts were not significantly different (P >0.05) between the three meat types. Compared to the Egyptian organization standards (EOS), 82.2 % of minced meat had unacceptable Aerobic mesophilic counts, while 79% showed higher levels of *S. aureus*. In addition, the highest loads of Psychrotrophic counts (5.62 log cfu / g), Coliform counts (3.98 log cfu/g) and Mold and Yeast counts (4.99 log cfu /g) were found, correspondingly, in frozen, chilled and fresh minced meat. The overall incidence of *Salmonella* sp. out of the three meat types, was 27.8 %. Salmonellae was also isolated from fresh (33.3%), chilled (26.7%) and frozen (23.3%) minced meat. *Salmonella* Enteritidis and *Salmonella* Typhimurium were the major isolated strains. Regarding protein content, 73.3%, 80% and 66.7% of fresh, chilled and frozen minced meat were approved comparable to EOS, while all samples were accepted for fat content. In conclusion, the results of the current study indicate that the three minced meat types sold in the Menofiya governorate were not adequately hygienic. The Egyptian food hygiene authorities shall therefore consider the implementation of a "pathogen-specific control system", beginning with primary production units, and strictly track compliance with hygiene measures during the preparation, processing and exhibiting of raw meat to obtain safe food.

Key words: Minced meat, Aerobic mesophilic counts, Psychrotrophic counts, coliform, *Salmonella*, *Staphylococcus aureus*, Mold/Yeast.

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1. INTRODUCTION

Food safety considered a priority for all stakeholders deal with the food supply and consumption. The intact tissues of healthy slaughtered animals are mostly sterile, but bacterial contamination of meat during processing is inevitable, especially when fresh meat ground and sold as such. Minced meat may be used for meat products containing additives and, in addition, usually cooked before consumption, or may be eaten raw in certain regions (Nørrung and Buncic, 2008). Preparation of minced meat requires the

degradation of meat tissue with the release of meat juices, thus spreading the bacteria usually present on the surface of the meat throughout the whole product (Narasimha Rao and Ramesh, 1988).

The meat may be contaminated during slaughtering from hands, workers, clothes, knives, hide, gut, fecal material on feet or from the environment (Tshabalala, 2011). Also, Cross-contamination during handling at food processing and retail sites has been identified as a causative agent of human illness (Perez-Rodriguez et al., 2010). Moreover, food equipment used during mincing, inadequate refrigeration, inadequate cooking or heating, or poor personal hygiene were reported as potential vehicles of *salmonella* and coagulase positive *S. aureus* contamination throughout the food (meat) chain *(Bintsis, 2017; Gounadaki et al., 2008)*. Thus, minced meat deserves particular attention because they provide an ideal environment for bacterial growth and can be the source of pathogenic and toxinogenic microorganisms.

Generally, harmful effects as spoilage of meat, reduced shelf-life of meat and public health hazard, as infection or intoxication, are the main sequalae of microbially contaminated meat (Eley, 1992; Park et al., 2015). Similarly, fungi occur in meat and meat products could cause various degrees of spoilage and/or render the affected meat hazardous by the production of mycotoxins (Feldsine et al., 2003; Ismail et al., 1995).

Many foodborne diseases, as food infection or intoxication, are caused among consumers by ingestion of meat contaminated with microbial pathogens (Eley, 1992). Of them, salmonellosis constituting a worldwide major public health concern and remains one of the most frequent food-borne zoonoses, (Antunes et al., 2016). The prevalence of Salmonella in food in tropical African countries was found to be at high level as compared to the rest of the world (Wikswo and Hall, 2012). Foods of animal origin, particularly meat, often implicated in human salmonellosis (Hussein and Bollinger, 2005; Todd, 1997). Around 20% and 50% of all human salmonellosis cases were attributed to meat (Nørrung and Buncic, 2008). The young children, the elderly, and immuno-compromised individuals are most at risk for complications, people at any age are susceptible to the diarrhea, intestinal cramping, and intestinal epithelial erosion associated with salmonellosis. The disease is often self-limiting, but can cause prolonged complications (Graham et al., 2000).

Moreover, children under 5 years of age were affected with a 40% of the foodborne disease. Worldwide, 18 million disability adjusted life years (DALYs) were attributed to foodborne diarrheal disease agents, particularly non-typhoidal *Salmonella* enterica (NTS), and the highest mortality rates because of foodborne diseases were in the African subregions (WHO, 2015).

The ability to produce enterotoxin and the possibility of subsequent food poisoning triggered the presence of *Staph. Aureus* in food may be considered as a public health hazard (Bintsis, 2017). The CDC reports that staphylococcal food poisoning in the US causes about 241,188 infections, 1,064 hospitalizations and 6 deaths each year (Food Drug Administration, 2012).

Of 1.5 million fungal species, around 300 fungal spp. are harmful to human health, could cause illnesses ranging from allergic reactions to life-threatening invasive infections (Hawksworth, 2001). Furthermore, World Health Organization (WHO) globally reported that fungi or their byproducts may be responsible for Some food-borne diseases (WHO, 2015).

To counteracting the food poisoning and high economic losses, the prevalence of such meat related pathogens and fungal contamination shall be detected at the early stages, to properly handle and control this meat. So, the current study focused to evaluate the microbiological and nutritional quality of fresh, chilled and frozen minced meat produced at market level in Menofiya governorate.

2. MATERIALS AND METHODS

2.1. Collection of samples:

A total of 90 fresh, chilled and frozen minced meat samples were collected from various butcher shops and supermarkets in Menofia Governorate. Each sample was wrapped separately in sterile polyethylene bag and transferred directly to the laboratory without delay in an ice box. In the laboratory, the following examinations were performed at once.

2.2. Preparation of samples:

Aseptically, 25 grams were taken of each sample, and then, separately homogenized in 225 ml of 0.85 % sterile saline for 1 min at room temperature. For detection of *Salmonella*, portion of the minced meat was mixed in 0.1 % sterile buffered peptone water for preenrichment.

2.3. Bacteriological Analysis:

Total aerobic mesophilic Count (AMC), Total Psychrotrophic count and coliform Count were determined according to (Sabike et al., 2014) and ISO 21528 – P2 :2004 (ISO, 2004). Briefly, a serial 10-fold dilution of sample were prepared using sterile normal saline, then dilutions of each sample were inoculated in duplicate in to aerobic plate count agar and Violet red bile agar (VRB) agar. The plates were then incubated at 37°C for 24 h before colonies were counted. The plates of Total Psychrotrophic count were incubated at fridge temp. (7°C) for 7 days.

2.4. Total Staphylococci and *Staphylococcus aureus* counts:

Enumeration and identification of *Staphylococcus aureus* were carried out according to (Bennett and Lancette, 2001; Sabike et al., 2014). A 0.1 ml from each of previously prepared serial dilutions was spread over duplicated plates of Baired Parker agar using a sterile disposable glass spreader. The inoculated and control plates were incubated at 37°C for 48 hours. For detection of coagulase positive *S. aureus*, a 0.5 ml reconstituted coagulase plasma was added to 0.2-0.3 ml Brain heart infusion (BHI) broth, inoculated with suspect *S. aureus* colonies and incubated for18-24 h at 35-37°C, and mix thoroughly. Incubate at 35-37°C and examine periodically over 6 h period for clot formation. Only firm and complete clot that stays in place when tube is tilted or inverted is considered positive for *S. aureus*.

2.5. Total Mold and Yeast count:

Given counting the total mold and yeast, one ml of the previously prepared serial dilutions was aseptically transferred into double sterile Petri dish, and then ten ml of Sabouraud Dextrose agar media cooled at 45ºC, were added and thoroughly mixed. Then, the plates were incubated at 25 or 28ºC for 48 hours. (ISO, 2008)

2.6. Conventional isolation of *Salmonella* spp.*:*

According to ISO 6579:2002 protocol (InternationalOrganizationforStandardization(ISO), 2002), The homogenate specified for isolation of salmonella was incubated at 37°C for 22 h for pre-enrichment. After resuscitation, 0.1 mL was inoculated into 10 ml Rappaport-Vassiliadis medium and incubated at 42°C for 24 h. After enrichment,

a loopful of each enriched sample was streaked onto xylose lysine desoxycholate agar and incubated at 37°C for 24 h.

2.7. Serological Identification:

Presumptive positive samples of Salmonella were subcultured onto nutrient agar slopes and sent to the Animal Health Research Institute Laboratory (Dokki Giza, Egypt) for serotyping. *Salmonella* serology was done according to Kauffmann–White serotyping scheme (Grimont and Weill, 2007; InternationalOrganizationforStandardization(ISO), 2014) using slide agglutination tests with commercial polyvalent and monovalent somatic and flagellar antisera.

2.8. Chemical assessment:

The collected samples were subjected to the chemical examination to estimate the nutritional value of the examined minced meat samples (fresh, chilled and frozen) according to the specification stipulated by EOS (1694/2005). Protein content and Fat content were determined by Kjeldahl and Soxhlet Apparatus according to Horwitz (2000).

2.9. Statistical analysis:

Microsoft Excel 2016 (Microsoft Excel 2016 MSO, Microsoft Corp., Redmond, WA) was used to calculate prevalence, average and maximum values, in addition to the excel function titled “data analysis; Anova; Single factor” was used to look for significance differences between the three forms of meat, with a level of significance of $P < 0.05$.

3. RESULTS

The microbiological profile of fresh, chilled and frozen minced meat samples is presented in Table 1. The mean APC of fresh, chilled and frozen minced meat were 6.44, 6.07 and 5.94 log CFU/g, respectively. Statistically no significant difference ($P > 0.05$) was found between the three meat forms, however the calculated APC from the 82.6, 83,3, and 76.7% of fresh, chilled and frozen minced were significantly higher ($P < 0.05$) than the maximum acceptable level (6 log CFU/g) required by Egyptian standards (ES No. 4334:2004) (Table 4). In contrast, Psychrotrophic counts showed significant differences between the fresh (5.44 log CFU/g), chilled (5.56 log CFU/g) and frozen (5.62 log CFU/g) minced meat. Further, *Coliform* were also detected in the three minced meat types with mean counts of 3.67, 3.98, and 3.55 log CFU/g, respectively. The mean Staphylococci counts detected at the three different meat were 4.13, 4.30, and 4.61 log CFU/g, respectively. The mean Staphylococci, and *Staphylococcus aureus* counts in the fresh meat were not significantly different ($P> 0.05$) from the other two types, chilled and frozen, but both counts in the three types were also greater than EOS criteria. Concerning Mold &yeast count, significant difference ($P > 0.05$) was found between the three minced meat types, and the counts were 4.99, 4.85, and 4.47 log CFU/g, respectively (Table 1). In comparison to Egyptian standards (No. 4334:2004), fresh meat shall be negative for coliform count, Enterobacteriaceae count, and Mold and yeast.

Table (1) Mean values of different microbial counts of the examined minced meat samples (n=30 of each):

Parameter	Fresh		Chilled		Frozen		***P* value**
	Mean (log CFU/g	+ve sample %	Mean (log CFU/g	+ve sample %	Mean (log CFU/g	+ve sample %	
Aerobic mesophilic count	6.44	100	6.07	100	5.94	100	0.27
Psych. Count	5.44	100	5.56	100	5.62	100	0.00
Coliform count	3.67	50	3.98	60	3.55	33.3	0.05
Staphylococci count	4.13	86.6	4.30	93.3	4.61	70	0.27
S. aureus count	3.33	70	3.80	76.7	3.63	60	0.33
Mould and yeast count	4.99	100	4.85	100	4.47	100	0.00

Percentage was recorded according to the total numbers of examined samples.

Table (2): Incidence and serotyping of Salmonellae isolated from the examined minced meat samples (n=30).

Salmonella Strains	Fresh		Chilled		Frozen	
	No.	%	No.	%	No.	%
Salmonella enterica serovars Enteritidis	3	10	2	6.67	3	10
Salmonella enterica serovars Typhimurium	4	13.33	2	6.67	1	3.33
***salmonella** anatum*	2	6.67	2	6.67	1	3.33
Salmonella enterica serotype Muenster	1	3.33	1	3.33	1	3.33
Salmonella haifa	-	-	1	3.33	1	3.33
Total	10	33.3	8	26.67	7	23.3

Percentage was recorded according to the total numbers of examined samples.

Table (3): Statistical analytical results of chemical quality parameters in the examined minced meat samples (n=30).

Parameter	Fresh		Chilled		Frozen	
	Mean%	Accepted samples%	Mean%	Accepted samples%	Mean%	Accepted samples%
Protein%	17.79±1.45	66.7	18.01±0.85	73.3	18.91±0.69	80
Fat %	11.05±0.63	100	12.19±0.75	100	11.55±0.48	100

Percentage was recorded according to the total numbers of examined samples; Acceptance according to EOS (2005).

Table (4): Acceptability of examined minced meat samples according to EOS (2005)

Parameter	MPL[A]	Accepted Fresh		Accepted chilled		Accepted frozen		Total	
		No.	%	No.	%	No.	%	No.	%
Aerobic mesophilic count	<6 log	4	13.33	5	16.7	7	23.3	16	17.8
S. aureus count	2 log	9	30	7	23.3	12	40	28	31.1
Salmonella	0	20	66.7	22	73.3	23	76.7	65	72.2
Protein %	>18%	20	66.7	22	73.3	24	80	66	73.3
Fat %	<20%	3	100	30	100	30	100	90	100

[A]*M.P.L. maximum acceptable limit according to EOS (2005) No.1694/2005 for minced meat.*

4. DISCUSSION

Food and their products, especially meat, shall be microbiologically surveyed to find opportunities for improving food safety, and to utilize the generated data to validate regulatory systems and further to define the performance standards that are integrated into regulations (Phillips et al., 2006). Similarly, recent study aimed to evaluate fresh, chilled and frozen minced meat samples collected from various butcher shops and supermarkets in Menofia Governorate, and to clarify different practices which may add more microbial load to minced meat, and to check the commitment with good hygienic practices recommended by Egyptian General Organization of Veterinary Services (GOVS) to produce safe meat.

Generally, microbial flora of meat is reflective of the slaughtering and processing environments (Jay et al., 2005). In current study, minced meat showed over counts of AMC, coliform, Staphylococci and Mold and yeast comparable to Egyptian standards (ES No. 4334:2004) (EOS, 2004) and to international standards, European and Australian standards, for fresh meat criteria.

In details, the AMC ranged from a low of 4.69 to a high of 6.46 log CFU/g. which considered acceptable according to European standards (EC, 2007) (5.7-6.7 log CFU/g) for AMC in fresh meat, but in comparison to Egyptian standards (< 6 log CFU/g) it will be unsatisfactory. This range is lower than the ranges reported by Daly et al. (1976) (5.54 to 9.11 log CFU/g) and Zerabruk et al. (2019) (5.0 to 7.44). Also, Duitschaever et al. (1973) found that Mesophilic and Psychrotrophic counts on 64% of the raw refrigerated ground beef were in excess of 7 log CFU/g. however, current results were higher than the total aerobic mesophiles (3.4 log CFU/g), and psychrotrophs (3.3 log CFU/g) recorded earlier by Pao and Ettinger (2009).

Coliform one of the indicator organism, whereas their presence usually provides evidence of poor hygiene, and post-process fecal contamination of foods. Coliform comprises a number of important foodborne pathogens such as pathogenic *Salmonella* species (Baylis et al., 2011). The prevalence of Coliform in the fresh, chilled and frozen minced meat were 50, 60, and 33.3%, with a mean of 3.67, 3.98, and 3.55 log CFU/g, respectively, and the count ranged between 3.0 to 4.86 log CFU/g, which is similar to the data of Narasimha Rao and Ramesh (1988). However, this result is significantly lower than previously recorded, where 95% of the samples had coliform count higher than 2 log CFU/g (Duitschaever et al., 1973) and the counts varied from <10 to 100,000 per gram. Similarly, Zerabruk et al. (2019) and ERDEM et al. (2014) published higher ranges of coliform counts, 5.30 to 7.28 log CFU/g and 4.43 to 8.30 log CFU/g, respectively.

Also, Egyptian Standards (ES No. 4334:2004), required that fresh meat shall be free of *Salmonella* species, but the results in Table 2 and 4 showed that 33.3% of fresh, 26.7% of chilled and 23.3% of frozen minced meat were *Salmonella*-positive (Table 2). *Salmonella enterica* serovars Enteritidis and Typhimurium, accounted for approximately 60% (15/25) of detected strain (Table 2), which in agreement with previous report that both strains were the most frequent serovars related to human illnesses (EFSA Panel on Animal Health Welfare, 2012) while 20 % (5/25), 12 % (3/25), 8 % (2/25) were the occurrence rates for *Salmonella anatum*, *Salmonella enterica serotype Muenster*, and *salmonella haifa* in minced meat samples, respectively. The data of *Salmonella* sp. indicate that 27.8 % (25/90) of minced meat samples tested in current investigation considered unsatisfactory and doesn't meet Egyptian standards (EOS, 2004), and is

significantly higher than reported earlier (Siriken, 2004).

In USA, *Salmonella* classified the most common bacterial cause of foodborne outbreaks, caused 62.6% (149/238) outbreaks and 3,944 illnesses, *Salmonella* Enteritidis was the most common incriminated serotype, accounted for 51 outbreaks (CDC, 2017). Similarly, in England and Australia nontyphoidal *Salmonella* spp. was the leading cause of foodborne diseases (Scallan et al., 2011). Worldwide, nontyphoidal Salmonella, comprising invasive nontyphoidal Salmonella (iNTS), resulting in the highest foodborne disability adjusted life years (DALYs), 4.07 million (WHO, 2015).

Totally, 68.9% (62/90) of meat samples contained *Staphylococcus aureus*, which is greater than recorded earlier (17%) (Duitschaever et al., 1973). In contrary, it was lower than what calculated by ERDEM et al. (2014), 96.6 %.The highest incidence (76.7%, 23/30) found in chilled meat, followed by fresh (70 %, 21/30), and then frozen meat (26.7%, 8/30) (Table 1). The high contamination rate of total Staphylococci (83.3%, 75/90), with a count varied from 3.56 to 4.88 log CFU/g, indicates the bad hygiene during the manipulation of meat. Unfortunately, many staphylococci other than *S. aureus* are able to produce Enterotoxins (Becker et al., 2001), and meat is, among the optimum growth media, frequently implicated in Staphylococcal food poisoning outbreaks. In the United States, *S. aureus* considers a significant cause of food-borne disease, accounted for 241,000 illnesses/ year (Scallan et al., 2011), further worldwide it is the second leading cause of food poisoning outbreaks (Yao et al., 2015).

All minced meat products (100 %) were positive for yeasts and molds counts and significant differences were found between the three products ($P < 0.05$), (Table 1). In comparison to the counts in previous studies, 1.4 log CFU/g (Pao and Ettinger, 2009), the yeasts and molds counts recorded here were high, but (ERDEM et al., 2014) found similar incidence rate and wider range (3.85-8.60 log CFU/g). Recently, fungal infections have emerged as a significant health problem associated with immunocompromised people (Pérez-Torrado and Querol, 2016). Hence, it is significantly important to prevent fungal contamination to avoid mycotoxin production and human infection (Vesković-Moračanin et al., 2009).

Protein of animal sources, containing all essential amino acids, and therefore provide a complete source of protein. Evaluation of the protein is important in assessing its appropriateness in the human diet. Proteins with inferior content and digestibility should be recognized and restricted or limited in the diet (Hoffman and Falvo, 2004). The obtained results in table (3) revealed that the frozen minced meat samples were higher in protein content, but fresh samples had lower protein content. There were significant differences between the examined samples ($P <0.05$). Also 66.7%, 73.3% and 80 % of examined fresh, chilled and frozen minced meat samples were accepted on the basis of protein percent according to EOS (2005). In terms of fat content, table (3) showed that refrigerated minced meat samples were higher in fat content, but fresh samples had the lowest fat content with significant differences ($P < 0.05$) between the samples examined. It was clear that 100 percent of minced meat samples were approved according to EOS (2005) for a fat content of less than 20 per cent.

Eventually, many unhygienic practices and habits were observed during current survey, which may contribute to higher microbial contamination of minced meat. These involving: the use of low-quality meats

cuts for mincing, unclean mincing equipment, keeping the mincing tools at room temp., low personal hygiene during processing and cleaning and disinfection of equipment and food contact surface are not routinely applied.

The microbial load and the flora composition of minced meat directly related to the bacteriological quality of the meats used for mincing, cleanliness of equipment, time and temperature of storage, and hygiene practices during the whole meat cutting and deboning procedures. Microbial cross-contamination through the hands and utensils and transfer of bacteria from the surface of the meat to the inner parts occurs during meat cutting and deboning operations due to the relatively intense handling and processing of the meat, which significantly increases microbial risks (Narasimha Rao and Ramesh, 1988; Nørrung and Buncic, 2008).

Conclusively, most of the hygienic measures examined in the current study did not fall within the acceptable ranges provided by EOS. Of course, infectious illnesses, hospitalization and economic losses will be a significant consequence of the ingestion of contaminated meat with these pathogens and/or their toxins. Current studies can improve the awareness of food safety authorities on the potential transfer of foodborne pathogens via minced meat. All Egyptian food safety organizations and authorities shall therefore pay more attention to the microbiological testing of minced meat sold in Egypt to ensure that their food safety standards relating to minced meat / meat preparations are enforced on the markets and to verify that the produced meat is free of foodborne pathogens and safe for human consumption.

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