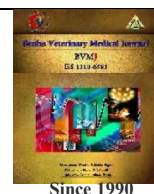




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Evaluation of the microbiological status of some retailed bovine meat and dairy-products with an improvement trial for the beef mince using acetic and lactic acids

Gehan S. A. Eltanani^{1*}, Seham Elbadry², Hend S. Nada³, Samah S. Abd Ellatif⁴, Heba Mohamed M. El-Sherbiny⁵

¹Department of Food Hygiene and Control (Meat Hygiene and Technology), Faculty of Veterinary Medicine, Benha University, Egypt

²Central Laboratory, Faculty of Veterinary Medicine, Zagazig University, 44511, Egypt

³Microbiology Department, Faculty of Veterinary Medicine, Zagazig University, 44511, Egypt

⁴Milk Hygiene and Technology, Department of Food Control, Faculty of Veterinary Medicine, Zagazig University, Sharkia Governorate, Zagazig City, 44511, Egypt

⁵Educational Veterinary Hospital (Meat Hygiene and Technology), Faculty of Veterinary Medicine, Mansoura University, Egypt

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ABSTRACT

Bovine meat and dairy-products are among the important sources for animal-derived protein, vitamins, and minerals. Meat and dairy products can be contaminated during processing, distribution, and storage, and can be implicated in the transmission of many foodborne pathogens world-wide. This study was undertaken to investigate the microbiological status of some bovine meat products (beef mince, sausage, luncheon, and basterma), and some dairy products (raw milk, dried milk powder, yoghurt, and kariesh cheese) retailed in the food markets in Zagazig city, Egypt. Evaluation of the sanitary status of these products were done via estimation of total bacterial count (TBC), total psychrophilic count (TPsC), coliforms count, total Staphylococci count (TSC) and total mold count (TMC). A trial for improvement of the microbiological status of the beef mince was conducted using acetic and lactic acids at different concentrations. The achieved results indicated unsatisfactory sanitary status of the examined products in the present study, in terms of high microbial counts. A clear and significant reduction for the microbial load was achieved after treatment of the beef mince with acetic and lactic, particularly at 2%.

1. INTRODUCTION

Bovine meat products such as beef mince, sausage, luncheon, and basterma, and dairy products as milk, cheese, dried milk, and yoghurt are considered as primary sources for the animal derived protein with high bioactive peptides, vitamins and provide humans with part of their needs from energy (Morshdy et al., 2018).

Microbial spoilage of meat and dairy products is determined by the hygienic practices adopted from the moment of milking, or slaughtering, dressing, evisceration, and further processing. Contamination of meat and dairy products may occur during processing or may be due to the use of contaminated raw materials or collecting containers (Amberle et al., 2001; Darwish et al., 2015).

Testing of some spoilage markers is of a significant importance from the microbiological, food safety and keeping quality points of view. Spoilage markers to judge the hygienic measures adopted during handling and manufacturing processes include estimation of total bacterial counts (TBC), total psychrophilic counts (TPsC), coliforms count, total staphylococci count (TSC), and total mold counts (TMC) (APHA, 2001).

Chemical antimicrobials are compounds that are used in the food industry for delaying the microbial growth or even cause microbial death in the food products. Organic acids such as acetic, and lactic acids are generally recognized as safe compounds and are broadly used in the food industry to reduce bacterial contamination. These chemicals have been found to be of high value in reducing or even prevention of some foodborne pathogens such as *Listeria monocytogenes*, *E. coli* O157:H7, and *Salmonella typhimurium* associated with food industry (Fabrizio et al., 2002).

Therefore, this study was undertaken to investigate the microbiological status of four bovine meat products: beef mince, sausage, luncheon, and basterma. And in four bovine dairy products including raw milk, dried milk powder, kariesh cheese, and yoghurt was further examined. Furthermore, the antimicrobial properties of acetic and lactic acids (1% and 2%) were tested using the beef mince as a food matrix.

* Corresponding author: Gehan S. A. Eltanani, Department of Food Hygiene and Control (Meat Hygiene and Technology) Faculty of veterinary medicine Benha University – Moshtohor, Kalyobiya 13736, Egypt.

2. MATERIAL AND METHODS

2.1. Collection of Samples:

A grand total of 160 random meat and dairy product samples including beef mince, sausage, luncheon, basterma, raw milk, kariesh cheese, dried milk powder, and yoghurt (20 of each) were collected from retail markets and grocery stores in Zagazig city, Egypt. Samples (50 g of each) were transferred cooled directly without delay to the laboratory for microbiological examination.

2.2. Organoleptical examinations:

Organoleptical examination for the examined samples was conducted using the method of Varnam and Sutherland (1995).

2.3. Microbiological examinations:

Samples were prepared according to the technique recommended by APHA (2001).

2.3.1. Determination of total bacterial count (TBC):

Total bacterial count was estimated using the method of APHA (2001) using plate count agar (Difco, USA) and incubation of the Petri dishes at inverted position for 48 h at $35 \pm 2^\circ\text{C}$.

$\text{TBC/g} = \text{average No. of colonies} \times \text{reciprocal of dilution}$
Counted colonies expressed as $\log_{10} \text{cfu/g}$.

2.3.2. Determination of total psychrophilic count (TPsC):

For estimation of the total psychrophilic count, the pour plate technique recommended by APHA (2001) was applied using standard plate count agar medium and incubated at 7°C for 10 days.

2.3.3. Determination of most probable number (MPN) of Coliforms:

Three tubes most probable number (MPN) method (APHA, 2001) was adopted.

2.3.4. Determination of total Staphylococcus count (TSC):

Regarding *Staphylococcus* count, culture was done according to the method of Quinn et al. (2011) using Baird Parker agar (Biolife, Italy) supplemented with egg yolk-tellurite emulsion (Himedia, India).

2.3.5. Determination of total mold count (TMC):

Total mold counts were determined by the pour plate technique using Sabouraud's dextrose agar media (Oxoid, UK) supplemented with chloramphenicol 100 mg/L followed by incubation in dark at 25°C for 5-7 days (Vanderzant and Splittstroesser, 2001).

2.3.6. Inoculation of the organic acids to the beef mince:

In a trial for reduction of the microbial load of the beef mince, acetic and lactic acids were used at two concentrations (1%, and 2%). Collected minced meat samples (Five samples, 300g/each) were formulated as meat balls ($n=5$, 50g/each). Formulated meat balls were grouped into 6 groups. Group 1 was immersed in clean distilled water for 30 min and served as a control; group 2 was immersed in acetic acid 1%; group 3 was immersed in acetic acid 2%; group 4 was immersed in lactic acid 1%; group 5 was immersed in lactic acid 2%, and group 6 was immersed in a mixture of both acetic and lactic acids at 2%(1:1). The exposure duration for all treatments lasted 30 min at room temperature (Darwish et al., 2015). Microbiological examination was conducted as mentioned before.

2.4. Statistical analysis:

All values were expressed as means \pm SD, and all measurements were carried out in duplicates. Microbial counts were converted into base logarithms of colony forming units per g ($\log_{10} \text{cfu/g}$). Statistical significance was evaluated using One way analysis of variance (ANOVA), followed by the Tukey-Kramer HSD post hoc test. In all

analysis, $P < 0.05$ was taken to indicate statistical significance (Gomez and Gomez, 1984).

3. RESULTS

Organoleptic examination of the collected samples revealed that all samples had normal sensory parameters. Microbiological examination of the collected samples revealed that all examined samples (100%) were contaminated with bacteria except for the dried milk powder where only 60% of the examined samples were contaminated (Fig. 1). The mean values of TBC for the examined samples were 6.41 ± 0.25 , 4.39 ± 0.26 , 4.16 ± 0.34 , and 4.79 ± 0.42 - $\log_{10} \text{cfu/g}$ for the examined beef mince, sausage, luncheon, and basterma, respectively (Fig.2A). For the examined raw milk, yoghurt, kariesh cheese, and dried milk powder these values were 6.46 ± 0.61 , 5.12 ± 0.63 , 4.97 ± 0.42 , and 2.75 ± 0.27 - $\log_{10} \text{cfu/g}$, respectively (Fig. 2B).

All examined samples (100%) were positive for psychrophilic bacteria, except for dried milk powder, where only 15% of the examined samples were positive for psychrophilic counts (Fig. 1). The mean concentrations of TPsC in the examined beef mince, sausage, luncheon, and basterma were 3.37 ± 0.28 , 3.09 ± 0.28 , 3.33 ± 0.29 , and 3.26 ± 0.29 - $\log_{10} \text{cfu/g}$, respectively (Fig. 3A), while these counts for the examined raw milk, yoghurt, kariesh cheese, and dried milk powder were 3.63 ± 0.23 , 3.23 ± 0.19 , 3.40 ± 0.25 , and 2.87 ± 0.15 - $\log_{10} \text{cfu/g}$, respectively (Fig. 3B).

Coliforms were detected in all of the examined products except for dried milk powder, where only 20% of the examined samples showed positive results (Fig. 1). The average values of coliforms were 4.82 ± 0.39 , 3.68 ± 0.18 , 3.43 ± 0.30 , and 3.77 ± 0.24 - $\log_{10} \text{MPN/g}$ in the examined beef mince, sausage, luncheon, and basterma, respectively (Fig. 4A). These values for the examined raw milk, yoghurt, kariesh cheese, and dried milk powder were 4.77 ± 0.13 , 3.48 ± 0.20 , 3.53 ± 0.23 , and 2.61 ± 0.15 - $\log_{10} \text{cfu/g}$, respectively (Fig. 4B).

Total *Staphylococcus* counts were further estimated in the examined samples in a percentage of 100%, 85%, 90%, 100%, 100%, 80%, 100%, 35% of the examined beef mince, sausage, luncheon, basterma, raw milk, yoghurt, kariesh cheese, and dried milk powder, respectively (Fig. 1). The average total *Staphylococci* counts in these sample were 3.73 ± 0.62 , 3.13 ± 0.31 , 2.99 ± 0.29 , 3.45 ± 0.40 , 3.70 ± 0.24 , 3.05 ± 0.29 , 3.16 ± 0.29 , and 2.61 ± 0.14 - $\log_{10} \text{cfu/g}$, respectively (Fig. 5A, B).

Mold contamination rates in the examined samples were 100%, 80%, 80%, 100%, 100%, 65%, 100%, 40% of the examined beef mince, sausage, luncheon, basterma, raw milk, yoghurt, kariesh cheese, and dried milk powder, respectively (Fig. 1). The average TMC in these sample were 3.84 ± 0.35 , 3.07 ± 0.17 , 2.93 ± 0.16 , 3.50 ± 0.24 , 3.63 ± 0.15 , 2.97 ± 0.34 , 2.89 ± 0.22 , and 2.90 ± 0.13 - $\log_{10} \text{cfu/g}$, respectively (Fig. 6A, B).

In an improvement trial for the microbial status of the beef mince, acetic and lactic acids at 1%, and 2% were used. The achieved results in Table 1 declared that TBC in the formulated meat ball samples was significantly reduced by 10.68%, 14.44%, 12.40%, 17.74%, and 24.80% after treatment with acetic acid (1%, and 2%), lactic acid (1%, and 2%), and a mixture of both acetic and lactic acids 2%, respectively. These treatments reduced TPsC by 5.95%, 10.12%, 5.36%, 11.31%, and 18.45%, respectively. These treatments improved the coliforms count by 9.11%, 18.22%, 15.18%, 20.39%, and 20.46%, respectively; TSC by 15.15%, 20.98%, 17.02%, 20.98%, and 29.60%, respectively; and TMC by 9.65%, 15.59%, 12.62%, 18.56%, and 24.50%, respectively.

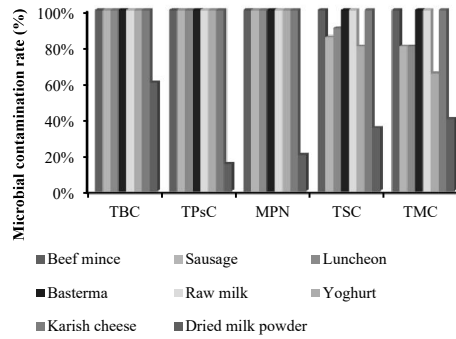


Figure 1: Microbial contamination rate (%) of the examined bovine meat and dairy products

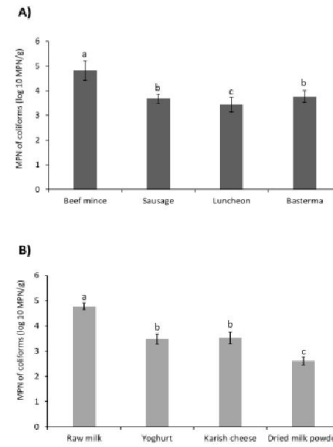


Figure 4: Coliforms count in retail bovine meat and dairy products.

Most probable number of coliforms in retail A) meat products, B) dairy products. Values represent means \pm SD (Log 10 MPN/g) of bovine meat and dairy products (n = 20/ each). Columns carrying different superscript letter differ significantly among examined samples at $P < 0.05$.

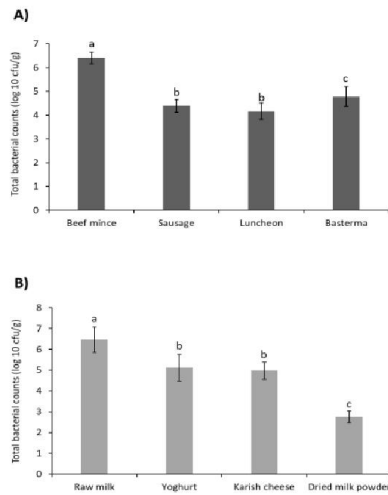


Figure 2: Total bacterial counts in retail bovine meat and dairy products

Total bacterial counts in retail A) meat products, B) dairy products. Values represent means \pm SD (Log 10 cfu/g) of bovine meat and dairy products (n = 20/ each). Columns carrying different superscript letter differ significantly among examined samples at $P < 0.05$.

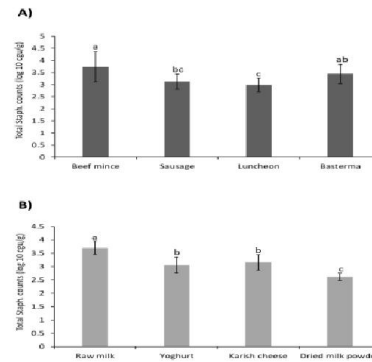


Figure 5: Total *Staphylococcus* counts in retail bovine meat and dairy products

Total *Staphylococcus* counts in retail A) meat products, B) dairy products. Values represent means \pm SD (Log 10 cfu/g) of bovine meat and dairy products (n = 20/ each). Columns carrying different superscript letter differ significantly among examined samples at $P < 0.05$.

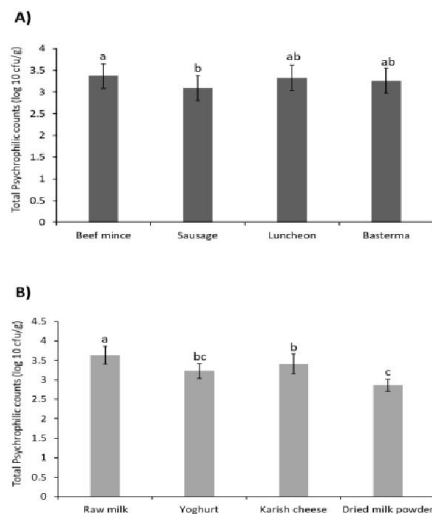


Figure 3: Total psychrophilic counts in retail bovine meat and dairy products

Total psychrophilic counts in retail A) meat products, B) dairy products. Values represent means \pm SD (Log 10 cfu/g) of bovine meat and dairy products (n = 20/ each). Columns carrying different superscript letter differ significantly among examined samples at $P < 0.05$.

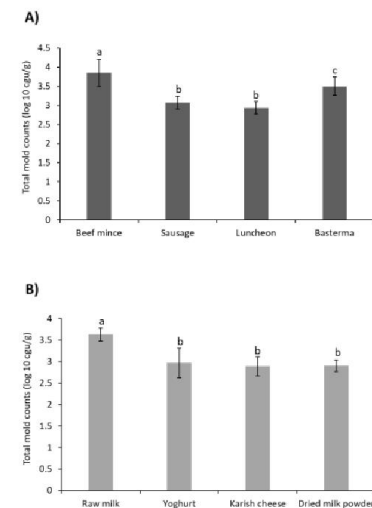


Figure 6: Total mold counts in retail bovine meat and dairy products.

Total mold counts in retail A) meat products, B) dairy products. Values represent means \pm SD (Log 10 cfu/g) of bovine meat and dairy products (n = 20/ each). Columns carrying different superscript letter differ significantly among examined samples at $P < 0.05$.

Table 1: The microbial status of the beef mince treated with acetic and lactic acids

	TBC		TPsC		Coliforms count		TSC		TMC	
	Mean \pm SD	Reduction %	Mean \pm SD	Reduction %	Mean \pm SD	Reduction %	Mean \pm SD	Reduction %	Mean \pm SD	Reduction %
Control	6.37 \pm 0.36 ^a	0	3.36 \pm 0.26 ^a	0	4.61 \pm 0.67 ^a	0	4.29 \pm 0.67 ^a	0	4.04 \pm 0.26 ^a	0
Acetic acid 1%	5.69 \pm 0.09 ^b	10.68	3.16 \pm 0.15 ^{ab}	5.95	4.19 \pm 0.46 ^{ab}	9.11	3.64 \pm 0.05 ^b	15.15	3.65 \pm 0.05 ^b	9.65
Acetic acid 2%	5.45 \pm 0.11 ^{bc}	14.44	3.02 \pm 0.23 ^{abc}	10.12	3.77 \pm 0.07 ^{bc}	18.22	3.39 \pm 0.09 ^{bc}	20.98	3.41 \pm 0.07 ^c	15.59
Lactic acid 1%	5.58 \pm 0.10 ^{bc}	12.40	3.18 \pm 0.18 ^{ab}	5.36	3.91 \pm 0.05 ^{bc}	15.18	3.56 \pm 0.07 ^{bc}	17.02	3.53 \pm 0.05 ^{bc}	12.62
Lactic acid 2%	5.24 \pm 0.15 ^c	17.74	2.98 \pm 0.13 ^{bc}	11.31	3.67 \pm 0.06 ^{bc}	20.39	3.39 \pm 0.09 ^{bc}	20.98	3.29 \pm 0.08 ^c	18.56
Acetic + Lactic acids 2%	4.79 \pm 0.09 ^d	24.80	2.74 \pm 0.07 ^c	18.45	3.39 \pm 0.09 ^c	26.46	3.02 \pm 0.23 ^c	29.60	3.05 \pm 0.08 ^d	24.50

Values within the same column carrying different superscript letter are significantly different at $P < 0.05$.

4. DISCUSSION

Bovine meat and dairy products are among the important sources for the animal-derived protein. Meat and dairy products including mince, sausage, luncheon, basterma, milk, cheese, yoghurt, and dried milk powder are preferred by a large section of the population because of their specific aroma and flavor, high nutritive values, and their easy preparation. Hygienic measures adopted during handling and processing of such products affect their initial microbiological load and subsequently affect their safety, and keeping (Tang et al., 2020). A major task for the food hygiene and food microbiology sectors is to confirm their microbial status of the retailed meat, milk and their and meat and dairy products. Therefore, it is highly recommended to perform continuous screening studies to investigate the hygienic status of the retailed bovine meat and dairy products on a regular basis. In the present work, microbial indicators for the sanitary status of such products were investigated. Such tests included TBC, TPsC, MPN of coliforms, TSC, and TMC. These indicators enable us to give correct decision about the hygienic practices adopted during product handling and processing, and subsequently accepting or rejecting the final products (Mossel et al., 1995).

In particular, beef mince and raw milk had significantly ($P < 0.05$) the highest counts among the examined samples. Similarly, Morshdy et al. (2018) reported high microbial counts with occurrence of *Staphylococcus aureus* and *Salmonella* Enteritidis contamination in the retailed meat products in Zagazig city, Egypt. Unsatisfactory hygienic measures for the retailed yoghurt, cream, kariesh cheese, Ras cheese, and Tallaga cheese were reported in dairy products retailed in Beni-Suef city, Egypt (Hassan et al., 2019).

The high bacterial counts in the beef mince and raw milk are reasonable as such products are sold raw without any heat treatments. In addition, the mincing process of meat with ingredients other than the meat itself may lead to increasing the microbiological load of

the produced mince. Furthermore, mincing machine is considered as a possible source of transferring food-borne pathogens from contaminated meat to non-inoculated ones. While dried milk powder had the lowest microbial rates compared to the other tested samples. Similarly, low rates of microbial contamination on dried milk powder were reported in Dakahlia Governorate, Egypt (Deeb et al., 2010). One possible reason is the drying process of the milk and this is considered as a major preservation method to reduce the water activity and subsequently affect the microbial growth (Papadopoulou et al., 2012). The recorded results in the current study go in agreement with those reported by Weistein, (1991) who reported that poor personal hygiene caused more than 90% of the sanitary problems in the food service industry. In addition, improper hand washing alone accounts for more than 25% of all foodborne diseases. Similarly, poor hygiene at meat and dairy products manufacturing facilities resulted in higher contamination, which may be due to dirty walls, cutting boards, unhygienic handling, and lack of knowledge of hygienic practices (Tambekar et al., 2008). Fungal contamination of the examined meat and dairy products was clear in the present study. Similar high mold contamination was reported in the retailed chicken meat cuts and giblets in Egypt (Darwish et al., 2014). This kind of contamination might lead to serious implications on both the food quality and public health. In a trial to reduce the microbial load in the beef mince, organic acids such as acetic and lactic acids were used. Interestingly, a significant reduction for the microbial load was achieved, in terms of reduction of TBC, TPsC, coliforms, TSC, and TMC. Both of the used acids improved the microbial quality in a concentration-dependent manner, with the highest reduction rate achieved with a mixture made from equal volumes of the acetic and lactic acids at 2% without any change in the sensory characters (firm in consistency, fresh odor, and brick red in color) of the final meat-product. Similarly, Menconi et al. (2013) evaluated the effects of wash solutions made from different combinations of organic acids (acetic, citric, and propionic acid) in reducing the microbial load in

raw chicken skin and to inhibit the growth of spoilage bacteria during refrigerated storage. A possible explanation for this reduction is due to lowering the pH value of the food matrix producing unfavorable media for growth and multiplication of the bacteria. In line with this assumption, Koutsoumanis et al. (2006) recorded a significant effect of the meat pH on the growth kinetics of *pseudomonads*, *B. thermosphacta*, and *Enterobacteriaceae*. Although the reduction in the pH value was narrow (6.2 to 5.0) but it significantly reduced the bacterial load.

4. CONCLUSION

In conclusion, strict hygienic precautions should be adopted during handling, processing, transportation and distribution of the bovine meat and dairy products. In addition, treatment of products with organic acids are of value in improving their microbial quality.

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