The 4th Int. Sci. Conf., The role of veterinary medicine for community, application of biotechnology in veterinary medicine., 5-6 April 2005, Mansoura, Egypt.

DETERMINATION OF SOME HEALTH SIGNIFICANT BACTERIAL CONTAMINANTS OF BEEF LIVER SOLD IN DAMIETTA CITY AND STUDYING THE EFFECT OF VINEGAR TO IMPROVE QUALITY AND SAFETY

By

DAWOUD, A.S.; MAROUF, H.A.; ABDALLAH, M.I.M. AND BAZALOU, M. S.

Food Inspection Lab., Damietta Sea' Port, Animal Health Research Institute, Agricultural Research Center, Giza, Egypt.

ABSTRACT

The present study aimed to give an available, easy, safe and effective solution to reduce bacterial load of some our popular foods. Thirty beef liver samples that randomly collected from different retail meat shops at Damietta City, were bacteriologically examined to determine their contamination by some bacterial contaminants that of health significance, total bacterial (mesophilic), coliforms, pathogenic E. coli, Staphylococcus aureus and anaerobic gas producing sporeformers counts were performed. The obtained results showed that the concerned bacteria were determined in 100.00; 63.33; 43.33; 20.00 and 20.00 %, respectively. The respective mean values were $48\times10^5 \pm 28\times10^5$; 30.8 ± 15.52; 3.2 ± 1.08; 240.67 ± 154 and 91.1 ± 50.18 cell/gm. Studying the effectiveness of vinegar solution 2.5% acetic acid by immersion technique in reducing bacterial load, to improve quality and safety showed reduction by 92.21; 70.72; 38.44; 90.31 and 72.52 % respectively. The public health significance was declared.

INTRODUCTION

Human health is highly attractive word. So, food safety remains a major challenge to food producers and to legislators endeavoring to adequate consumer protection.

Beef liver is a very popular meal in developing countries (**Polska Norma**, **2000**). Due to tradition and consumption habits, the liver among the offal products are consumed in large amounts. On the other hand, these products due to specific recipes of production, very short shelf-life, storage conditions and sometimes inappropriate management in the house and shops might be undesirable for consumption.

Most reports of outbreaks of food borne diseases indicate that mishandling occurred in food service establishment and home (**Simmon**, **1972**).

Raw liver is perceived to responsible for significant amount human illness because of the relatively high frequency of contamination with some indicator organisms as E-coli and staphylococcus aureus (**Delmore et al.**, **1999**).

The presence of indicator organisms provide a useful index for determining the hygienic quality of food and indicates the extent of sanitation, handling, and distribution, or processing, production and storage (**Erwa**, **1972**). Escherichia coli is the most involved coliforms in food items could taken as indication of feacal contamination, as they are commonly found in the alimentary tract of man and animals, hence the possible presence of enteric pathogens is expected (**ICMSF**, **1986** & **Varnam** and **Evans**, **1991**).

Staphylococcus aureus possess a public health hazards due to production of thermostable enterotoxin that is responsible for food poisoning throughout the world (**ICMSF**, **1986**).

Clostridia Gram-positive endospore-forming, are obligate anaerobic bacteria. Botulism is a sever food poisoning, result from ingestion of food containing botulinal toxin produced during growth of Cl. botulinum that produces a protein with characteristic neurotoxicity. Measures taken to prevent botulism include reduction of microbial contamination level, acidification, reduction of moisture level and whenever possible destruction of all botulinal spores in food (**Solomon** and **Lilly**, **1998**). Cl. perfringens possess a public health hazards, numerous exotoxins are responsible for food poisoning. Recently, necrotizing enteritis associated with Cl. perfringens type A has been reported (**Eley**, **1996**).

For at least 20 years, scientists have studied techniques to reduce the bacterial contamination on beef carcass and of beef variety meats as liver. These techniques are now used with Hazard Analytical and Critical Control Point (HACCP) system and have proven effective in reducing the level of contamination. However, there is need to investigate potential microbiological decontamination techniques for use on beef products in order to improve their microbiological status and so, quality and safety.

Acetic acid and its related salts are widely used as acidulants and antimicrobials (**Baird-Parker**, **1980** & **Doores**, **1983**). The activity of acetic acid varies with food products, environment and microorganisms. However, FSIS-USAD has currently approved the use of solution of acetic acid at concentration of 1.5-2.5 % as antimicrobial treatment to reduce bacterial contamination of meats and its products (**FSIS-USAD**, **1996**).

The objective of this study was monitoring some indicator bacteria and anaerobic gas-producing sporeforming bacteria and evaluate effectiveness of commercially available acetic acid (vinegar) in reducing bacterial count of beef liver sold in Damietta City.

MATERIAL AND METHODS

Sampling technique:

Thirty beef liver samples were randomly collected from different retail meat shops at Damietta City, samples placed in separate and labeled clean plastic bags and immediately transported in an insulated cooler to the laboratory for analysis. Samples, each approximately about 200 gm were divided into two parts. First part was for bacteriological examination before treatment; second one was examined bacteriologically after immersion for 10 sec. in vinegar solution 2.5% that was prepared by mixing 2 equal volumes of commercial vinegar (5% acetic acid) with tap water to give the appropriate solution of pH 3.

Immediately after application of the appropriate treatment, samples were placed into sterile sample bags and held 10 min. at ambient temperature to stimulate commercial practice prior to bacteriological analysis.

Bacteriological analysis:

A surface rinsing procedure (using phosphate buffer) was employed to dislodge bacteria from the surface of each liver sample. Standard bacteriological techniques were used for analysis. The sample (10 gm) was transferred to an appropriate bag, made a 1:10 dilution (w/v) with sterile buffered peptone water and thoroughly homogenized in blender at 1500 rpm for 2 min.. Serial tenfold dilutions were prepared (**Elliot et al.**, **1978**) and following cultures were employed:

- Total bacterial count (mesophilic count): was applied according to (**FDA**, **1998**).
- Coliforms count (MPN/gm): according to (**ICMSF**, **1978**).
- Pathogenic E. coli: the technique recommended by (**Harrigan** and **McCance**, **1976**).
- Pathogenic staphylococcus aureus: was performed according to (**FDA**, **1998**).
- Anaerobic sporeformers count (MPN/gm): according to (**Varnam** and **Evans**, **1991**).

All analysis were carried in duplicates. Colonies were enumerated after proper incubation and recorded /gm.

Statistical analysis:

The mean and standard errors were calculated for all measurements by using statistical programmed (**SPSS**, **1993**).

Visual observation:

All samples were visually evaluated by laboratory technicians immediately following application of the decontamination treatment to determine its effect on liver appearance.

RESULTS AND DISCUSSION

Meat and meat products is an ideal media for many organisms to grow because it is high in moisture (potentially dangerous foodstuffs), rich in nitrogenous compounds (amino acids, peptides, proteins) and plentifully supplied with minerals and accessory growth factors. Furthermore, it has some fermentable carbohydrates, usually glycogen and keeps favorable pH for growth of most microorganisms (**Frazier** and **Westhoff**, **1988**) & (**Van Laack**, **1994**). If the microorganisms involved are pathogenic, their association with our food supply is critical from a public health point of view. Here, we attempt to prevent their entrance and growth in our food or eliminate them by processing (**Frazier** and **Westhoff**, **1978**).

Results listed in **table** (**1**) and **Fig**. (**1**) show that out of examined 30 beef liver samples, before immersion technique was applied, 30(100.00%); 19(63.33); 13(43.33%); 6(20.00%) and 6(20.00%) were contaminated with total bacteria; coliformes; E. coli; Staphylococcus aureus and anaerobic sporeformers gas producing bacteria with counts ranged from 75×10^2 to 88×10^6; 3 to 460; 3 to 28; 110 to 4100 and 23 to 1100 cell/gm, with mean values $48\times10^5 \pm 28\times10^5$; 30.8 ± 15.52, 3.2 ± 1.08; 240.67 ± 154 and 91.1 ± 50.18 cell/gm respectively.

Results tabulated in **table** (**1**) and **Fig**. (**1**) of examined beef liver samples, after immersion technique in vinegar solution 2.5% acetic acid (approval received FSIS-USAD) for 10-sec. with pH value 3 revealed that 30(100.00%); 18(60.00%); 12(40.00%) ; 5(16.66%) and 6(20.00%) were contaminated with total bacteria; coliformes; E. coli; Staphylococcus aureus and anaerobic sporeformers gas producing bacteria with mean counts $38\times10^3 \pm14\times10^3$; 9.0 ± 3.9; 1.97 ± 0.51; 23.33 ± 10.2 and 25.03 ± 11.36 cell/gm respectively.

Higher mean values were reported by **Katarzyna** and **Hanna** (**2003**) for total colony; coliformes; E. coli; and Staphylococcus auerus counts, while lower finding was mentioned by **Delmore et al.** (**1999**) for total colony count. Indeed, no available data overlap fairly with our findings for anaerobic sporeformers gas producing bacteria.

Reduction percentages of examined beef liver samples, as a sequel of immersion technique in vinegar solution 2.5% acetic acid of pH 3 which act as an antimicrobial agent were 92.21; 70.72; 38.44; 90.31 and 72.52 % respectively as shown in **table** (**2**).

Nearly similar finding was reported by **Delmore et al.** (**1999**) for total colony count reduction by immersion in acetic acid 2.0%, while in literatures there is no available data overlap fairly with our findings for using this technique in beef liver for coliformes; E. coli; Staphylococcus aureus and anaerobic sporeformers gas producing bacteria.

As far as we knew, little available literatures dealing with bacteriological quality and also with decontamination techniques of beef liver, it was hard to discuss the aforementioned results but generally several authors allover the world reported on the antimicrobial effect of acetic acid on different bacteria as **Gill** and **Badoni (2004)** evaluated that efficacies of antimicrobial solution of 4% acetic acid that held for 5 min. at 7+/-1 ºC was achieved reduction of number of aerobes, cloiforms or E.coli on chilled and raw meat. And, **Bell et al.**, **1986** showed that at 1.2 % as 10-sec., dip for meat in acetic acid reduced microflora such as S. typhimurium, Shigella sonni, Y. enterocolitica, E-coli, Pseudomonas aeruginosa and Streptococcus faecalis by an average of 65%. While, **Levine** and **Fellers** (**1939**) observed that Staphylococcus aureus was inhibited by using bacteriostatic

concentration of acetic acid at pH 5. In addition, **Minor** and **Marth**, **1970** reviewed that 99% of Staphylococcus aureus was inhibited by using acetic acid at pH 5 as antimicrobial agent.

Woolford (**1975**) studied the antimicrobial role of acetic acid at different pH degrees 4; 5 and 6, it was observed that Clostridia; Gram-negative and Gram-positive bacteria were inhibited.

A number of surveys have shown that the incidence of Cl. botulinum in meat and meat products to be low but variable (**Abrahamson** and **Riemann**, **1971**). Cl. perfringens is present in small number in a wide range of meat products. This is of no direct significance, but in raw one the level of contamination tends to reflect the standard of abattoir hygiene (**Roberts**, **1982**).

The results in this study indicate the unhygienic quality of sold beef liver which also, indicate the lack of satisfactory sanitary conditions and quality control during slaughtering and/or post handling of liver and in turn create possible hazards for health and food safety.

Visual observation:

All samples showed slight or negligible change in color and surface appearance when decontamination using technique involved in this study.

Public health significance:

The risk of contamination of beef liver with certain type of bacteria may affect its flavour and making it to be unsavory product as well as possible source of human hazards.

The public health importance of the coliforms have been reported by many authors **(Collins, 1984** and **ICMSF**, **1986)**. Escherichia coli in food items could also be taken as indication of faecal contamination as well as the possible presence of enteric pathogens **(ICMSF, 1986** & **Varnam** and **Evans**, **1991)**.

Staphylococcus aureus possess a public health hazard due to production of thermostable enterotoxin that is responsible for food poisoning (**ICMSF**, **1986)**.

Anaerobic sporeforming bacteria of Clostridium species are responsible for a number of diseases of man and animals, including food poisoning, tetanus and gas gangrene, wound and soft tissue infections, abscesses and pseudomemberanous colitis. Two species, Cl. botulinum and Cl. perfringens (type A) are primarily involved in food poisoning, although the production of botulinum toxin and consequent human disease by Cl. baratii and Cl. butyricum has been recorded (**Pierson** and **Reddy**, **1988**).

IMPLICATION

In spite of our knowledge of microbiology and implementation of safety producers, such as HACCP (Hazard Analysis & Critical Control Point), the worldwide incidence of food poisoning outbreaks are increasing in gravity (**Baird-Parker, 1994** & **Kelly et al., 1996**).

Use of acetic acid immersion as bacteriological hurdles in conjunction with a HACCP system would result in beef liver of improved quality and safety. This study documents that there are home decontamination treatment available to reduce the level of bacteria normally found on commercially sold beef liver.

ACKNOWLEDGMENT

Great private thanks to my colleague, **Dr. Amr El-gohary** also, **Dr. Fify Anees** and **Dr. Rania Salem** members of Food Inspection Lab, Damietta about their great help during this study.

Table (1): Statistical analytical results of bacteriological examination of beef liver samples.

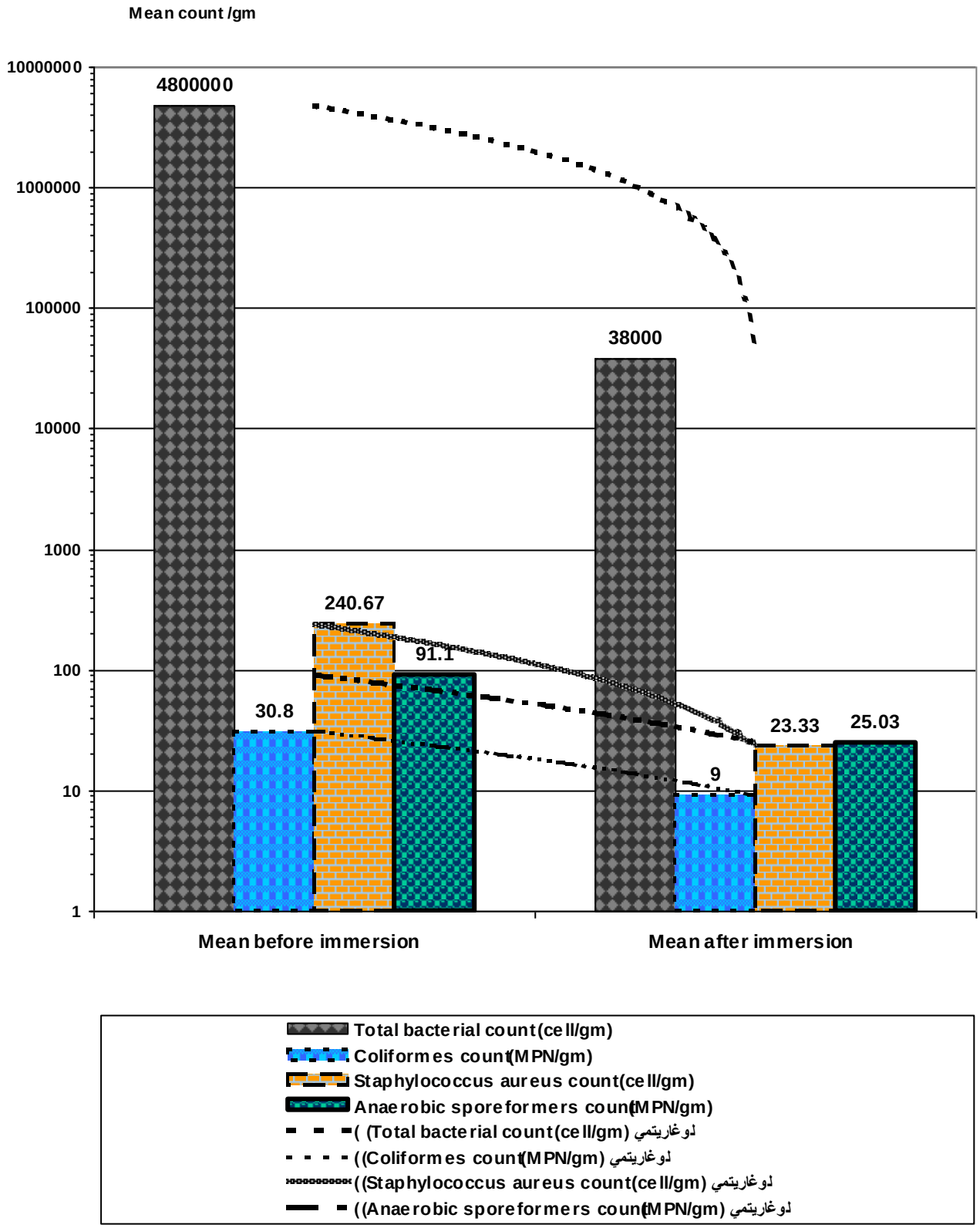
Bacterial Count/gm	Beef liver samples											
	Before immersion in vinegar solution 2.5% acetic acid						After immersion in vinegar solution 2.5 % acetic acid					
	Total No. of samples	Positive		Min.	Max.	Mean ± S.E.	Total No. of samples	Positive		Min.	Max.	Mean ± S.E.
		No.	%					No.	%			
Total bacterial count (cell/gm)	30	30	100.00	75×10^2	88×10^6	48×10^5 ± 28×10^5	30	30	100.00	5×10^2	3×10^5	38×10^3 ± 14×10^3
Coliformes count (MPN/gm)	30	19	63.33	3	460	30.8 ± 15.52	30	18	60.00	< 3	120	9.0 ± 3.9
E. coli count (cell/gm)	30	13	43.33	3	28	3.2 ± 1.08	30	12	40.00	< 3	9	1.97 ± 0.51
Staphylococcus aureus count (cell/gm)	30	06	20.00	110	4100	240.67 ± 154	30	05	16.66	100	200	23.33 ± 10.2
Anaerobic sporeformers count (MPN/gm)	30	06	20.00	23	1100	91.1 ± 50.18	30	06	20.00	15	240	25.03 ± 11.36

Table (2): Reduction percentages of decontamination technique of vinegar solution 2.5% acetic acid on beef liver samples (n=30).

Bacterial count/gm	Before immersion	After immersion	Reduction
	Mean ± S.E.	Mean ± S.E.	%
Total bacterial count (cell/gm) (MPN/gm)	$48\times10^5 \pm 28\times 10^5$	$38\times10^3 \pm 14\times10^3$	92.21
Coliformes count (MPN/gm)	30.8 ± 15.52	9.0 ± 3.9	70.72
E. coli count (cell/gm)	3.2 ± 1.08	1.97 ± 0.51	38.44
Staphylococcus aureus count (cell/gm)	240.67 ± 154	23.33 ± 10.2	90.31
Anaerobic sporeformers count (MPN/gm)	91.1 ± 50.18	25.03 ± 11.36	72.52

n = number of examined samples.

Fig.(1): Mean values of bacterial examination of beef liver samples before and after immersion in vinegar solution 2.5% acetic acid (n=30).



REFERENCES

Abrahamson, K. and **Riemann, H. (1971**): Prevalence of Clostridium botulinum in semipreserved meat products. Appl. Micro., 21, 543-544.

Barid-Parker, A.C. (1980): Organic acids. P. 126. In: Microbial Ecology of foods, Vol. 1. factors Affecting Life and Death of Microorganisms. International Commission on Specifications for Foods. Academic, New York.

Baird-Parker, A.C. (1994): Foods and Microbiological risk: Microbiology 140, 687- 695.

Bell, M.F.; **Marshall, R.T.** and **Anderson**, **M.E. (1986):** Microbiological and sensory tests of beef treated with acetic acid and formic acids. J. food Prot. 49:207.

Collins, C.H. (1984): Microbiological Methods. 5th Butler and Tanner Ltd. France and London.

Delmore, R.J.; **Sofos, J.N.Jr. Schmidt, G.R.**; **Belk, K.E.** and **Smith, G.C.(1999):** Reduction of microbiological counts on beef variety meats. Meat Science and Safety, Beef Program Report Department of Animal Science Colorado State University. pp. 201-8.

Doores, S. (1983): Organic acids. P. 75. In: Antimicrobials in Foods. Branen, A.L., and Davidson, P.M. (Eds.). Marcel Dekker, New York.

Eley, A. (1996): Microbial Food Poisoning 2nd Edition, Chapman and Hall, London.

Elliot, R.P.; **Clark, K.H.**; **Lewis, H.**; **Lundbeck, J.C.**; **Olson, J.R.** and **Simmonsen, B.(ed) (1978):** Microorganisms in Foods 1. Their Significance and Methods of Enumeration, 2ed. Ed. University of Toronto Press, Toronto. 118-119.

Erwa, H.E. (1972): Enterococci in diarrhea of neonates. Frances, R. Soc. Top. Med. administration. Hyg. 66: 539.

F.D.A. (1998): Food and drug administration. Bacteriological analytical manual 8th Edition, AOAC international, Gaithersburg, USA.

Frazier, W.C. and **Westhoff, D.C. (1978):** Food Microbiology. 3rd Ed. Mcgraw-Hill Book Company. New York, USA.

Frazier, W.C. and **Westhoff, D.C. (1988):** Contamination, preservation and spoilage of meats and meats products. Food Microbiology Fourth Ed. (McGraw-Hill, New York). pp. 218-242.

FSIS-USAD (1996): Food Safety and Inspection Service. Pathogen reduction; HACCP system. Code of Federal Regulations 9:308, 310, 318, 326, 327, and 381; Docket No. 93-106 pp. food safety and Inspection Service, US Department of Agriculture, Washington, DC.

Gill, C.O. and **Badoni, M. (2004):** Effects of peroxyacetic acid, acidified sodium chloride or lactic acid solutions on the microflora of chilled beef carcasses. Int. J. Food Microbiol. Feb. 15; 91 (1): 43-50.

Harrigan, W.F. and **McCance, M.E. (1976):** Laboratory methods in food and dairy microbiology. Academic Press London, New York, San Francisco.

ICMSF "International Committee on Microbiological Specifications for foods" (1978): Microorganisms in food: Their significance and methods of enumeration. 2nd Ed., Univ. of Toronto Press, Toronto and Buffalo, Canada.

ICMSF "International Committee on Microbiological Specifications for foods" (1986): Microorganisms in food. Sampling for microbiological analysis, principle and specific application. Blackwell Scientific Publications: Oxford.

Katarzyna Dovanska and **Hanna Rozanaska (2003):** Microbiological quality of polish edible offals processed meat products during storage: Influence on N-nitrosamines content. Bull. Vet. Inst. Pulawy, Poland, 47, 217-223.

Kelly, W.J. ; **Asmundson, R.V.** and **Huang, C.M. (1996):** Isolation and characterization of bacteriocin producing lactic acid bacteria from ready - to - eat food products. International J. Food Mirco., 33 : 209 - 218.

Levine, A.S. and **Fellers, C.R. (1939):** the inhibiting effect of acetic acid with sodium chloride and sucrose on microorganisms. J. Bacteriol. 39: 17.

Minor, T.E., and **Marth, E.H. (1970):** Growth of Staphylococcus aureus in acidified pasteurized milk. J. Milk Food Technol. 33: 516.

Pieson, M.D. and Reddy, N.R. (1988): Clostridum botulinum-Status summary. Fd. Tech., 42(4), 196-198.

Polska Norma, PN.A-82023 (2000): Meat and meat products. Terminology.

Roberts, D. (1982): Bacteria of public health significance. In Meat Microbiology, (Eds. Brown, M.H.), Applied Science Publishers LTD. London and New York.

Simmon, C. (1972): Hygiene in the meat trade No. 8, Gross contamination. Meat Trades J. No. 4408, Nov. 23.

Solomon, H.M. and **Lilly, T.Jr. (1998):** Clostridium botulinum. Ch. 17. In Food and Drug Administration Bacteriological Analytical Manual, 8th ed. (revision A), (CD-ROM version). R.L. Mèrker (Ed.). AOAC International, Gaithersburg, MD.

SPSS (1993): Statistical Package for Social Science. SPSS for windows release. Copy write, Version 6.0, SPSS Inc. 710808.

van Laack, R.L.J.M. (1994): Spoilage and preservation of muscle foods. Muscle foods: Meat; Poultry and Seafood technology ed. D.M. Kinsman; A.W. Kotula and B.C. Breidenstein. (Chapman & Hall, New York), pp. 873-405.

Varnam, A.H. and **Evans, M.G. (1991):** Food-borne Pathogens. An illustrated text. Published by Wolfe Publishing Ltd., England.

Woolford. (1975): Antimicrobial agents. Food Additives. pp. 93, cited after P. Michael and Vijay'k. Juneja. Univ. of Tennessee, Knoxville, Tennessee. Sepper Solminen (1989).

the 4th Int. Sci. Conf., The role of veterinary medicine for community, application of biotechnology in veterinary medicine., 5-6 April (2005), Mansoura, Egypt.

تحديد مدى تواجد بعض الملوثات البكتيرية ذات الأهمية الصحية للكبد البقري المُباع في مدينة دمياط ودراسة تأثير الخل لتحسين الجودة والأمان

د. عبد السلام احمد داود- د. حسن على محمد معروف- د. مدحت إبراهيم محمد عبد الله - د. محمد صالح البدري بزالو

معمل فحوص الأغذية بميناء دمياط البحري- معهد بحوث صحة الحيوان- مركز البحوث الزراعية- الجيزة- مصر

المُلخص

استهدف البحث تقديم حل متوفر و سهل و آمن لتقليل التلوث البكتيري في بعض أغذيتنا الشعبية. أجريت الدراسة على ثلاثين عينة عشوائية من الكبد البقري المباع حيث جمعت من محلات بيع اللحوم بالتجزئة بمدينة دمياط. تم إجراء الفحوص البكتيريولوجية التالية وهى العد الكلى للبكتيريا الهوائية و العد الكلى لبكتيريا المجموعة القولونية و عد بكتيريا الاشريشياكولاي الممرضة وعد بكتيريا المكور العنقودي الذهبي و كذا عد بكتيريا المجموعة اللاهوائية المتجرثمة المنتجة للغاز. دلت النتائج على أن نسب تواجدها كانت على التوالي ١٠٠ و ٦٣,٣٣ و ٤٣,٣٣ و ٢٠ و ٢٠ % و بمتوسط قدره ٤٨×١٠ ° ± ٢٨×١٠ ° و ٣٠,٨ ± ١٥,٥٢ و ٣,٢ ± ١,٠٨ و ٢٤٠,٦٧± ١٥٤ و ٩١,١ ± ٥٠,١٨ خلية لكل جرام على التوالي. كما أوضح البحث أن تأثير المعالجة بمحلول الخل ٢,٥% حمض خليك و المستخدم في هذا البحث بطريقة الغمس أدى إلى اختزال التلوث البكتيري بنسب كالآتي ٩٢,٢١ و ٧٠,٧٢ و ٣٨,٤٤ و ٩٠,٣١ و ٧٢,٥٢ % على التوالي. كما تم مناقشة الأهمية الصحية للمعزولات.