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Inhibitory activity of some functional cultures toward common microbial contaminants in soft cheese manufactured from raw milk

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ABSTRACT

The inhibitory activity of Lb. plantarum NRC AM10, Lb. curvatus NBIMCC 3452, Lb. paracasei NRRL B-4560 and Lb. gasseri NRRL B-14168 were investigated against the microbial contaminants in raw milk soft cheese. Cultures were added at the level of 5 % in T1, T2, T3 and T4 respectively, and the control treatment made without starters. Microbial counts in raw milk sample showed 10.25, 6.85, 5.89, 4.76, 4.42, 4.44, 3.77, 3.64 and 3.46 log cfu/ml for total viable count, total coliforms, Staphylococci, moulds and yeasts, Enterococci, fecal coliforms, spore forming bacteria, psychrotrophic and Salmonella respectively. Some of microbial groups in control cheese were increased in the first 5 days of storage and others were increased until 10 days then all microbial groups were gradually decreased with extending storage period. Staphylococci reached to the lowest counts 3.11 log cfu/ml after 20 days, on contrast moulds and yeasts reached to the highest count 5.22 log cfu/ml at the end of storage period. The inhibition rate % (IR) were influenced by the variety of strain added in cheese treatments and the highest IR 32.58 and 33.96 % against total coliforms and Enterococci were recorded in the treatment containing Lb. plantarum NRC AM10 while the highest IR 25.84 % against fecal coliforms was recorded in the treatment containing Lb. curvatus NBIMCC 3452. Meanwhile, the IR 35.19 and 15.84 % were recorded against Salmonella and spore forming bacteria with strain Lb. gasseri NRRL B-14168. The strain Lb. paracasei NRRL B-4560 showed IR 42.23 and 44.4 % against Staphylococci and psychrotrophic respectively. Moulds and yeasts talked a revers trend of IR because of increasing the counts by acidity development. In conclusion, the only addition of functional cultures to control the growth and survival of pathogens in raw milk products is not enough to produce safe product. Use of pasteurized milk, good hygienic and good manufacturing practices are essential to reduce the risk of microbial contamination and health hazards in dairy products.

KEYWORDS: *Raw milk, functional cultures, inhibition activity, soft cheese, health hazard*

INTRODUCTION

In recent decade, food safety has emerged as an important global issue to ensure safe food throughout the food chain from primary producer to the ultimate consumers. Production and consumption of locally white soft cheese is still under way especially those produced in most villages, small dairy units
and supermarkets distributed in our developing countries. In addition, small-scale dairies in south Europe still have been using raw milk for cheese production, especially those close to rearing

facilities [1, 2]. Meanwhile, in some world regions people continue to consume raw milk cheese and minimally processed foods even though numerous epidemiological studies have shown clearly that raw milk contaminated by a variety of microbial pathogens some of which are associated with human illness and disease [3, 4]. The food risks associated with raw milk and artisanal dairy products consumption vary considerably between developed and developing countries. It could be extremely risky and dangerous to people with weakened immune systems, older adults, pregnant women, and children due to carry harmful bacteria [5]. The consumption of raw milk cheese has been associated with foodborne outbreaks and this constitute a public health hazards [6, 7]. In this regard, the control and prevention of contamination with pathogens are of primary importance to ensure public health [8]. In general, pathogenic microorganisms can contaminate raw milk from various sources like the external surfaces of udder and teats, mastitis, milking equipment, air and environment [9]. Raw milk contains a complex of microbial community because of its high water content and neutral conditions which allow the growth of spoilage or potentially pathogenic species including the genera *Staphylococcus*, *Streptococcus*, *Bacillus*, *Micrococcus*, Coliforms and *Corynebacterium* that affect the quality of dairy products [10, 11]. Several studies confirmed that pathogenic bacteria have the ability to survive during manufacturing process and/or the ripening period [12]. Raw milk cheese samples (245 sample) were evaluated microbiologically for coagulase-positive *Staphylococci* were evidenced in all samples tested whereas *E. coli* was evidenced in 20 samples. The results suggested that they need improvement of good manufacturing practice and milking operations [13]. The impact of spoilage microorganisms and pathogens in foods will become even more challenging as consumer demand for food products without chemical additives increases and the population ages [14]. In recent years, consumers and food manufacturers not prefer the synthetic and chemical additives in foods. Dairy industry is going toward safe products and natural additives by applying different strategies like use of lactic acid bacteria and/or its metabolites for controlling the growth of spoilage and pathogenic bacteria in the products [15, 16]. Continuous good farming practices, good manufacturing and hygiene practices throughout the dairy chain production to reduce the risks associated with milk and dairy products. Pasteurization of milk is the basic treatment performed in order to eliminate pathogenic bacteria from the final product. In addition, recognition of smallholder dairying and informal markets through training and certification would be very significant in ensuring the safety of milk and dairy products. The outcome of all these would be a well-functioning dairy system that not only brings economic incentives, but also protects the health of the consumers [17]. The aim of the present study was planned to use functional cultures for inhibiting the common spoilage microorganisms and foodborne pathogens in soft cheese type produced from raw milk during storage period.

MATERIALS AND METHODS

Origin of multifunctional cultures

The probiotic cultures were as follows: *Lactobacillus paracasei* NRRL B-4560 and *Lactobacillus gasseri* NRRL B-14168 provided from Northern Regional Research Laboratory (NRRL), Illinois, USA, *Lactobacillus curvatus* NBIMCC 3452 supplied by National Bank for Industrial Microorganisms and Cell Cultures (NBIMCC), Sofia, Bulgaria and *Lactobacillus plantarum* NRC AM 10 was isolated, characterized and identified by [18].

Raw milk soft cheese manufacture

Raw milk sample was taken and bacteriologically analyzed before milk worming then cow's milk (15 kg 3.2 % fat) was heated to 30° C (for renneting) then divided into 5 equal portions (3 kg for each portion). The first portion was kept as a control without starter addition and the other portions (T1, T2, T3 and T4) were inoculated with protective starters at the level of 5 %. The starter cultures were activated in 12 % reconstituted sterile skim milk and incubated overnight then added to four portions as follows: *Lactobacillus plantarum* NRC AM10, *Lactobacillus curvatus* NBIMCC 3452, *Lactobacillus paracasei* NRRL B-4560 and *Lactobacillus gasseri* NRRL B-14168 respectively. The inoculated milk portions were stirred gently and incubated at 37° C for 1 hour after that, rennet powder was added at the rate of 2 g /100 kg of milk then all portions were held in incubator to fully coagulation. The curd was left to whey drain at room temperature after sodium chloride 3% was added between cheese layers. The resultant cheese cut and distributed into plastic containers (250 g) then stored in refrigerator at (5 ±

2 ° C) for 20 days. The resultant cheese were analyzed when fresh (zero time) 5, 10, 15 and 20 days of storage period for microbiological evaluation.

Microbiological analysis

Total viable bacterial counts

Total viable bacterial counts were determined by using standard plate count agar medium (Oxoid) according to [19].The plates were incubated at 32 ±2° C for 48 h.

Total and fecal coliform counts

Total coliform and fecal coliform counts were enumerated on violet red bile agar medium (VRBA) according to the method described by [20]. The plates were anaerobically incubated at 37° C for 48 h and at 44° C for 48 h for total coliform and fecal coliform respectively.

Mould and yeast counts

Moulds and yeasts count of all cheese samples were determined by using rose bengal chloramphenicol agar medium (Oxoid) according to **[**21]. The plates were incubated at 25° C for 3-5 days.

Aerobic spore forming bacteria, *Salmonella*

Aerobic spore forming bacteria, *Salmonella* and psychrotrophic bacteria were detected according to [22]. The plates were aerobically incubated at 37° C for 24 h for aerobic spore forming bacteria and *Salmonella* and at 7° C for 10 days for psychrotrophic bacteria.

Psychrotrophic enumeration

Psychrotrophilic bacteria were enumerated according to Standard Methods for the Examination of Dairy Products [23]. The plates were aerobically incubated at 7° C for 10 days.

***Staphylococci* counts**

Staphylococci counts bacteria were detected on Baird parker agar supplemented according to the method described by [24]. The plates were aerobically incubated at 37° C for 24 h.

***Enterococci* counts**

Enterococci were estimated using bile esculin azide agar according to the method described by [25]. The plates were incubated anaerobically at 37° C for 24 h.

The inhibition rate (%)

The inhibition rate percentage of functional cultures against microbial contaminants was calculated from these equations: Inhibition rate of pathogenic bacteria = CPc-CPt/CPc ×100. (1). Inhibition rate percentage of spoilage bacteria = CSc-CSt/CSc ×100. (2). Where CPc is the count of pathogens in control (Log cfu/g) and CPt is the count of pathogenic in treatment while CSc is the count of spoilage bacteria in control (Log cfu/g) and CSt is the count of spoilage bacteria in treatment.

RESULTS AND DISCUSSION

COMMON MICROBIAL COUNTS IN RAW MILK

The results of common microbial contamenants in raw milk sample used in cheese manufacture are presents in (Figure 1). The results showed that the common microbial contamenantsin raw milk sample had high bacterial load and the average of total viable bacterial counts is 10.25 log cfu/ml. The other microbial groups in milk sample were found as follows: 6.85, 5.89, 4.76, 4.42, 4.44, 3.77, 3.64 and 3.46 log cfu/ml for total coliforms, *Staphylococci*, mould and yeast, *Enterococci*, fecal coliforms, spore forming bacteria, psychrotrophic and *Salmonella* respectively. These results were similar with found by [26, 27]. They reported that white cheese may be subjected to spoilage by coliforms from raw milk or these microorganisms enter as post-pasteurization contaminants or from infected workers during cheese handling. Raw milk can contaminate from different sources and the high bacterial load could be due to the poor sanitation of the milking conditions, dirty udders, and unclean milk equipment. Moreover, the data indicated that there are several genera of pathogenic bacteria were found in raw milk *e.g. Staphylococci*, *Salmonella* and *Enterococci* spp., and *Bacillus*. These results are in the harmony with those reported in several studies, which have investigated the microbiological quality and outbreaks, caused by raw milk consumption [28 29, 30, 31].

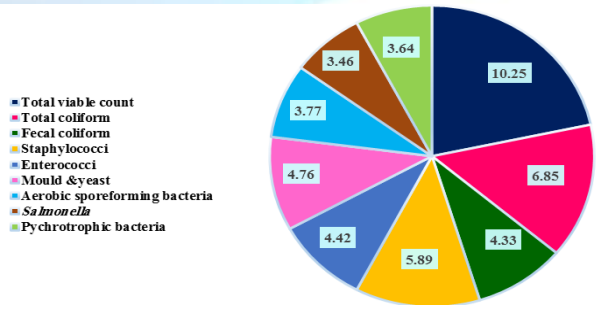

Figure 1: Common microbial counts (Log cfu/ml) in raw milk sample.

Behavior of common microbial contamenants (Log cfu/ml) in control raw milk soft cheese during storage time

As presented in (Figure 2) the results indicated that all microbial groups detected in raw milk

samples were transferred to the cheese product and survive during storage time. The total coliforms count started with 4.76 log cfu/g at zero time and the total coliforms count increased reached to the highest count 5.65 log cfu/g after 10 days of storage period. After that, the counts were gradually decreased with extending the storage period and recorded 4.14 log cfu/g at the final of storage period. Fecal coliforms count recorded 3.5 log cfu/g and decreased by one log cycle reached to 2.82 log cfu/g at the end of storage period. The decreasing in total and fecal coliform counts may be attributed to the acidity development in cheese and other metabolites, which lead to inhibit the coliform bacteria. The results were agreement with that reported by [32]. On the other hand, *Staphylococci* recorded at zero time 5.27 log cfu/g then the count gradually decreased to 3.11 log cfu/g by two log cycle at the end of storage period. The results were in agreement with those found by [33, 34 and 31]. He mentioned that the use of probiotic cultures leads to significant reduction in *Staphylococcus* counts in cheese products. Mould and yeast teak a gradually increase by two log cycle with prolonging storage time from 2.65 log cfu/g at zero time reached to 4.76 log cfu/g at the end of storage period. The highest count 5.74 log cfu/g of *Enterococci* spp. was found after 10 days of storage time. Aerobic spore forming bacteria recorded 3.12 Log cfu/g and psychrotrophic bacteria recorded 3.72 log cfu/g after 5 days of storage then both of them decreased by one log cycle [27, 35]. Finally, *Salmonella* count recorded 2.91 log cfu/g at zero time and still in the same log during all storage time. These results coincide with those found by [28, 36]. They reported that the cheese made from raw milk showed high microbial counts of *Bacillus cereus* and *E. coli*, *Salmonella* spp. and *Staphylococcus aureus* and the raw milk had been shown to be a potential source of cheese contamination.

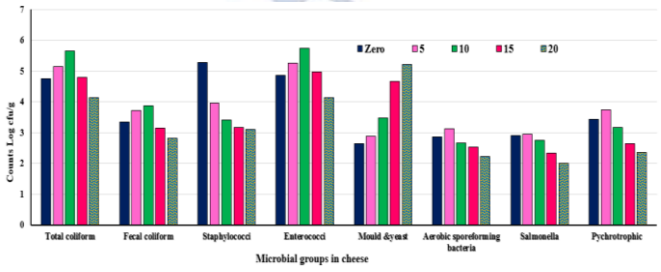

Figure 2: Behavior of common microbial contamenants (Log cfu/g) in control raw milk soft cheese during storage time.

Inhibition rate percentage of functional cultures against all microbial groups in cheese model during storage period.

Inhibition rate functional cultures against coliform group

The inhibition rate (IR) of functional cultures against coliform group in cheese model during storage period are presented in (Figure 3). The results indicated that coliform bacteria in cheese samples were inhibited because of adding functional cultures because of these strains able to produce inhibitory substances in cheese like organic acids and bacteriocins. The IR of all functional cultures strains against coliforms were increased with prolonging the storage time. At the end of storage time 20 days, the IR were recorded 32.58, 30.11, 28.54 and 27 % with strains *Lactobacillus plantarum* NRC AM 10, *Lactobacillus curva*tus NBIMCC3452, *Lactobacillus paracasei* NRRL B- 4560 and *Lactobacillus gasseri* NRRL B-14168 respectively. These results were similar with those obtained by [37, 38].

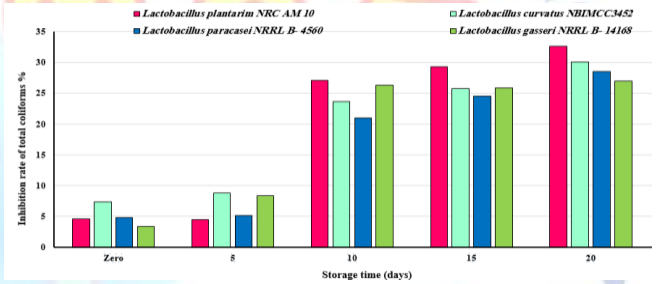

Figure 3: Inhibition rate of functional cultures against coliform group in cheese model during storage period.

Inhibition rate of functional cultures against fecal coliform

Moreover, the IR of functional cultures against fecal coliform in cheese model are illustrates in (Figure 4). The results indicated that a good IR of all functional cultures against fecal coliforms in cheese. The IR of all functional cultures against fecal coliforms were increased with prolonging the storage time. The higher IR 25.84 and 23.16 % were recorded with *Lactobacillus curva*tus NBIMCC3452 and strains *Lactobacillus plantarum* NRC AM 10 respectively. The data were coincide with found by [12]. They mentioned that the levels of *E. coli* O157:H7 detected in Gouda and Cheddar manufactured from raw milk after 94 and 108 days in respectively.

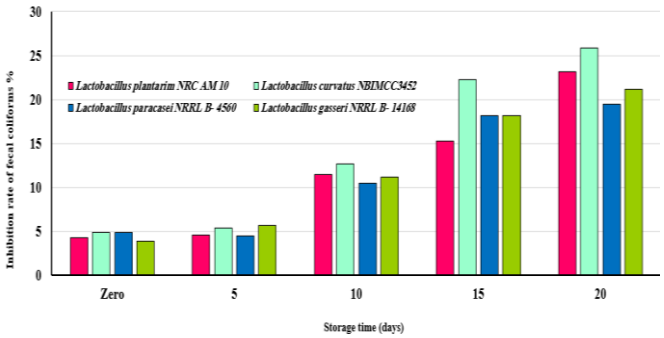

Figure 4: Inhibition rate of functional cultures against fecal coliforms in cheese model during storage period.

Inhibition rate of functional cultures against *Staphylococci*

All functional cultures showed higher IR against *Staphylococci* in cheese model as illustrates in (Figure 5). The IR % against *staphylococci* in cheese at the end of storage period were 42.23, 41.12, 40.61 and 39.8 % with *Lactobacillus paracasei* NRRL B- 4560, *Lactobacillus curva*tus NBIMCC3452 *Lactobacillus gasseri* NRRL B-14168 and *Lactobacillus plantarum* NRC AM 10 respectively [24, 37].

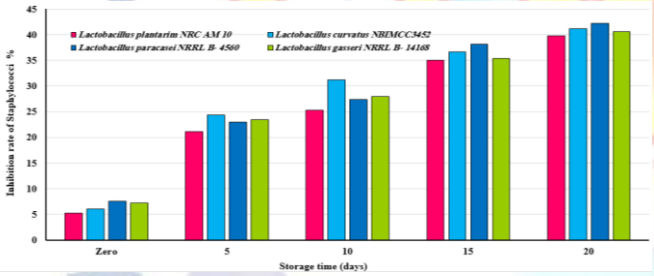

Figure (5). Inhibition rate of functional cultures against *Staphylococci* in cheese model during storage period.

Inhibition rate of functional cultures against mould and yeast

As shown in (Figure 6) the IR of functional cultures against mould and yeast in cheese model the data indicated that the IR of all functional cultures against mould and yeast in cheese toke a revere trend with increasing the storage time because of reducing. The IR were decreased with prolonging the storage time due to the acidity development in cheese that the good condition for mould and yeast growth. The highest IR 16.5 % was recorded with *Lactobacillus paracasei* NRRL B-4560 after 5 days then all strains showed low IR tell the end storage. The results are in agreement with findings of by [26, 34 and 31] they mentioned that these microorganisms enter as post-pasteurization contaminants from raw milk**.**

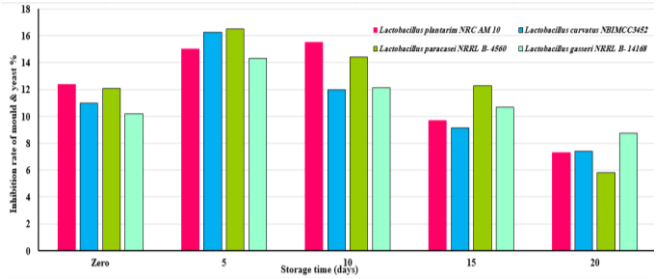

Figure 6: Inhibition rate of functional cultures against mould and yeast in cheese model during storage period.

Inhibition rate of functional cultures against *Enterococci*

The results are presents in (Figure 7) indicated that the IR % functional cultures against *Enterococci* in cheese model was increased with prolonging the storage time reached to 33.96, 32.1m 30.19 and 28.11% in the treatments containing strains *Lactobacillus plantarum* NRC AM 10, *Lactobacillus curva*tus NBIMCC3452 *Lactobacillus gasseri* NRRL B-14168 and *Lactobacillus paracasei* NRRL B- 4560 respectively. The data in similar with obtained by [39, 31] they mentioned that, the presence of *Enterobacteriaceae* in cheese generally indicates fecal contamination of raw the milk or the product during processing. In addition, these microorganisms survived in embedded cheese manufactured from raw milk.

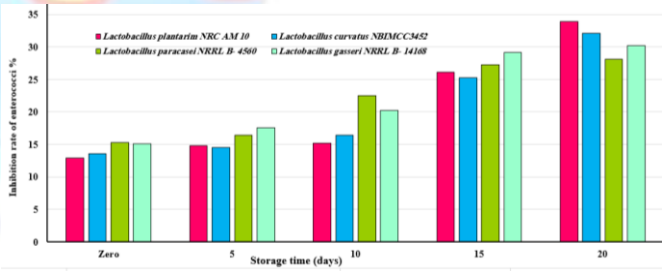

Figure 7: Inhibition rate of functional cultures against *Enterococci* in cheese model during storage period.

Inhibition rate percentage against psychrotrophic and spore forming bacteria

The IR of functional cultures against psychrotrophic and spore forming bacteria in cheese model are illustrates in (Figures 8 and 9). The results showed that the high IR of all functional cultures were observed against psychrotrophic bacteria. The IR of all strains were increased with prolonging the storage time reached to 44.4 % in the treatment containing *Lactobacillus paracasei* NRRL B- 4560, 41.35 % with *Lactobacillus plantarum* NRC AM 10, 38.57 with *Lactobacillus gasseri* NRRL B-14168 and 34.25 in the treatment containing *Lactobacillus curva*tus NBIMCC3452 at the end of storage time. The

results were confirmed the information mentioned by [40, 41, 42 and 43]. They reported that, spore-forming bacteria bacterial communities are widely distributed in dairy farms and are easily introduced into raw milk often present in dairy products, are able to survive different heat treatments and form biofilms within pipes and stainless steel equipment. Also, The results indicated that the IR against spore forming bacteria were increased with prolonging the storage time reached to 14.34 % after 15 days in the treatment containing *Lactobacillus gasseri* NRRL B-14168. Increasing in IR % were recorded at the end of storage time reached to 15.11, 11.7, 12.3 and 15.84 in the treatments containing *Lactobacillus paracasei* NRRL B- 4560, *Lactobacillus curva*tus NBIMCC3452, *Lactobacillus plantarum* NRC AM 10 and *Lactobacillus gasseri* NRRL B-14168 respectively. The results are in the harmony with those reported by [27, 44]. They mentioned that psychrotrophic bacteria were found in raw milk cheeses due to inadequate pasteurization, sanitizing milking equipment and utensils of milk or use of unpasteurized milk. Psychrotrophic bacteria can cause quality defects in dairy products and lose in milk fats and proteins and influence milk and milk products' shelf life.

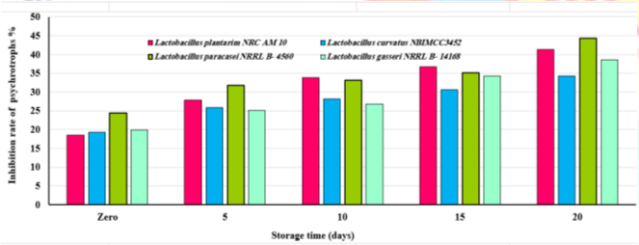

Figure 8: Inhibition rate of functional cultures psychrotrophic in cheese model during storage period.

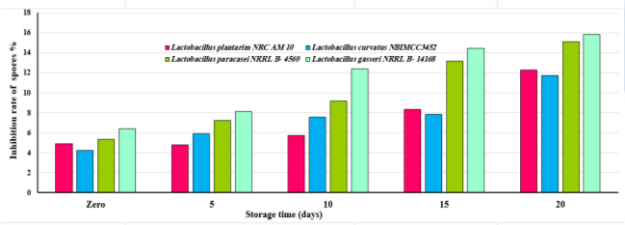

Figure 9: Inhibition rate of functional cultures against spore forming bacteria in cheese model during storage period.

Inhibition rate functional cultures against *Salmonella*

The IR of functional cultures against *Salmonella* in cheese model are illustrates in (Figure 10). The results showed that high IR of all functional cultures were observed against *Salmonella*. The IR of all functional cultures against *Salmonella* were increased with prolonging the storage time reached to 35.19 % in the treatment containing *Lactobacillus gasseri* NRRL B-14168, 33.65 % with *Lactobacillus curva*tus NBIMCC3452, 31.22% with *Lactobacillus plantarum* NRC AM 10 and 29.43 % with *Lactobacillus paracasei* NRRL B-4560 at the end of storage time. The results are in agreement with findings of [31].

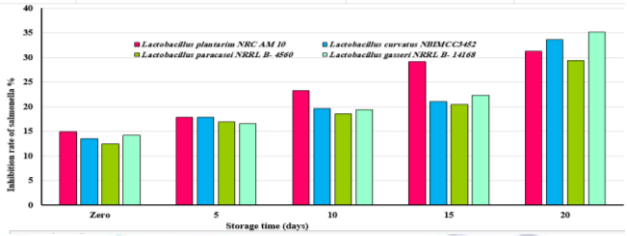

Figure 10: Inhibition rate functional cultures *Salmonella* in cheese model during storage period.

REFERENCES

[1] De Buyser ML, Dufour B, Maire M, Lafarge V (2001). Implication of milk and milk products in food-borne diseases in France and in different industrialised countries. International Journal of Food Microbiology. 67: 1-17.

[2] Costanzo N, Carlotta C, Adriano S, Maria T C and Francesco C (2020). Food borne Pathogen Assessment in Raw Milk Cheeses. International Journal of Food Science. 1-5.

[3]Oliver SP, Kathryn JB, Steven CM and Shelton EM (2009). Food Safety Hazards Associated with Consumption of Raw Milk. Food borne Pathogens and Disease. 6(7):793-804.

[4] Claeys WL, Sabine C, Georges D, JanDe B, Koen D, Katelijne D, Lieven D, André H, Hein I, Pierre T, Yvan V, Lieve H (2012). Raw or heated cow milk consumption: Review of risks and benefits. Food Control. 31(1): 251-262.

[5]Latorre AA, Van KJS, Karns JS, Zurakowski MJ, Pradhan AK, Zadoks RN, Boor KJ, Schukken YH, (2009). Molecular ecology of *Listeria monocytogenes*: Evidence for a reservoir in milking equipment on a dairy farm. Applied and Environmental Microbiology. 75: 1315- 1323.

[6]Kousta M, Mataragas M, Skandamis P, and Drosinos EH (2010). Prevalence and sources of cheese contamination with pathogens at farm and processing levels. Food Control. 21(6): 805–815.

[7]Todaro A, Adly FA and Omar OAH (2013): History, Processing and quality enhancement of traditional Egyptian Kariesh cheese. J. Food Science and Technology. 1: 1-6.

[8]Farrokh C, Jordan K, Auvray F, Glass K, Oppegaard H, Raynaud S, Thevenot D, Condron R, De Reu K, Govaris A, Heggum K, Heyndrickx M, Hummerjohann J, Lindsay D, Miszczycha S, Moussiegt S, Verstraete K, and Cerf O (2013). Review of Shiga-toxin-producing *Escherichia coli* (STEC) and their significance in dairy production. Int. J. Food Microbiol. 162:190-212.

[9]Verraes C, Vlaemynck G, Van Weyenberg S, De Zutter L, Daube G, Sindic M, Uyttendaele M (2015). A review of the microbiological hazards of dairy products made from raw milk. International Dairy Journal. 50: 32-44.

[10]Angulo FJ, LeJeune JT, Rajala-Schultz PJ (2009). Unpasteurized milk: A continued public health threat. Clin. Infect. Dis. 48: 93-100.

[11]Mendonça Moraes P, Nogueira Viçosa G, Keizo Yamazi A, Tassinari Ortolani MB, Nero LA (2009). Foodborne pathogens and microbiological characteristics of raw milk soft cheese produced and on retail sale in Brazil. Food borne Pathogens and Disease. 6(2): 245-249.
[12]D'Amico DJ, Druart MJ, and Donnelly CW (2010). Behavior of *Escherichia coli* O157:H7 during the Manufacture and Aging of Gouda and Stirred-Curd Cheddar Cheeses Manufactured from Raw Milk. Journal of Food Protection. 73(12): 2217-2224.
[13]Costanzo, N, Carlotta C, Adriano S, Maria T C and Francesco C (2020). Food borne Pathogen Assessment in Raw Milk Cheeses. International Journal of Food Science. 1-5.
[14]Ben Said L, Helene G, Laurent D, Miche T, and Ismail F (2019). Bio protective Culture: A New Generation of Food Additives for the Preservation of Food Quality and Safety. Industrial biotechnology 15 (3):138-147.
[15]Sarkar S (2016). Microbiological Safety Concerns of Raw Milk. Journal of Food Nutrition and Dietetics. Food Nutri Diete. 1(2): 1-7.
[16]Pal, M, Devrani M, and Pinto S (2018). Significance of Hygienic processing of Milk and Dairy products. Madridge J Food Tech. 3(2): 133-137.
[17]Owusu-Kwarteng J, Fortune A, Dominic A and Lene J (2020). Microbial Safety of Milk Production and Fermented Dairy Products in Africa. Microorganisms 8(5): 752.
[18]Mabrouk AMM, Effat BAM and Sadek ZIM (2019). Isolation, Identification and Antibiotic Susceptibility of Probiotic *Lactobacillus plantarum* NRC AM10. International Journal of Biology, Pharmacy and Allied Sciences, 8(3): 528-538.
[19]Houghtby, G. A.; L. J. Maturin and E. K. Koening (1992). Microbiological count methods. In: Standard Methods for the Examination of Dairy Products. (16th Ed.), R. T. Marshal (Ed.), mer. public Health Association, Washington D.C., U.S.A, 1992, p.213-246.
[20] El-Nasri NA, Sirag SO and El-Shafei HEH (2012). Packaging type and their effects on the chemical and microbial quality of Sudanese white cheese (*Gibna bayda)* J. Toxicol. and Environ. Health Sci. 4(10): 185-191.
[21]Jarvis B (1973). Comparison of an improved Rose Bengal-Chlorotetracycline agar with other media for the selective isolation and enumeration of molds and yeasts in foods. J. Appl. Bacteriol. 36:723 -727.
[22]Stulova I, Adamberg S, Krisciunaite T, Kampura M, Blank L and Laht TM (2010). Microbiological quality of raw milk produced in Estonia. Letters in applied microbiology. 51: 683-690.
[23]Marshall RT (1992). Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, DC.
[24]Beliockova E, Tkacikova L, Nass HT, Vargova M, Ondrasovic M, Ondrasovicova O, Obstinikova D and Toth L (2001). *Staphylococci* plate counts in foods of milk origin. Vet. Med. Czech., 46 (1): 24-27.
[25] Mennane ZK, Khedid A, Zinedine M, Lagzouli MO and Elyachioui M (2007). Microbiological characteristics of Klila and Jebn traditional Moroccan cheese from raw cow's milk. World Journal of Dairy and Food Sciences. 2 (1): 23-27.
[26]Sadek ZI, Hosn IM, El-Kholy WL and El-Dairouty RK(2009). Comparative Investigation for detection of food borne microorganisms in Egyptian hard Cheese, Ras using conventional and fast biochemical tests. Global Vet., 3 (3): 189-195.
[27]El-Leboudy A, Amr A, Amer A, Mohamed EN and Shaimaa ME (2015). Occurrence and Behavior of *Pseudomonas* Organisms in White Soft Cheese. Alexandria Journal of Veterinary Sciences. 44: 74-79.
[28]Sayed M, Abdel-Hameid A and Shabaan W (2011). Microbiological evaluation of some Egyptian white soft cheeses. Benha Veterinary Medical Journal. 1: 1- 6.
[29]Giacometti F, Andrea S, Guido F, Paolo D, Marina NL, Marco T, Andrea G, Roberto M, Raffaela R and Renato G Z (2012). Field handling conditions of raw milk sold in vending machines: experimental evaluation of the behaviour of *Listeria monocytogenes*, *Escherichia coli* O157:H7, *Salmonella typhimurium* and *Campylobacter jejuni*. Italian Journal of Animal Science.11: 132-136.
[30]Peng S, Hummerjohann J, Stephan R, and Hammer P (2013). Short communication: Heat resistance of *Escherichia coli* strains in raw milk at different subpasteurization conditions. J. Dairy Sci. 97:6623-6623.
[31]Haddad M.A and Yamani M.I (2017). Microbiological Quality of Soft White Cheese Produced Traditionally in Jordan. J Food Process Technol. 8(12): 1-6.
[32] Awad R. A, Farahat AM and Salama WM (2012). Production and in vivo nutritional evaluation of functional soft cheese supplemented with broccoli. World J. of Dairy and Food Sci. 7 (2): 150 -159.
[33]Mabrouk AMM, Effat BAM, Sadek ZIM, Tawfik NF, Hassan ZMR and Magdoub MNI (2014). Antibacterial activity of some lactic acid bacteria isolated from Egyptian dairy products. International Journal of Chem Tech Research 6 (2): 1139 -1150.
[34]Ibrahim G, Sharaf O and Azzat BAE (2015). Microbiological Quality of Commercial Raw Milk, Domiati Cheese and Kareish Cheese. Middle East Journal of Applied Sciences.
[35]Júnior J, Ribeiro C, De Oliveira AM, Silva FG, Tamanini R, De Oliveira ALM, and Beloti J V (2018). The main spoilage-related *psychrotrophic* bacteria in refrigerated raw milk. J. Dairy Sci. 101:75-83.
[36]Heikal GI, khater DF and Al-wakeel SA (2014). Bacteriological hazard of white cheese processed in some small primitive plants (dairy shops) in Tanta city. Benha veterinary medical journal. 26 (1):185- 194.
[37]Elbagory AM, Elshazly SE and Fathalla K E (2015). Impact of Probiotic Strains on Growth of Some Food Poisoning Bacteria from Milk and Soft Cheese. 1(2):1-6.
[38]Arena MP, Amandine S , Giovanni N , Francesco G , Djamel D , Giuseppe S , Daniela F (2016). Use of *Lactobacillus plantarum* Strains as a Bio-Control Strategy against Food-Borne Pathogenic Microorganisms. Frontiers in Microbiol. 7: 464.
[39]Alemdar S and Ağaoğlu S (2016). Behavior of *Escherichia coli* o157:H7 during the ripening of herby cheese manufactured FROM RAW MILK. Journal of Food and Health Science 2(1): 49-56.
[40]MagnussonM, Christiansson A, Svensson B (2007). *Bacillus cereus* spores during housing of dairy cows: Factors affecting contamination of raw milk J. Dairy Sci., 90: 2745-2754.
[41]RanieriML, Huck JR, Sonnen M, Barbano DM, Boor KJ (2009). High temperature, short time pasteurization temperatures inversely affect bacterial numbers during refrigerated storage of pasteurized fluid milk J. Dairy Sci., 92: 4823-4832.
[42]Watterson MJ, Kent D, Boor K, Wiedmann M, Martin N (2014). Evaluation of dairy powder products implicates thermophilic sporeformers as the primary organisms of interest J. Dairy Sci., 97: 2487-2497.
[43]Doyle CJ, Gleeson D, Jordan K, Beresford TP, Ross RP, Fitzgerald GF and Paul DC (2015). Anaerobic spore formers and their significance with respect to milk and dairy products. Int. J. Food Microbiol. 197: 77–87.
[44]Yuan L, Burmølle M, Wang NI, and GUoqing HE (2019). Insights into *Psychrotrophic* Bacteria in Raw Milk: A Review J. Food Prot. 82 (7): 1148-1159.