Journal Tikrit Univ. For Agri. Sci. Vol. (18) No.(3) – 2018
ISSN-1813-1646

Effect of Yoghurt Starter Culture and Pasteurization on Some Pathogenic Microorganisms in Domestic Soft Cheese

Abdulbaset Kufah Abdullah al-Jubouri[1] and Atallah Hameed Abdulghani
Department of Food Science, College of Agriculture, Tikrit University

Key words:
Rural soft cheese, LAB, *Staphylococcus aureas*, *Escherichia coli*

Corresponding author:
Abdulbaset K. A. al-jubouri
Email:
basetpr@yahoo.com

Received: 9/8/2017
Accepted: 17/12/2017

ABSTRACT

This work was conducted to determine the microbial contamination in soft cheese processed in laboratory. Pure yoghurt starter culture consists of *Lactobacillus bulgaricus and Streptococcus thermophillus* and domestic starter of yoghurt were used in making of soft cheese in laboratory. Both starters were individually added to raw and pasteurized cheese milk before adding rennet. Samples of manufactured soft cheeses were stored at 7°C for 1, 7, 14 and 21 days. The results showed an increase in the total aerobic count in all treatments to which the starter cultures were added. Yeasts & molds count was lower in pasteurized milk cheese compared to raw milk cheeses. Coliform count was observed to increase more rapidly in raw milk cheese in comparison with pasteurized milk cheese. *Escherichia coli and Staphylococcus aureas* were not found in pasteurized milk cheese, in contrast with raw milk cheese in which their counts rose rapidly during storage especially in control raw milk cheese. Influence of pure yoghurt starter culture was higher on microbial load in soft cheese compared to domestic starter.

تأثير بادئ اللبن الرائب والبسترة في محتوى الجبن الطري المحلي المصنع مختبرياً في بعض المسببات المرضية

عبد الباسط كفاح عبدالله الجبوري وعطاالله حميد عبدالغني
قسم علوم الأغذية/كلية الزراعة/جامعة تكريت

الخلاصة

أجري هذا البحث لدراسة التلوث المايكروبي في الجبن الطري المصنع مختبرياً. استخدم بادئ اللبن الرائب النقي المكون من *Lactobacillus delbrueckii subsp. Bulgaricus* و *Streptococcus thermophiles* وبادئ اللبن المحلي في تصنيع جبن طري محلي مختبرياً. أضيف البادئان كل على إنفراد الى معاملات الحليب البقري الخام والمبستر قبل إضافة المنفحة. خزنّت نماذج من معاملات الجبن المصنّع لمدة 1 و7 و14 و21 يوما عند 7 م°. بينت النتائج ارتفاع العدد الكلي للبكتريا الهوائية في جميع المعاملات التي اضيف لها البادئ. كانت أعداد الخمائر والاعفان منخفضة في المعاملات المصنعة من الحليب المبستر ومرتفعة في معاملات الحليب الخام. لوحظ تزايد عدد بكتريا القولون في معاملات الحليب الخام بنسبة أعلى من أعدادها في الجبن المصنع من الحليب المبستر. ولوحظ خلو الجبن المصنّع من الحليب المبستر من *Escherichia coli* و*Staphylococcus aureas* طوال مدة الخزن بينما لوحظ وجودهما في معاملات الجبن المصنّع من الحليب الخام وارتفعت اعدادها خلال مدة الخزن مع ملاحظة ان نسبة ارتفاعها في المعاملات المضاف لها بادئ كانت اقل مقارنةً بعينة السيطرة. وكان تأثير البادئ النقي في المحتوى المايكروبي للجبن الطري كان اعلى من تأثير البادئ المحلي.

الكلمات المفتاحية:
الجبن الطري الريفي ، بكتريا حامض اللاكتيك *Staphylococcus aureus* *Escherichia coli*
للمراسلة:
عبدالباسط كفاح عبدالله الجبوري
البريد الإلكتروني:
basetpr@yahoo.com
الاستلام: 9 / 8 / 2017
القبول: 17 / 12 / 2017

Introduction:

Soft cheese is well-known not only in our region but in all agricultural countries. It is very nutritional, cheap and has distinctive flavour. It contains high level of proteins and lipids. Also, it is distinguished by high level of moisture (50%) and neutral pH compared to other types of ripened cheeses (Nelson *et al*, 2009).

[1] This research is a part of MSc. Thesis for the first author.

In Iraq, soft cheese is manufactured in large amounts mostly by farmers, but the procedure they follow in cheese making is poor and risky due to using raw milk with high microbial load and unsanitary utensils and conditions that increase probably cheese contamination. In addition, marketing of soft cheese occurs in unhealthy conditions. Despite of these circumstances , soft cheese is consumed widely fresh and without ripening (Ali *et al*, 2013).

Cheese manufactured from raw milk has a better taste comparatively with the same cheese manufactured from pasteurised milk due to active indigenous enzymes in the milk and some bacteria, particularly non-starter lactic acid bacteria (NSLAB) present in the raw milk play a positive role in cheese ripening. This is an important marketing merit for raw milk cheeses. Nevertheless, it is clear that soft cheeses can be problematic and those made from raw milk particularly so. *Staphylococcus aureus* is a common cause of mastitis in dairy cows and, hence, its existence and consequently their enterotoxins in most raw milks is probable. Also, *Escherichia coli* is likely to be present in raw milk (Fox *et al*, 2017, Giraffa *et al*, 2010).

Soft cheese provides an optimal for the growth of many pathogenic micro-organisms at low temperatures. It is a potential means for some foodborne diseases such as *Staphylococcus aureus, Escherichia coli, Listeria monocytogenes, Bacillus spp., Salmonella spp.* and *Brucella spp.*, particularly, if the soft cheese is made of unpasteurized milk. Also, soft cheeses are very susceptible to fungal spoilage, which causes economic losses and is a public health concern due to the possible production of mycotoxins, which are capable of causing disease and death in both humans and animals (Fernandez *et al*, 2017). This type of cheeses is mostly linked to some foodborne outbreaks even if it was kept in refrigerators, consequently great precautions should be taken through all steps of cheese making (Choi *et al*, 2016, Fox *et al*, 2017, Fox, 2011).

Biological preservation is one of the methods used widely to face food deterioration especially that caused by microorganisms. It is an easy means, cheap, active and applicable in dairy manufacturing and above all, it is safe. Lactic acid bacteria (LAB) have been used for a long time in food processing and its use is considered one of the dependable methods to keep unpasteurized food items safe. LAB is applicable vastly in fermented dairy and cheese processing due to its positive effects on flavour, texture and nutritional value (Fernandez *et al*, 2017: Kongo and Malcata, 2016).

Starter culture in cheese manufacturing decreases the pH of the medium which affects the growth of pathogenic and deteriorate contaminants in cheese, besides of producing many enzymes leading to wide range of flavour compounds which enhance the organoleptic characteristics of the final product (Broome *et al*, 2011).

Lactobacillus delbrueckii subsp. bulgaricus and *Streptococcus thermophiles* are one of the most important types of LAB. They are widely used in many fermented dairy products and can be considered as a safe and natural component in food (Rizzello and De Angelis, 2011: Fernandez *et al*, 2017).

The main objective of this work was to study the capability of yoghurt starter culture to control the growth of some pathogenic contaminants in soft cheese manufactured in laboratory and then after applying the idea in Iraqi villages as yoghurt culture is available in all villages and the farmers are very familiar with using it.

Materials and methods:

Full cream raw milk was divided as in Table 1. Pure yoghurt starter culture consists of *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus* (1:1) and domestic yoghurt culture obtained from some villages in Tikrit, were used to inoculate cheese milks and incubating the milks for 3 h. at 42°C prior to rennet adding to all treatments and then cheese making was completed. Both starter cultures were activated at least three times before using it in cheese milks' inoculation. Soft cheese blocks of all treatments were kept in refrigerator at 7 °C for 1, 7, 14, 21 days. Starter cultures were added before applying rennet according to a simple experiment carried out previously to select the incubation time properly before acid coagulation (Figure 1).

Table 1: Treatments of the cheese milk assigned for current study.

Raw milk	Pasteurized 63°C for 30 min.	Treatment 1 (T1)	Pure yoghurt starter culture added
		Treatment 2 (T2)	Domestic yoghurt starter culture added
		Treatment 3 (T3)	Control 1
	Unpasteurized	Treatment 4 (T4)	Pure starter culture added
		Treatment 5 (T5)	Domestic yoghurt starter culture added
		Treatment 6 (T6)	Control 2

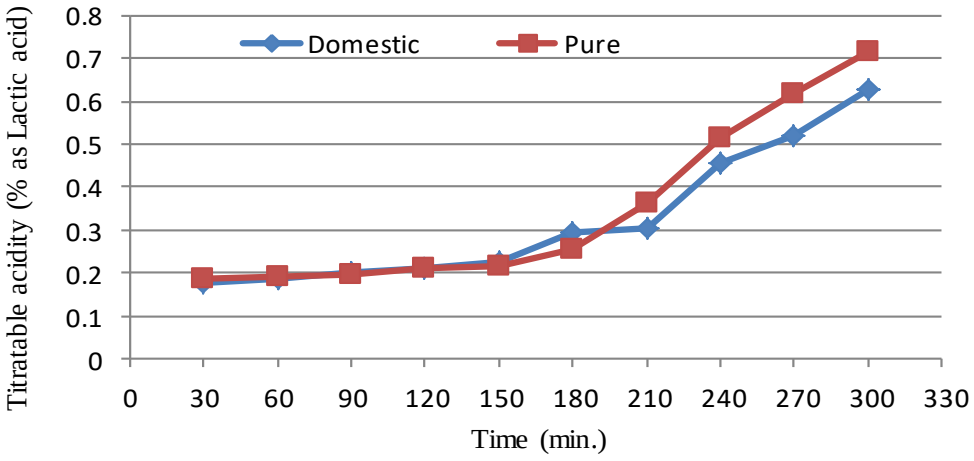


Figure 1 Growth and Titratable acidity % of Pure yoghurt starter culture (*Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus*, 1:1) and domestic yoghurt culture.

Sample preparation:

Cheese samples of 25 g were collected from many places of the blocks, mixed well and 225 ml of sodium citrate solution (2%) was added, mixed again until the sample fully dissolved. This solution was considered as first dilution. Other dilutions were prepared with peptone solution (0.1%) (Graham, 2004). The following tests were done during the storage period:

Determination:

Total Aerobic Bacterial Count:

This test was done according to AOAC (2010) with Nutrient agar and the plates were incubated at 37 °C for 48 h.

Yeasts & mold counts:

Potato dextrose agar (PDA) medium was used. Petri-dishes were incubated at 25 °C for 5 days (Frank and Yousef, 2004).

Coliform count:

Coliform bacteria count in cheese samples was done on MacConkey Agar. Petri dishes were cultivated upside-down in an incubator at 37 °C for 48 h. (AOAC, 2010).

Escherichia coli:

E. coli count was conducted as described by Davidson *et al*, (2004) on Violet Red Bile Agar. Petri dishes were put upside-down in an incubator at 44 °C for 48 h.

Staphylococcus aureas:

Mannitol salt agar was used to count *Staphylococcus aureus* (Henning *et al*, 2004). After spreading, petri-dishes were incubated at 37 °C for 48 h.

Biochemical Identification tests:

IMVIC tests:

Indole, methyl red, Voges-Proskauer and citrate utilization tests (IMVIC) were conducted according to Tille (2013).

Carbohydrate fermentation tests:

Purple broth with selected carbohydrates (Xylose, Rhamnose, Mannitol and Glucose) used to achieve these tests. The media were incubated at 35 °C for 48 h. (Andrews and Hammack, 2011).

Hemolysis test:

Sheep blood agar was used (Brown and Smith, 2015). After striking the media, plates were kept at 37 C for 24 h.; Clear zone was evidence to complete hydrolysis and green zones around colonies to partial hemolysis.

Catalase test:

According to Brown and Smith (2015), hydrogen peroxide (3%) was used to test the capability of bacteria to produce catalase.

Coagulase test:

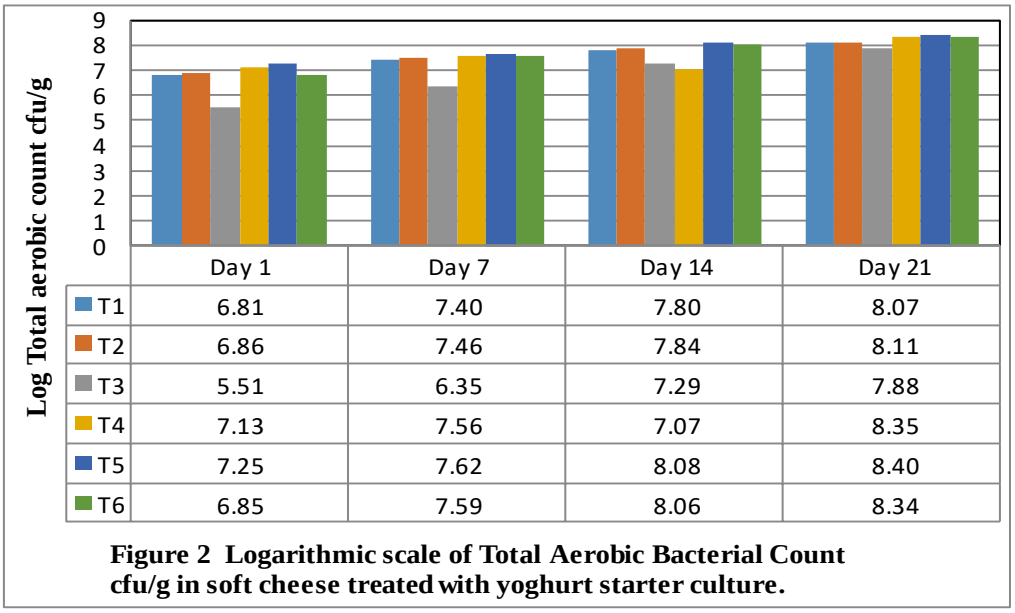
According to Berkowitz and Jerris (2015), three drops of blood plasma on a slide was inoculated by some of the bacterial growth. If the plasma coagulates within 15 s, the test is considered positive.

Results and discussion:

Microbial content of manufactured soft cheese:

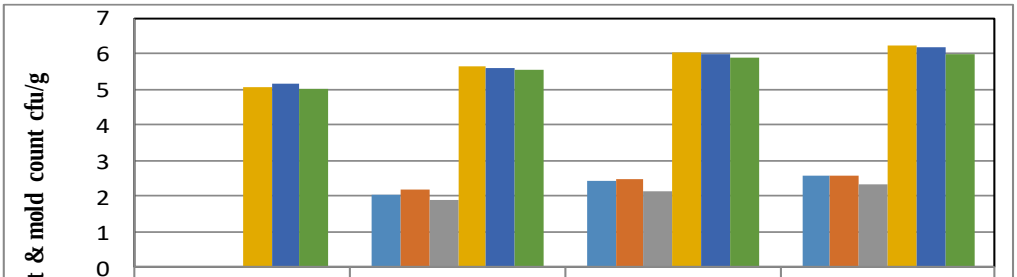
Total aerobic bacterial count increased in all treatments which yoghurt starter cultures (T1, T2, T3 and T4) were added, but the increasing rate was lower in pasteurized milk soft cheeses (T1, T2 and T3) in comparison with raw milk soft cheeses (T4, T5 and T6). After the first day of storage (7 °C), T3 showed the lowest TAC (5.51 cfu/g) whereas the highest count was in T5 (7.25 cfu/g). TAC increase was observed to develop during the cold storage period. This development was in agreement with Briggiler-Marcó *et al*, (2007) who reported that TAC in the soft cheese in which starter culture was added, is attributed to the culture itself which its count might reach 10^8 cfu/g.

Yeast & mold counts were nil after first day of storage for pasteurized milk cheeses (T1, T2 and T3) whereas the range of Yeast and mold counts in raw milk cheeses was high (5.0-5.15 cfu/g; Figure 3). Although the counts were low in T1, T2 and T3 compared to T4, T5 and T6, both increased through the storing period. These results reflect that raw milk had already high load of yeast and mold, but pasteurization, hygienic storing and handling of pasteurized milk soft cheeses were all efficient factors to lessen increasing rates (Al-Nazal, 2016). In addition, the effectiveness of lactic acid bacteria as an inhibitor of the proliferation of yeast and mold was obvious, especially in pasteurized milk soft cheese during storage period which continued for 21 days (Fernandez *et al*, 2017).



	Day 1	Day 7	Day 14	Day 21
T1	6.81	7.40	7.80	8.07
T2	6.86	7.46	7.84	8.11
T3	5.51	6.35	7.29	7.88
T4	7.13	7.56	7.07	8.35
T5	7.25	7.62	8.08	8.40
T6	6.85	7.59	8.06	8.34

Figure 2 Logarithmic scale of Total Aerobic Bacterial Count cfu/g in soft cheese treated with yoghurt starter culture.

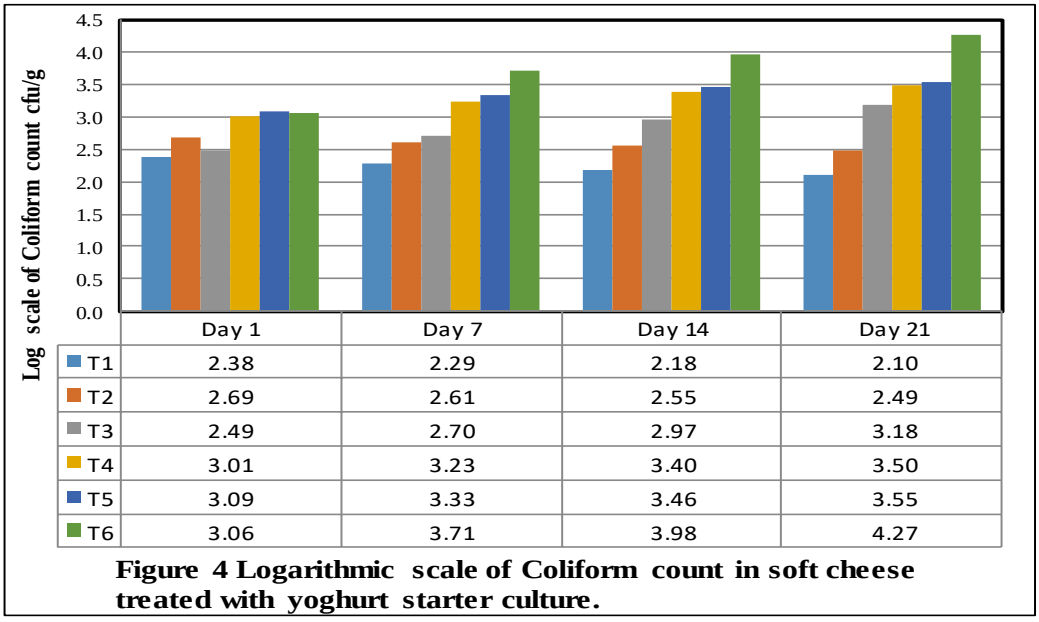


	Day 1	Day 7	Day 14	Day 21
T1	0.00	2.02	2.45	2.57
T2	0.00	2.19	2.46	2.58
T3	0.00	1.90	2.14	2.34
T4	5.04	5.65	6.02	6.25
T5	5.15	5.61	5.98	6.19
T6	5.00	5.53	5.87	6.00

Figure 3 Logarithmic scale of Yeast & mold count in soft cheese count treated with yoghurt starter culture.

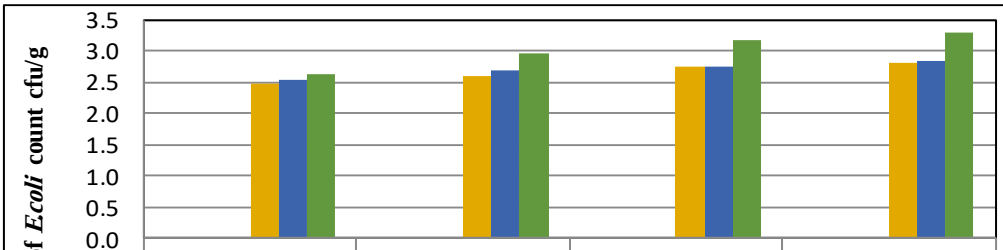
Figure (4) shows coliform counts in soft cheeses. The counts were low for pasteurized milk cheeses (T1, T2 and T3) after 1st day of refrigerated storage as the counts ranged in log scale between 2.38-2.69 cfu/g, whereas the counts in raw milk cheeses (T4, T5 and T6) were higher and ranged from 3.01-3.09 cfu/g. Higher counts in the latter can be attributed to the initial microbial load of raw milk, while lower counts in the former cheeses is due to the effect of pasteurization (Hadal, 2010).

Figure (5) shows that Escherichia coli did not appeared in pasteurized milk cheeses through the storage period (7 C, 21 d). But its counts in raw milk cheeses ranged 2.46-2.64 cfu/g after 1st day and reached (2.82-3.29 cfu/g) at the end of the period. the increase in the counts occurred during the storage period in all T4, T5 and T6 but its rate in Control Treatment of raw milk cheeses (T6) was higher than others, mostly due to the activity of starter cultures in producing lactic acid (Akpinar *et al*, 2011). All *E. coli* grown colonies that were counted identified and characterized by IMVIC tests which were positive to Indole and Methy Red tests and negative to Gram, Citrate and Voges-Proskauer tests (Davidson *et al*, 2004: Sandle, 2014).



	Day 1	Day 7	Day 14	Day 21
T1	2.38	2.29	2.18	2.10
T2	2.69	2.61	2.55	2.49
T3	2.49	2.70	2.97	3.18
T4	3.01	3.23	3.40	3.50
T5	3.09	3.33	3.46	3.55
T6	3.06	3.71	3.98	4.27

Figure 4 Logarithmic scale of Coliform count in soft cheese treated with yoghurt starter culture.



	Day 1	Day 7	Day 14	Day 21
T1	0.00	0.00	0.00	0.00
T2	0.00	0.00	0.00	0.00
T3	0.00	0.00	0.00	0.00
T4	2.46	2.61	2.74	2.82
T5	2.55	2.69	2.76	2.85
T6	2.64	2.97	3.16	3.29

Figure 5 Logarthmic Scale of *Escherichia coli* count in soft cheese treated with yoghurt starter culture.

Staphylococcus aureus was absent in all pasteurized milk cheeses (T1, T2 & T3) at the first day and through storage period (Figure 6), but it appeared in raw milk cheeses (T4, T5 and T6). Its counts at day one were 2.77, 2.80 and 2.80 cfu/g, respectively. Also the counts increased during the storage and reached 3.02, 3.05 and 3.43 cfu/g, respectively. Results showed that the rise in the colony counts of *Staphylococcus aureus* was higher in T6 (Control) compared to T4 and T5 in which starter cultures were added during cheese making, mostly due to the activity of the starter cultures (Rizzello and De Angelis, 2011).

All *Staphylococcus aureus* grown colonies that were counted identified and confirmed by many tests; Gram, urease, Catalase, Coagulase and Hemolysis tests, which were positive to all tests according to Gillaspy and Iandolo (2009) and Leboffe and pierce (2012).

Conclusion:

The results showed the effectiveness of pasteurization and applying yoghurt starter cultures (both pure and domestic). Although pasteurization of raw milk inhibits all pathogenic microorganism in milk, but the consumers prefer raw milk cheese rather than pasteurized milk cheese. Despite of marketing advantages, the farmers have no intention to change their traditional method in cheese making because of the problems of the heat treatment, e.g. poor coagulation of caseins and yield precents. Therefore, applying LAB in cheese making is very productive, easy, available and safe. Nevertheless, the combination between the two factors is the best because heat treatment inhibits pathogens and LAB can acidify the medium and improve coagulation due to release of Ca++. In addition, the enzymes of LAB will produce distinctive flavour to the cheese. Also, keeping quality of soft cheese will be improved.

References:

AKPINAR, A., YERLIKAYA, O. & KILICCEDIL, S. 2011. Antimicrobial activity and antibiotic resistance of Lactobacillus delbrueckii ssp. bulgaricus and Streptococcus thermophilus strains isolated from Turkish homemade yoghurts. African Journal of Microbiology Research, 5, 675-682.

AL-NAZAL, A. I. 2016. Isolation and characterization of some microbial and chemical contaminants in refrigerated raw milk in some regions of salahuddin governarate. Tikrit Journal for pure sciences, 21, 36-41.

ALI, M. Y., KHAZEL SH ABDULLAH & S.MOHAMMED, H. 2013. Production of Semi Like Aushari Cheese by using Probiotic Bacteria and Compare it with some Native Species to some Areas in Northern Iraq Journal of Tikrit University For Agriculture Sciences 13, 20-24.

ANDREWS, W. & HAMMACK, T. 2011. Salmonella in Bacteriological Analytical Manual. Federal Drug Administration, USA.

BERKOWITZ, F. E. & JERRIS, R. C. 2015. Practical Medical Microbiology for Clinicians, John Wiley & Sons.

BRIGGILER-MARCÓ, M., CAPRA, M. L., QUIBERONI, A., VINDEROLA, G., REINHEIMER, J. A. & HYNES, E. 2007. Nonstarter Lactobacillus Strains as Adjunct Cultures for Cheese Making: In Vitro Characterization and Performance in Two Model Cheeses. Journal of Dairy Science, 90, 4532-4542.

BROOME, M. C., POWELL, I. B. & LIMSOWTIN, G. K. Y. 2011. CHEESE - Starter cultures: specific properties Academic Press - Elsevier.

BROWN, A. & SMITH, H. 2015. Benson's microbiological applications: Laboratory manual in general microbiology, McGraw-Hill Higher Education.

CHOI, K.-H., LEE, H., LEE, S., KIM, S. & YOON, Y. 2016. Cheese Microbial Risk Assessments — A Review. Asian-Australasian Journal of Animal Sciences, 29, 307-314.

DAVIDSON, P., ROTH, L. & GAMBREL-LENARZ, S. 2004. Coliform and other indicator bacteria. Standard methods for the examination of dairy products, 187-226.

FERNANDEZ, B., VIMONT, A., DESFOSSÉS-FOUCAULT, É., DAGA, M., ARORA, G. & FLISS, I. 2017. Antifungal activity of lactic and propionic acid bacteria and their potential as protective culture in cottage cheese. Food Control, 78, 350-356.

FOX, P. F. 2011. Cheese - Overview. In: FUQUAY, J. W., FOX, P. F. & MCSWEENEY, P. L. (eds.) Encyclopedia of dairy sciences. 2 ed.: Academic Press – Elsevier.

FOX, P. F., GUINEE, T. P., COGAN, T. M. & MCSWEENEY, P. L. H. 2017. Pathogens in Cheese and Foodborne Illnesses. Fundamentals of Cheese Science. Boston, MA: Springer US.

FRANK, J. F. & YOUSEF, A. E. 2004. Chapter 8, Tests for Groups of Microorganisms. In: FRANK, J. F. (ed.) Standard Methods for the Examination of Dairy Products. American Public Health Association.

GILLASPY, A. F. & IANDOLO, J. J. 2009. Staphylococcus- Introduction. . In: SCHIECHTER, M. (ed.) in: Encyclopedia of Microbiology. 3rd ed. Oxford: Academic Press.

GIRAFFA, G., CHANISHVILI, N. & WIDYASTUTI, Y. 2010. Importance of lactobacilli in food and feed biotechnology. Research in Microbiology, 161, 480-487.

GRAHAM, T. 2004. Sampling Dairy and Related Products. In: WEHR, H. M. & FRANK, J. F. (eds.) Standard Methods for the Examination of Dairy Products. 17th ed. Washington,: American Public Health Association.

HADAL, A. 2010. Effect of Heat Treatment on the White Cheese Produced From Raw Milk on Microbiological Content and Some Chemical Properties. Journal of Damascus University for agricultural sciences, 26, 261-272.

HARNETT, J., DAVEY, G., PATRICK, A., CADDICK, C. & PEARCE, L. 2011. Lactic Acid Bacteria / Streptococcus thermophilus. In: FUQUAY, J. W., FOX, P. F. & MCSWEENEY, P. L. (eds.) in: Encyclopedia of dairy sciences. Academic Press – Elsevier.

HENNING, D. R., FLOWERS, R., REISER, R. & RYSER, E. T. 2004. Pathogens in Milk and Milk Products. In: WEHR, H. M. & FRANK, J. F. (eds.) Standard Methods for the Examination of Dairy Products. 17 ed.: American Public Health Association

INTERNATIONAL, A., HORWITZ, W. & LATIMER, G. W. 2010. Official methods of analysis of AOAC International, Gaithersburg, MD., AOAC International.

KONGO, J. M. & MALCATA, F. X. 2016. Cheese: Processing and Sensory Properties.

LEBOFFE, M. J. & PIERCE, B. E. 2012. A photographic atlas for the microbiology laboratory, Morton Publishing Company.

NELSON, L., GADOTTI, C. & DIEZ, F. 2009. Risk Assessment of Fresh Hispanic Cheeses and Technologies to Improve their Safety. women, 500, 2001.

RIZZELLO, C. G. & DE ANGELIS, M. 2011. Lactic Acid Bacteria - Lactobacillus spp.: Lactobacillus delbrueckii Group. In: FUQUAY, J. W., FOX, P. F. & MCSWEENEY, P. L. (eds.) Encyclopedia of dairy sciences. 2 ed.: Academic Press – Elsevier.

SANDLE, T. 2014. BIOCHEMICAL AND MODERN IDENTIFICATION TECHNIQUES| Food-Poisoning Microorganisms. In: BATT, C. A. & TORTORELLO, M.-L. (eds.) Encyclopedia of food microbiology. 2nd ed.: Academic Press-Elsevier.

TILLE, P. 2013. Bailey & Scott's Diagnostic Microbiology-E-Book, Elsevier Health Sciences.