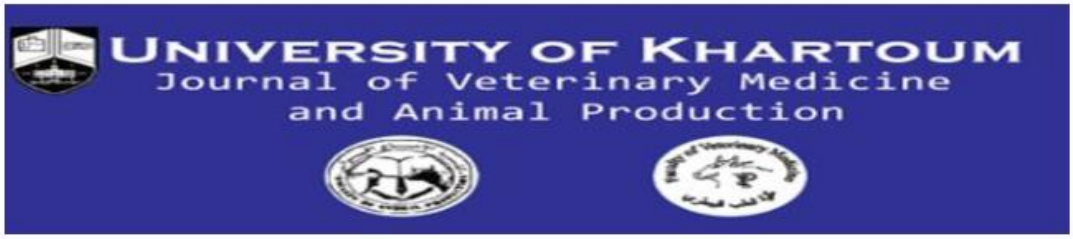
U. of K. J. Vet. Med. Anim. Prod., Vol.11, Issue 1&2 (2020) page 353-362
ISSN: 243456



Microbial Load of Mish during Storage Period at Retailers' Shops in Khartoum State, Sudan

Mazza A. E. Barakat[1] and Ibtisam E. M El Zubeir[2*]

[1]Animal Production Research Center, Kuku
[2]Department of Dairy Production, Faculty of Animal Production, University of Khartoum, P. O. Box 321, Khartoum, Sudan
**Corresponding author: Ibtisamelzubeir17@gmail.com*

Abstract

Mish is traditional fermented milk that might be consumed alone or with the meals. Currently mish is produced by different dairy companies and stored in groceries for a period of 21 days (shelf life) in a condition, which might affect its microbiological load. This study aimed to evaluate and compare the effect of storage period on the microbiological load of mish collected from three different dairy companies (C1, C2 and C3). Ten samples of mish were collected from each company during day 2 and day 20 randomly from groceries for microbiological evaluation (total viable bacteria, coliform and yeast and mold counts). The microbiological analysis results showed that log total viable bacterial count (6.120) and coliform count (5.872) revealed non-significant ($P>0.05$) differences between those companies. The total viable bacteria and coliform showed log 6.090 and log 5.858 for mish samples collected during day 2 respectively, while the respective values were log 6.149 and 5.886 for mish samples during day 20. Yeast and mold counts showed highly significant differences ($P<0.001$) between mish producing companies, which recorded a mean of log 5.326. Also it increased from day 2 (log 5.034) to day 20 (log 5.619) during the storage periods. This study remarked that there were variations between and within mish producing companies in their microbial properties. This might be related to the variations of raw materials, the processing methods and the storage conditions in retailers. Hence this study recommended consumption of fresh mish, in addition the methods of its preservation should be considered.

Keywords: Mish, microbiological evaluation, storage period

المستخلص

المش من الألبان المتخمرة التي يمكن ان تستهلك لوحدها أو مع الوجبات. يتم إنتاج المش حاليا بواسطة شركات مختلفة لمنتجات الالبان و يتم تخزينه في البقالات لمدة 21 يوم (فترة الصلاحية) في ظروف قد تؤثر علي حمولته الميكروبيولوجية. هدفت الدراسة إلى تقييم ومقارنة تأثير فترة التخزين على الحمولة الميكروبيولوجية لعينات المش التي تم جمعها من ثلاث شركات ألبان (C1، C2 و C3). جمعث 30 عينة من المش في اليوم الثاني واليوم العشرون خلال فترة التخزين عشوائياً لإجراء التقييم الميكروبيولوجي) أعداد البكتيريا الكلية وبكتريا القولون والخمائر والأعفان) . أظهرت نتائج التحليل الميكروبيولوجي لعينات المش أنه لا توجد فروق معنوية ($P>0.05$) بين تلك الشركات حيث بلغ متوسط أعداد البكتيريا الكلية (لو6.120) وأعداد بكتريا القولون (لو5.872). بينما بلغ مجموع البكتيريا الكلية وبكتريا القولون لو 6.090 و 5.858 في اليوم الثاني على التوالي، في حين كانت القيم المعنية لو 6.149 و 5.886 لليوم العشرون علي التوالي من فترة التخزين. أظهرت أعداد الخمائر والأعفان فروقات معنوية عالية ($P<0.001$) بين الشركات المنتجة قيد الدراسة، والتي سجلت متوسط لوغريثم 5.326 بينما أظهرت فترة التخزين زيادة في أعداد الخمائر والأعفان من اليوم الثاني (لو 5.034) مقارنة باليوم العشرون (لو 5.619) من فترة التخزين. خلصت هذه الدراسة إلي أن هنالك إختلاف بين الشركات المنتجة للمش في خصائص المش الميكروبيولوجية. هذا الإختلاف قد يرجع إلي المادة الخام التي تدخل في تكوين المش و طريقه تصنيعه وتخزينه وحفظه في المحال التجارية وعليه توصي الدراسة بإستهلاك المش الطازج مع مراعاة طرق حفظه.

الكلمات المفتاحية: المش، التقيم الميكروبيولجي، فترة التخزين.

Introduction

Final characteristics of fermented milk depend on milk composition, heat treatment, fermentation conditions and the starters cultures (Chammas *et al*., 2006). Traditional food fermentation can take potentially hazardous substances as raw materials, such as raw milk and transform them into products with both improved keeping qualities and reduced risk of causing illness (Beukes *et al*., 2001). Many pathogenic microorganisms were isolated from traditionally fermented dairy products in different parts of the world (Beukes *et al*., 2001; Lore *et al*., 2005; Uzeh *et al*., 2006 and Suliman and El Zubeir, 2016). To eliminate the hazard of disease transmission, fermented dairy products should be prepared only from high-grade milk that has been adequately pasteurized, followed by cooling to ensure that the product is safe and free from post pasteurization contaminants pathogenic microorganisms (Abdalla and El Zubeir, 2006).

The main reason for the isolation of pathogenic organisms from traditionally fermented dairy products is the method of manufacture which involves the use of unpasteurized milk (Abdalla and Hussain, 2010). Abdalla and El Zubeir (2006) reported that coliform bacteria should not be present in commercial fermented dairy products prepared from adequately pasteurized milk, as their presence suggests inadequate pasteurization or recontamination after heating.

Yeasts and molds are the main spoilage organisms found in cultured milk products, since the high acidity of such products inhibits many bacteria (Viljoen *et al.*, 2003 and Mayoral *et al*., 2005). Gadage *et al*. (2000); Carbo *et al.* (2001) and Gadage *et al.* (2001) linked the increasing presence of yeasts and molds in fermented dairy products to insufficient hygiene during the production, sanitation of the equipment, air contamination, insufficient heat treatment or inadequate microbiological quality of the supplements used. However, several herbs have therapeutic properties and antimicrobial activities. Therefore, fortification of dairy foods with herbs and spices could help to provide functional dairy products with nutritional and medicinal values (El-Sayed and Youssef, 2019). Moreover, Abdalla and El Zubeir (2006) found low average of bacterial counts in the mish samples produced after

pasteurization of milk. Abdel Gadir *et al.* (2013) found the total bacterial count, yeasts and moulds counts and coliform count of mish samples produced by traditional procedure were lower ($2.27\times10^5\pm1.08\times10^5$, $4.96\times10^4\pm2.19\times10^4$ and $9.48\times10^3\pm5.5\times10^3$ for the respectively) compared to mish produced by modern procedure ($5.2\times10^4\pm1.7\times10^4$, $1.4\times10\pm0.7\times10$ and 6.75 ± 5.3, respectively).

Mish is usually store in the groceries for a period of 21 days, thus it is subjected to fluctuation of electricity supply, which might affect its microbiological load. Hence the aim of this study was **e**valuation and comparison of microbial content of commercial mish produced by three different dairy companies at the beginning and the end of storage period.

Materials and methods

Area of study

Mish samples produced by three different dairy factories were collected randomly during August to October 2016 from groceries during the beginning of manufacturing period (day 2) and the end of storage period (day 20) from El Greif west, southern of Khartoum.

Samples collection

Thirty samples of mish that processed by three different dairy factories in Khartoum were purchased randomly including 5 batches (duplicate samples) from groceries located at El Greif west, southern of Khartoum. The samples were transported cooled (5 °C) to the laboratory of Department of Dairy Production, Faculty of Animal Production – University of Khartoum for estimating the microbiological load.

Microbiological analysis

All media were obtained in dehydrated forms and prepared according to the manufacture instructions.

Plate count agar medium was used to determine the total bacterial count according to Houghtby *et al.* (1992). MacConkey agar medium was used to determine the coliform count (Christen *et al.*, 1992) and yeast extract agar medium was used for determining the total yeast and molds counts (Frank *et al.*,1992).

Sterilization

Glass wares such as test tubes, pipettes, Petri-dishes, flasks and bottles were sterilized in a hot oven at 180º C for one hour, whereas media, ringer solution and tips were sterilized by autoclaving at 121º C for 15 minutes (Marshall, 1992).

Culturing methods

One ml of the mish sample was transferred aseptically by sterile pipette to 9 ml sterile ringer's solutions. This procedure was repeated to make tenfold dilutions from 10^{-1} – 10^{-4} according to Rishardson (1985).

One ml from each selected dilution was cultured at 32º C for 48 hours using pour plate technique for total bacterial count, while the coliform count was incubated at 37º C for 24 hours and 25° C for 5 days was estimated for the yeasts and molds count. The plates containing 30– 300 cfu were enumerated (Marshall, 1992).

Statistical analysis

The data were statistically analyzed using Statistical Analysis System (SAS version, 9.1.3). General liner model was used for statistical analysis and means were separated by Duncan Multiple Range Test at $P<0.05$.

Results

The effect of storage period on microbiological content of mish samples

Table 1 showed the effect of storage period on microbiological load of mish samples collected from three dairy companies included total bacterial count, coliform count and yeast and mold counts. Total bacterial count of mish samples recorded log 6.090 during day 2 and log 6.149 during day 20 of storage period. Moreover, the results showed non-significant ($P<0.05$) variation between the two periods of storage (Table 1 and Figure 1), There were also no significant ($P>0.05$) difference in coliform count of mish samples between day 2 and day 20 that revealed log 5.858 for day 2 and log 5.886 for day 20 (Table 1 and Figure 2).

Table 1: The effect of storage period on microbiological properties of mish samples collected from three companies

Microbial load (log)	Day 2	Day 20	L S
Total bacterial count	$6.090^{a} \pm 0.31$	$6.149^{a} \pm 0.18$	**N S**
Coliform count	$5.858^{a} \pm 0.21$	$5.886^{a} \pm 0.17$	**N S**
Yeast and mold count	$5.034^{b} \pm 0.36$	$5.619^{a} \pm 0.14$	***

Means in the same row bearing the same superscripts letters are not significantly different $P>0.05$

** = $P<0.01$

*** = $P<0.001$

LS= level of significant.

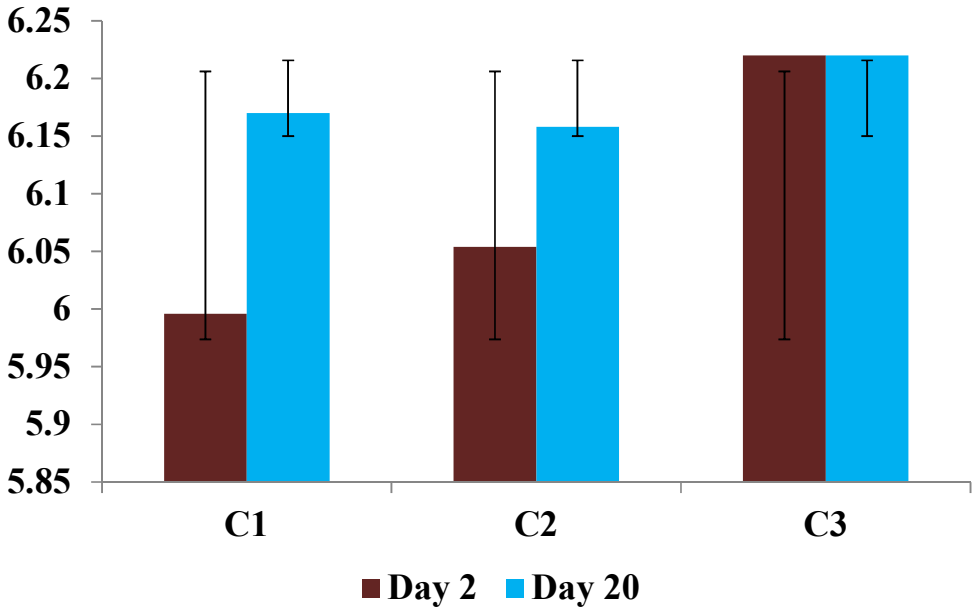


Figure 1: Total viable bacteria count of mish samples collected from three companies during storage

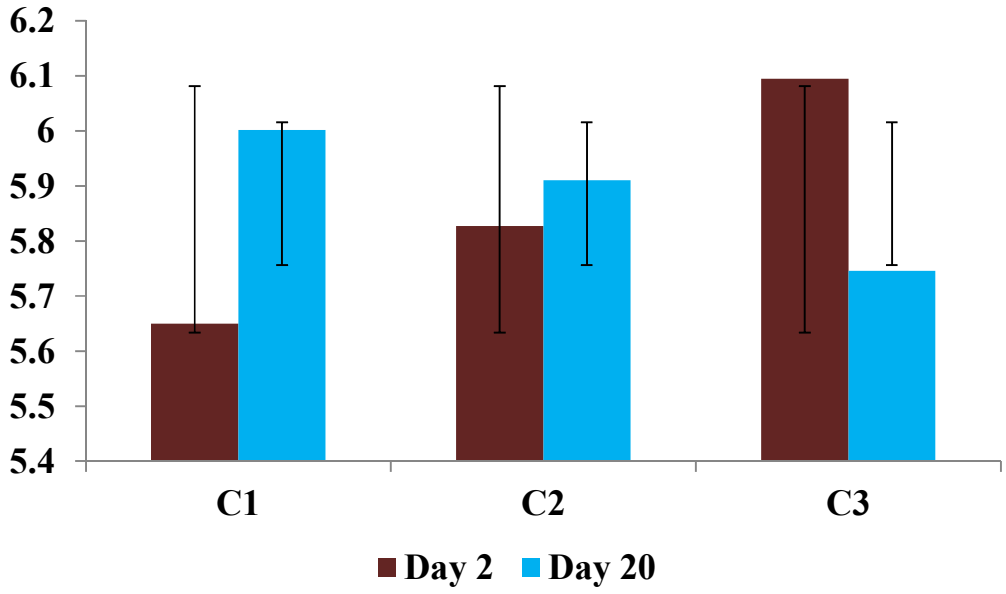


Figure 2: Coliform count of mish samples collected from three companies during storage

However highly significant (P<0.001) variations were obtained for the counts of yeast and molds showed for the mish samples during the two periods of storage, which increased from log 5.034 during day 2 to log 5.619 during day 20 (Table 1 and Figure 3).

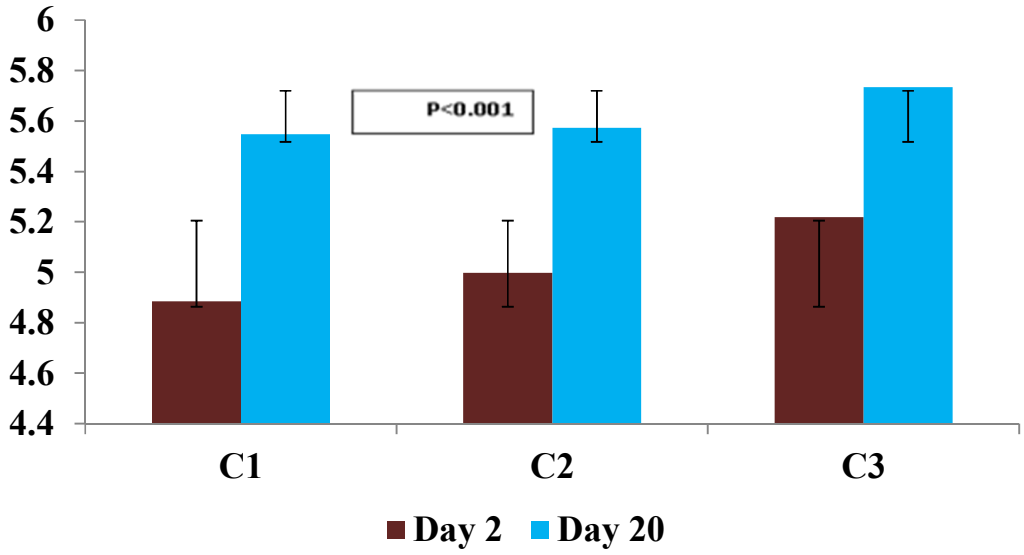


Figure 3: Yeast and mold count in mish samples collected from three companies during two periods of storage

Variations of microbial load in mish samples produced by different companies

Figure 1, 2 and 3 showed the variations of microbiological load in mish samples collected from three dairy companies; C1, C2, C3 during the storage period that included total bacterial count, coliform and yeast and molds. The result doesn't show significant variation ($P>0.05$) between mish producing companies; C1, C2 and C3 (Figure 1) for total bacterial count that revealed means of log 6.084, log 6.106, and log 6.169, respectively. However, the mean values of coliform count in the mish samples showed significant ($P<0.05$) variation between companies under study (Figure 2), it revealed log 5.827 for C1, log 5.869 for C2 and log 5.921 for C3. Moreover, the results of yeast and molds, which was presented in Figure 3, showed highly significant ($P<0.001$) variation between different mish companies; C1, C2 and C3, a mean value for C3 (log 5.477) was higher than those of C2 (log 5.285) and C1 (log 5.217).

Discussion

Total viable bacterial count showed non-significant ($P>0.05$) difference between the two periods of storage; day 2 and day 20 (Table 1 and Figure 1). This could be due to the use of spices in mish was approved as preservative (El Zubeir et al., 2005). This might also be because the fermentation process of traditional fermented milk depends on the end products of lactic acid bacteria formed during the fermentation of lactose (Rattanachaikunsopon and Phumkhachorn, 2010). Moreover, the lactic acid bacteria (LAB) are recognized for their fermentative ability and thus enhancing food safety, improving organoleptic attributes, enriching nutrients and increasing health benefits (Gemechu, 2015). However, the means for the total bacterial count of mish samples produced by the modified procedure and traditional procedure were $5.2\times10^4\pm1.7\times10^4$ and $2.27\times10^5\pm1.08\times10^5$, respectively (Abdel Gadir *et al*., 2013). The variation in the total viable bacterial count may be related to the initial microflora prevailing in the raw milk for mish preparation, since lactic acid bacteria are responsible for many microbial transformations found in fermented milk (Franz *et al*., 1999). Also Abdalla and Abdel Nabi (2010) reported that most of total viable bacterial count in mish may be due to lactic acid bacteria.

Data presented in Figure 1 showed non-significant variations between total viable bacterial counts of mish produced by the different companies (C1, C2 and C3). Total viable bacterial count presented was lower than those reported by Abdalla and Abdel Nabi (2010) who recorded log 9.47±0.121 and log 8.89±0.113 respectively, for samples collected during the beginning and the end of the storage periods and higher than those found by Abdel Gadir *et al.* (2013) who recorded on their study 5.20×10^4. The addition of spices such as black cumin inhibits the growth of some pathogenic bacteria (Abdalla and El Zubeir, 2006). Also garlic (*Allium sativum*) as aromatic plant is commonly used in ayran (*Turkish traditional yoghurt drink*), yoghurt and various cheese types because of their taste, natural preservative additives due to its antimicrobial and antioxidant activity (Kaptan and Sivri, 2018). Moreover, the major roles of fermentation include preservation of food through formation of inhibitory metabolites such as lactic acid, etc., often in combination with decrease of water activity (Ross *et al*., 2002 and Gaggia *et al*., 2011). In addition to

improving food safety through inhibition of pathogens (Adams and Mitchell, 2002 and Adams and Nicolaides, 2008).

Coliform bacteria showed non-significant (P>0.05) variation between the two different periods (Table 1 and Figure 2). The counts were higher than these reported for other traditional dairy products (Benkerroum and Tamime, 2004; Al-Tahiri, 2005; Lore *et al.*, 2005; El-Baradei *et al.*, 2008 and Suliman and El Zubeir, 2016) and lower than those reported by Uzeh *et al.* (2006) and Abdalla and Abdel Nabi (2010). In addition to that coliform bacterial count decreased during storage period and this is due to the lactic acid bacteria (Al-kadamany *et al.,* 2002). Moreover, high number of coliforms is a sign of unsanitary conditions and /or post processing contamination (Abdalla and Abdel Nabi, 2010) as well as lower standards of hygiene in the dairy plants (Attita Allah *et al.*, 2011 and Abdel Gadir *et al.*, 2013).

The coliform count of mish samples that was obtained during this study showed non-significant variation (Figure 2). Coliform count recorded had lower count comparable with Abdalla and Abdel Nabi (2010) and Abdel Gadir *et al.* (2013) who recorded log 6.78±0.155 and log 6.75±0.53, respectively.

In a previous study, yoghurt produced by some of dairy factories was found to be contamination with high load of yeast and mould (El bakri and El Zubeir, 2009; Attita Allah *et al.*, 2011 and Mohammed and El Zubeir, 2011). Moreover, Attita Allah *et al.* (2011) concluded that quality of set yoghurt produced by the selected dairy factory need to be improved, as was indicated by the high count of TBC and coliform bacterial count, which exist in the product up to 7 days of storage.

Thus, in order to eliminate the hazard of disease transmission, fermented dairy products should be prepared only from high-grade milk that has been adequately pasteurized, followed by cooling to ensure that the product is safe and free from post pasteurization contaminants pathogenic microorganisms (Abdalla and El Zubeir, 2006). Yeast and mold revealed highly significant (P<0.001) differences between the date of storage period day 2 and 20 (Table 1 and Figure 3). Results was supported Abdalla and Abdel Nabi (2010) and higher than those reported by Abdel Gadir *et al.* (2013). The high numbers of yeast and mold reflect unsanitary hygienic conditions during processing and distribution of the product, despite the fact that the product under investigation was superior to all similar fermented products except that it had high acidity compared to these products (Abdalla and Abdel Nabi, 2010). The present results are expected since the product chemically deteriorated towards the end of storage, which supported Samolada *et al.* (1998).

The average of yeast and mold counts obtained showed highly significant (P<0.001) difference for mish samples produced by C1, C2, and C3. Viljoen *et al.* (2003) and Mayoral *et al*. (2005) reported that yeasts and molds are the main spoilage organisms found in cultured milk products, since the high acidity of such products inhibits many bacteria.

The mish samples from C3 were higher in yeast and mold counts than those obtained from other companies under comparison (Table 2 and Figure 3). These counts were higher than those reported by Abdel Gadir *et al.* (2013) who recorded 1.4± 0.7 for mish samples. However, less variation was observed when compared to the result of Abdalla and Abdel Nabi (2010). These results might be due to the ability of yeast to grow over rather wide range of pH, alcohol and sugar concentrations (Jay, 1986). Abdel Gadir *et al*. (2013) attributed the significant (P<0.001) variation between two manufacturers of mish in

microbiological properties to the differences of manufacture's procedure and the hygienic status of the plant. They added that the total bacterial count, coliform count and yeast and mould counts were increased during the storage period with noticeable higher counts for the mish that produced using the traditional methods.

Conclusion

The results of this study concluded that mish showed significant variation for microbiological quality between different dairy plants and within the two periods of storage. Hence this study recommended that mish must be prepared under good quality control measures that include raw materials, production conditions, storing and monitoring of the product until it reach the consumer.

References

Abdalla, M. O. M. and Abdel Nabi, S. Z. A. (2010). Evaluation of microbiological quality of Sudanese fermented dairy product Mish' during storage. Adv. J. Food Sci. Technol., 2: 155-158.

Abdalla, M. O. M. and Hussain, S. I. K. (2010). Enumeration and identification of microflora in roub, A Sudanese traditional fermented dairy product. Br. J. Dairy Sci., 1: 30-33.

Abdalla, W.M. and El Zubeir, I.E.M. (2006). Microbial hazard associated with fermented milk (roub and mish) processing in Sudan. Int. J. Dairy Sci., I (1): 21- 26.

Abdel Gadir, M.K.H.; El-bakri, J.M. and El Zubeir, I.E.M. (2013). The physicochemical and microbiological quality of the Sudanese yoghurt Mish produced by traditional and modernized methods. International Journal of Dairy Technology, 10: 1471- 0307.

Adams, M. and Mitchell, R. (2002). Fermentation and pathogen control: A risk assessment approach. International Journal of Food Microbiology,79:75-83.

Adams, M.R. and Nicolaides, L. (2008). Review of the sensitivity of different food borne pathogens to fermentation. Food Control, 8: 227-239.

Al-kadamany, E.I.; Toufeili, M.; Khattar, Y.; Abou- Jawdeh, S. and Harakeh, T.H. (2002). Determination of shelf life of concentrated yoghurt (labneh) produced by in-bag straining of set yoghurt using hazard analysis. J. Dairy Science, 85 (5): 1023-1030.

Al-Tahiri, R. (2005). Comparison on microbial conditions between traditional dairy products sold in Karak and same products produced by modern dairies. Pak. J. Nutr., 4(5): 345-348.

Attita Allah, A.H.; El Zubeir, I.E.M. and El Owni, O.A.O. (2011). Microbiological quality of set yoghurt from reconstituted whole and mixed milk powder. Annals of Food Science and Technology, 12(1): 63-70.

Benkerroum, N. and Tamime, A.Y. (2004). Technology transfer of some Morocan traditional dairy products (lben, jben and smen) to small industrial scale. Food Microbial., 21: 399-413.

Beukes, E.M.; Bester, B.H. and Moster, J.F. (2001). The microbiology of South African traditional fermented milk. Int. J. Food Microbial. , 63: 189-197.

Carbo, R. M.; Lanciottl, R.; Albenzio, M. and Sinigaglia, M. (2001). Occurrence and characterization of yeasts isolated from milk and dairy products of Apulia region. Int. J. Food Microbial. ,69: 147- 152.

Chammas, G.I.; Saliba, R.; Corrieu, G. and Béal, C. (2006). Characterisation of lactic acid bacteria isolated from fermented milk "laban". Int. J. Food Microbiol., 110: 52-56.

Christen, G.L.; Davidson, P.M.; McAllister, J.S. and Roth, L.A. (1992).

Coliform and other indicator bacteria. In: Standard method for the examination of dairy products. Edited by Marshall, R.T American Public Health Association, 16th ed., Washington D.C., pp 247-267.

El-Baradei, G.; Delacroix-Buchet, A. and Ogier, C.J. (2008). Bacterial biodiversity of traditional Zabady fermented milk. Int. J. Food Microbial., 121:295-301.

El bakri, J.M. and El Zubeir, I.E.M. (2009). Chemical and microbiological evaluation of plain and fruit yoghurt in Khartoum State, Sudan. International Journal of Dairy Science, 4: 1-7.

El Zubeir, I.E.M.; Abdalla, W.M. and El Owni, O.A.O. (2005). Chemical composition of fermented milk (Rob & Mish) in Sudan. Food Control, 16: 633-637.

El-Sayed, S.M. and Youssef, A.M. (2019). Potential application of herbs and spices and their effects in functional dairy products. Heliyon, 5(6): e01989.

Frank, J.F.; Christen, G.L. and Bullerman, L.B. (1992). Tests for groups of microorganisms. In: Standard Methods for the Examination of Dairy Products, 16th edition, Ed., R.T. Marshall. American Public Health Association. Washington, DC, USA.

Franz, C.M.A.P.; Holzapfel W.H. and Stiles, M.E. (1999). Enterococci at the crossroads of food safety. International Journal of Food Microbiology, 47: 1-24.

Gadage, T.H.; Mutukumira, A.N. and Narrhus, I.A. (2000). Enumeration and identification of yeast isolated from Zimbabwean traditional fermented milk. Int. Dairy J., 10: 456-466.

Gadage, T.H.; Mutukumira, A.N. and Narrhus. J.A. (2001). The growth and interaction of yeast and lactic acid bacteria isolated from Zimbabwean naturally fermented milk in UHT milk. Int. Food Microbial., 68:21-32.

Gaggia, F.; Di Gioia, D.; Baffoni, L. and Biavati, B. (2011). The role of protective and probiotic cultures in food and feed and their impact on food safety. Trends in Food Science and Technology, 22: S58-S66.

Gemechu, T. (2015). Review on lactic acid bacteria function in milk fermentation and preservation. African Journal of Food Science, 9(4):170-175.

Houghtby, G.A.; Maturin, L.J. and Koenig E.K. (1992). Microbiological Count Methods. In: Standard Methods in Examination of Dairy Products. Marshall, R.T. (ed.), 16th edition, Port City Press, Baltimore, Washington.

Jay, M.J. (1986). Modern Food Microbiology, 3rd ed, Van. Nostrand Reinhold Company, USA, New York.

Kaptan, B. and Sivri, G.T. (2018). Utilization of Medicinal and aromatic plants in dairy products. .J Adv. Plant Sci., 1: 207

Lore, A.T.; Mbugua, K.S. and Wango, H.J. (2005). Enumeration and identification of microflora in suusac, a Kenyan traditional fermented camel milk product. Lebensm-Wiss.u. - Technol., 38: 125-130.

Marshall, R.T. (1992). Media. In: Standard Methods for the Examination of Dairy Products. Marshall, R. T (ed), 16th edition, Port City Press, Baltimore, Washington.

Mayoral, M.B.; Martin, Rjsanz, A.; Hemarndez, P.E.; Gonzalez, 1. and Carcia T. (2005). Detection of Klogveromyces marxinus and other spoilage yeasts in yogurt using a PCR culture technique. Int. J. Food Microbial., 105:27-34.

Mohammed, E.E.B. and El Zubeir, I.E.M. (2011). Chemical composition and microbial load of set yoghurt from fresh and recombined milk powder in Khartoum State. International Journal of Dairy Science, 6 (3): 172-180.

Rattanachaikunsopon, P. and Phumkhachorn, P. (2010). Lactic acid bacteria: Their antimicrobial compounds and their uses in food production. Annals of Biological Research, 1 (4): 218-228.

Richardson, H.G. (1985). Standard Methods for Examination of Dairy Products, 5th ed., PP: 133– 150 American Public Health Association, Washington D.C.

Ross, R.P.; Morgan, S. and Hill, C. (2002). Preservation and fermentation: Past, present and future. International Journal of Food Microbiology, 79: 3-16.

Samolada, M.; Litopoulou-Tzanetaki, E.; Xanthopoulos, V. and Tzanetakis, N. (1998). Changes in microbial flora during manufacture of traditional fermented milk from ewe's milk. Food Microbial., 15: 43-50.

Suliman, E.S.K. and El Zubeir, I.E.M. (2016). Microbial loads of Gariss collected during movement and settlement of nomadic camel herders in Algadarif State, Sudan. Food Science and Nutrition Technology, 1(2):1-7.

Uzeh, E.R.; Ohenhem, E.R. and Rojugbokan, K.A. (2006). Microbiological and nutritional quality of dairy products: Nono and Wara. Nature and Science, 4: 37- 40.

Viljoen, B.C.; Lourens, H.A.; Ikalafeng, B. and Peter, G. (2003). Temperature abuse initiating yeast growth in yoghurt. Food Research International, 36(2): 193-197.