Bacteriological Evaluation of Yoghurt in Misurata City, Libya

ABUSHAALA A. F.[1], Alwoshesh M. M.[2]

[1] Microbiology Department, Faculty of Science, Misurata University

[2] Microbiology Department, Faculty of pharmacy, Misurata University

Abstract

Laban raieb is one of the important fermented milk consumed by different ages in Middle east and throughout the world and characterized by high nutritive value, maintenance of normal intestinal microflora. The aim of this study to evaluate the bacterial content of yoghurt in Misurats's markets, 20 samples have been collected for plain, fruit, and flavored yoghurt. These samples were transported to the lab to perform microbial examinations. First of all, we measured the pH in the yoghurt samples which were 4.58 ± 0.27 in plain yoghurt, 4.61± 0.23 in fruit yoghurt and 4.61± 0.22 in flavored yoghurt. we investigated the presence of coliform species in each type of the yoghurt samples which were *Enterobacter cloacae, Citrobacter freundii*, *Enterobacter agglomerans, Klebsiella panticola, E. coli* and *Enterobacter aerogenes* in the plain yoghurt samples, While *Klebsiella panticola*, *Klebsiella oxytoca, Klebsiella pneumonia, E. coli* and *Enterobacter areogenes* present in the flavored yoghurt samples. However, in the fruit yoghurt samples we isolated *Klebsiella oxytoca, Klebsiella pneumonia*, and *E. coli* only. Besides coliform, we were able to isolate *Staphylococcus aureus* and *Staphylococci epidermidis* of all the yoghurt samples.

Keywords: Plain yoghurt, Flavored yoghurt, Fruit yoghurt

Introduction

A large variety of fermented food products are produced and consumed around the world. Fermentation serves to preserve raw foods and increases the diversity of available food products **[27, 32].** Cereals, oil seeds, milk, fish, meat and vegetables are raw foods that are fermented world-wide **[19, 23].** As part of the human diet, fermented foods can play an important role in maintaining a healthy intestinal tract and increase the acceptability of dairy products to lactose intolerant individuals **[8, 9].**

Natural or plain yoghurt is the traditional type of fermented milk with a sharp acidic taste, while fruit yoghurt is made by the addition of fruits and sweeting agents to natural yoghurt **[29**].

Fruit yoghurt usually have stabilizers incorporated to reduce whey separation during distribution many of the stabilizers are complex carbohydrates which providing "a bulking agent" so stimulating intestinal peristalsis and avoiding some of the risk of colonic malfunction. It also absorb some of the potentially toxic chemicals that may be formed in the large intestine as a result of bacterial action. This unavailable carbohydrates acting to further delay the diffusion of sugar to the intestinal wall that could help lactose intolerant patients and those prone to post prandial hyperglycemia **[30, 36].**

MATERIALS AND METHODS

Collection of samples:

A total of 60 random samples plain, flavored and fruit yoghurt (20, 20 and 20 respectively) collected from different localities in Misurata city, from 10-1-2017 to 4-3-2017.

Preparation of samples:

On arrival to the laboratory each sample was perfectly mixed before being divided into two sub-samples. The first one used for determination of pH, while the second was examined bacteriologically.

Determination of pH: *Standard method* [5].

Was used to determine pH by using pH meter (Jenway- model:3505, Made in UK).

Microbiological examination:

Preparation of serial dilution [5].

After thoroughly mixing of a sample, weigh 1 ml. were transferred into sterile, wide mouthed container, containing 9 ml of sterile water, shake well until a homogeneous dispersion of 1:10 dilution obtained, withdraw appropriate amounts of this dilution for plating or further decimal dilution.

Total bacterial count: [20].

1. Using aseptic technique, transfer 1 ml of sample to a 9 ml sterile distilled water to made 10^{-1} dilution.
2. Immediately after the 10^{-1} dilution, it has been shaken, uncap it and aseptically transfer 1 ml to a second 9ml sterile distilled water. Since this is a 10^{-2} dilution, and else to produce a 10^{-14} dilution.
3. Then Shook the 10^{-1} diluted sample again and aseptically transfer 1.0 ml to one petri plate and 1 ml to another petri plate. All the samples were done in similar way.
4. Removed one agar pour tube from the 48 to 50° C water bath and aseptically pour the agar into it. The agar and sample are immediately mixed gently moving the plate in a figure-eight motion or a circular motion while it rests on the tabletop.
5. After the pour plates have cooled and the agar has hardened, they are inverted and incubated at 37° C for 24 hours.
6. Calculated the number of bacteria (C.F.U) per milliliter of sample by dividing the number of colonies by the dilution factor multiplied by the amount of specimen added to liquefied agar.

Enumeration and isolation of Coliforms (MPN\ml): [5].

A series of 3 fermentation tubes containing 9 ml. MacConkey's broth (Oxoid, 1990) with inverted Durham's tube were inoculated with 1 ml for each of the previously prepared decimal dilutions as well as from the original sample after thorough mixing.

Inoculated and control tubes were incubated at 37°C \ 24 hours.

API 20E kits for identification of Enterobacteriaceae (Biomerieux, France)

1. Preparation of the strip:

The incubation box (tray and lid) was prepared by distributing 5 ml of distilled water into the honey-combed wells of the tray to create a humid atmosphere.

The strain reference was recorded on the elongated flap.

The strip was removed from individual packaging and placed in the incubation box.

2. Preparation of the inoculums

The organism was cultivated onto nutrient agar 18-24 hours at 37°C.

About four to five colonies were transferred to the API 20E.

The turbidity was then adjusted to match a McFarland 0.5 barium sulphate standard ($1.5x10^8$ CFU).

3. Inoculation of the strip

The micro tube were filled with the inoculated API 20E medium by using a pipette, then the capules of ADH, LDC, ODC and URE were filled with mineral oil to ensure anaerobic condition, after that the capules of CIT, VP and GEL were completely filled by suspension and placed in, then Closed the incubation box and incubate at 37^{o}C for 18-24 hours.

4. Reading and interpretation

Read the strip after 18-24 hours at 37^{o}C, the following reagents were added as the follows:

VP: 1 drop **VP1** and 1 drop **VP2** reagents

TDA: 1 drop **TDA** reagent

IND: 1 drop Kovacs reagent **\James** reagent

Enumeration and isolation of Staphylococci: [5]

0.1 ml from the previously prepared decimal dilutions of the examined samples was transferred and evenly spread on the dry surface of Mannitol salt agar (Oxoid, 1998) medium plates using a sterile bent glass rod. Inoculated plates were incubated at 37°C for 24 hours and Staphylococci count were calculated and recorded.

Identification of Staphylococcus by Biochemical reactions: [10].

1. Catalase test:

A loopful of the tested culture was suspended in a drop 3% Hydrogen peroxide (H_2O_2) on a slide. Evolution of bubbles within one minute was recorded as positive.

2. Coagulase tube test:

In a sterile tube, 0.1 ml of 24 hours nutrient Cultures were transferred to 0.3 ml of plasma. The tubes were incubated at 37°C for 4 hours before being examined for clot formation. Extent of coagulase reaction (1-4+) was recorded. Tubes were left at room temperature for an additional 20 hours and then re-examined for clot formation. The extent of coagulation of the plasma was reported after 4 and 24 hours.

Statistical analysis: Done by ANOVA one way and t- test

Results and Discussion

The results in table (1) and figure (1) show that the pH in examined plain yoghurt samples was ranged from 4.2 to 5.1 with a mean value of 4.58 ± 0.27 and in examined fruit yoghurt samples was ranged from 4.2 to 5.1 with a mean value of 4.61± 0.23.

Table (1): Statistical analytical results of pH in examined yoghurt samples.

Product	No.	Minimum	Maximum	Mean ± S.D	p. value
Plain	20	4.2	5.1	4.58 ± 0.27	0.902
Fruit	20	4.2	5.1	4.61± 0.23	
Flavored	20	4.3	5.0	4.61± 0.22	

While in examined flavored yoghurt samples was in a range from 4.3 to 5.0 with a mean value of 4.61± 0.22. Nearly similar data were obtained by **[22, 24].**

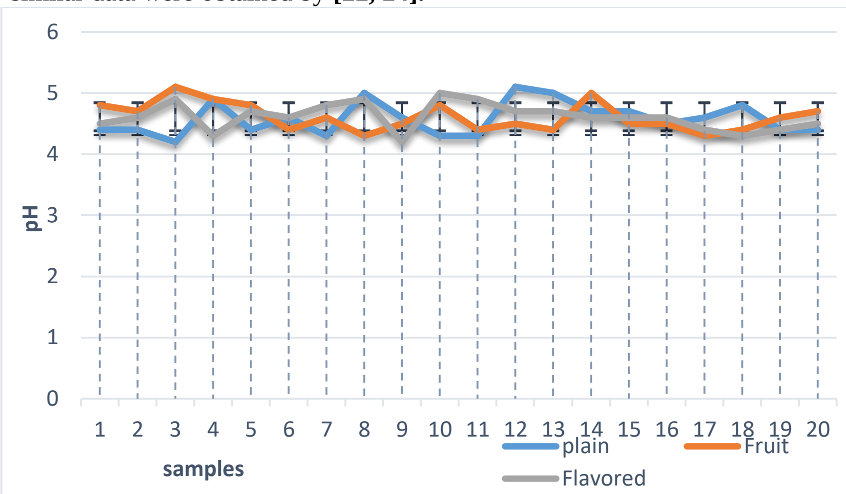

Figure (1): pH in examined yoghurt samples

A careful inspection of table (2) reveals that according to frequency distribution of examined yoghurt samples based on pH. The highest frequency distribution of examined plain yoghurt samples (25%) lies within the range of 4.31- 4.4, while the highest frequenc0y distribution of examined fruit yoghurt samples (20%) lies within the range of 4.31- 4.4 and 4.41- 4.5, while the highest frequency distribution of examined flavored yoghurt samples (25%) lies within the range of 4.51- 4.6.

Table (2): Frequency distribution of examined yoghurt samples based on their pH

Intervals	Plain Yoghurt		Fruit yoghurt		Flavored yoghurt	
	No.	%	No.	%	No.	%
4.21-4.3	4	20%	2	10%	3	15%
4.31-4.4	5	25%	4	20%	2	10%
4.41-4.5	1	5%	4	20%	2	10%
4.51-4.6	3	15%	2	10%	5	25%
4.61-4.7	2	10%	2	10%	3	15%
4.71-4.8	1	5%	3	15%	1	5%
4.81-4.9	1	5%	1	5%	3	15%
4.91-5.0	2	10%	1	5%	1	5%
5.01-5.1	1	5%	1	5%	0	0%
Total	20	100%	20	100%	20	100%

The results in table (3) and figure (2) reveal that the total bacterial count in examined plain yoghurt samples was ranged from $1.32X10^2$ to $1.30X10^6$ with a mean value of $9.63X10^4 \pm 2.88X10^5$ and from $1.27X10^2$ to 1.97 $X10^6$ with a mean value of $1.67X10^5 \pm 4.69X10^5$ for examined fruit samples.

Table (3): Statistical analytical results of total bacterial count\ml in examined yoghurt samples.

Examined yoghurt samples	No. of exam. samples	Positive samples		Count\ml		Mean ± S.D	p. Value
		No.	%	Min.	Max.		
Plain	20	20	100%	$1.32X10^2$	$1.30X10^6$	$9.63X10^4 \pm 2.88X10^5$	0.888
Fruit	20	20	100%	$1.27X10^2$	$1.97\ X10^6$	$1.67X10^5 \pm 4.69X10^5$	
Flavored	20	20	100%	$1.19X10^2$	$5.8X10^6$	$3.9X10^5 \pm 1.3X10^5$	

While in examined flavored yoghurt samples was ranged from $1.19X10^2$ to $5.8X10^6$ with a mean value of $3.9X10^5 \pm 1.3X10^5$. These results were comparable with **[12].** Results confirm no differences in the 3 types of yoghurt samples which may due to the use of the same starter for the products.

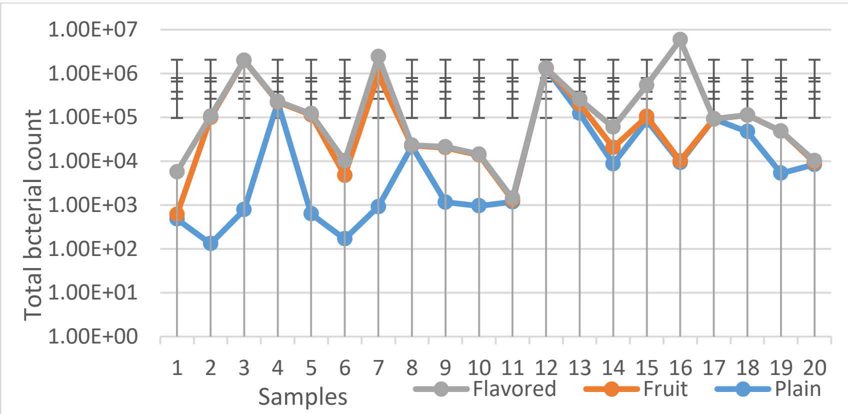

Figure (2): Total bacterial count\ml in examined yoghurt samples

Figure (3, 4, 5) show positive relation between pH and total bacterial count in the three products.

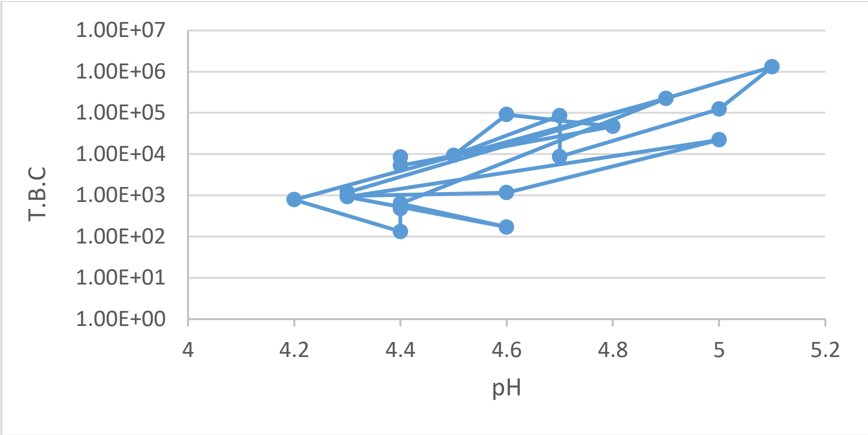

Figure (3): Relation between pH & T.B.C in plain yoghurt

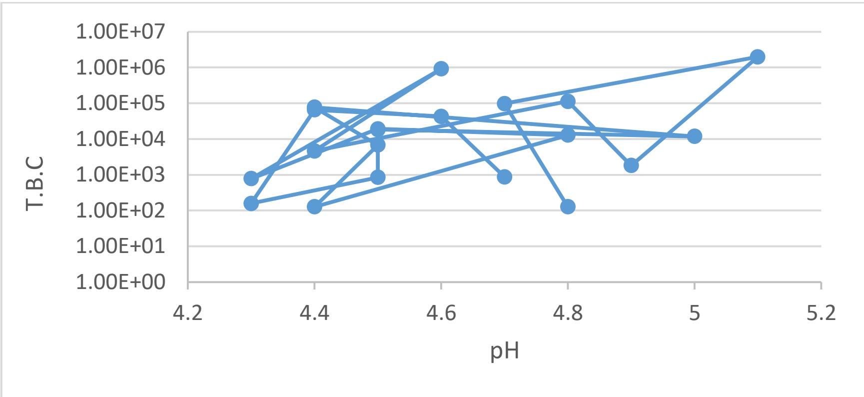

Figure (4): Relation between pH & T.B.C in Fruit yoghurt

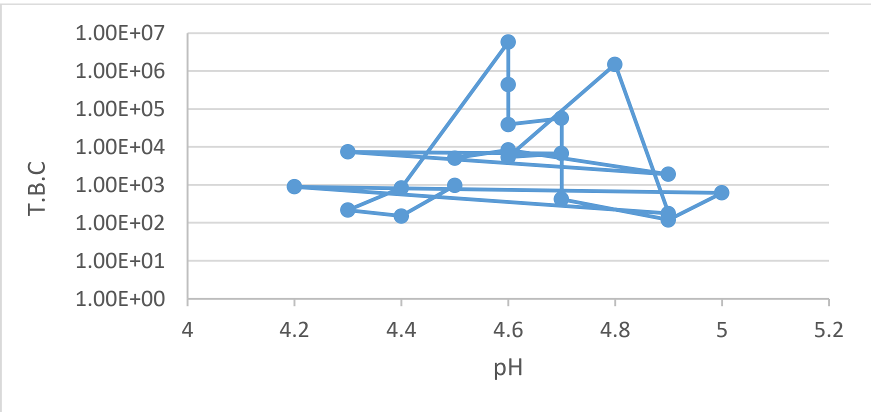

Figure (5): Relation between pH & T.B.C in Flavored yoghurt

The results in table (4) displays that nearly about (35%) of examined plain yoghurt samples based on their total bacterial count lies within the range of $1.0X10^2$-$1.0X10^3$ and the majority (40%) of examined fruit yoghurt samples lies within the range $1.01X10^4$-$1.0X10^5$ and nearly the half of examined flavored yoghurt (45%) lies within the range $1.0X10^2$-$1.0X10^3$.

Table (4):Frequency distribution of examined yoghurt samples based on their total bacterial count.

Intervals	Plain yoghurt samples		Fruit yoghurt samples		Flavored yoghurt samples	
	No.	%	No.	%	No.	%
$1.0X10^2$-$1.0X10^3$	7	35%	6	30%	9	45%
$1.01X10^3$-$1.0X10^4$	6	30%	3	15%	6	30%
$1.01X10^4$-$1.0X10^5$	4	20%	8	40%	2	10%
$1.01X10^5$-$1.0X10^6$	2	10%	2	10%	1	5%
$1.01X10^6$-$1.0X10^7$	1	5%	1	5%	2	10%

The results in table (5) shows that total coliform count (MPN\ml) of examined plain yoghurt was only 20% of samples were contaminated and ranged from $2.30x10^3$ to $4.0X10^5$ with a mean value of $1.09X10^5 \pm 1.93X10^5$.

Table (5): Statistical analytical results of Coliform count\ml in examined yoghurt samples.

Examined yoghurt samples	No. of examined Samples	Positive samples		Count\ml		Mean ± S.D	p. value
		No.	%	Min.	Max.		
Plain	20	4	20%	$2.30x10^3$	$4.0X10^5$	$1.09X10^5 \pm 1.93X10^5$	0.104
Fruit	20	2	10%	$2.10X10^4$	$1.40X10^5$	$8.05X10^4 \pm 8.41X10^4$	
Flavored	20	2	10%	$1.10X10^3$	$2.30X10^4$	$1.21X10^4 \pm 1.54X10^4$	

while in fruit yoghurt 10% of samples were positive and ranged from $2.10X10^4$ to $1.40X10^5$ with a mean value $8.05X10^4 \pm 8.41\ X10^4$, While in flavored yoghurt also 10% of samples were contaminated and ranged from $1.10X10^3$ to $2.30X10^4$ with a mean value $1.21X10^4 \pm 1.54X10^4$. These findings substantiate those reported by **[11, 25, 33].** Lower values were obtained by **[1, 7, 35].** While higher counts were declared by **[3, 13, 15, 18, 28]. [26]** Concluded that 97% of examined yoghurt samples were free from coliform bacteria, while **[31]** reported that Enterobacrteiaceae failed to be detected in examined yoghurt samples.

The results listed in table (6) reveals that the highest frequency distribution based on their coliform counts of examined plain yoghurt samples (50%) lies within the range of $1.01X10^4$-$1.0X10^5$, in examined fruit yoghurt samples (50%) lies within the range $1.01X10^4$-$1.0X10^5$ and $1.01X10^5$-$1.0X10^6$ also in examined flavored yoghurt samples (50%) lies within the range $1.0X10^3$-$1.0X10^4$ and $1.01X10^4$-$1.0X10^5$.

Table (6): Frequency distribution of examined Yoghurt samples based on their coliform counts.

Interval	Plain yoghurt		Fruit yoghurt		Flavored yoghurt	
	No.	%	No.	%	No.	%
$1.0X10^3$-$1.0X10^4$	1	25%	0	0%	1	50%
$1.01X10^4$-$1.0X10^5$	2	50%	1	50%	1	50%
$1.01X10^5$-$1.0X10^6$	1	25%	1	50%	0	0%
Total	4	100%	2	100%	2	100%

The results reported in table (7) and figure (6) present that *Enterobacter cloacae, Citrobacter freundii*, *Enterobacter agglomerans*, *Klebsiella panticola, E. coli* and *Enterobacter aerogenes* were isolated from the examined plain yoghurt samples in the percentage of 37.5%, 12.5%, 12.5%, 12.5%, 12.5% and 12.5% respectively.

Table (7): Incidence of isolated Coliforms in examined yoghurt samples.

Isolates	Yoghurt samples					
	Plain yoghurt		Fruit yoghurt		Flavored yoghurt	
	Positive	%	Positive	%	Positive	%
Citrobacter freundii	1	12.5%	0	0%	0	0%
Enterobacter agglomerans	1	12.5%	0	0%	0	0%
Enterobacter cloacae	3	37.5%	0	0%	0	0%
Klebsiella panticola	1	12.5%	0	0%	1	20%
Klebsiella oxytoca	0	0%	1	25%	1	20%
Klebsiella pneumonia	0	0%	1	25%	1	20%
E. coli	1	12.5%	2	50%	1	20%
Enterobacter areogenes	1	12.5%	0	0%	1	20%
Total	8	100%	4	100%	5	100%

While in fruit yoghurt only *Klebsiella oxytoca*, *Klebsiella pneumoniae* and *E. coli* were isolated by the percentage of 25, 25 and 50% respectively. While in flavored yoghurt *Klebsiella panticola*, *Klebsiella oxytoca*, *Klebsiella pneumonia*, *E. coli* and *Enterobacter areogenes* were isolated by the percentage 20, 20, 20, 20 and 20% consecutively. The results obtained are nearly similar to that reported by **[2, 7, 14, 17, 37].** On other hand, **[37]** said *Citrobacter* spp. was not found in any of the analyzed samples**.**

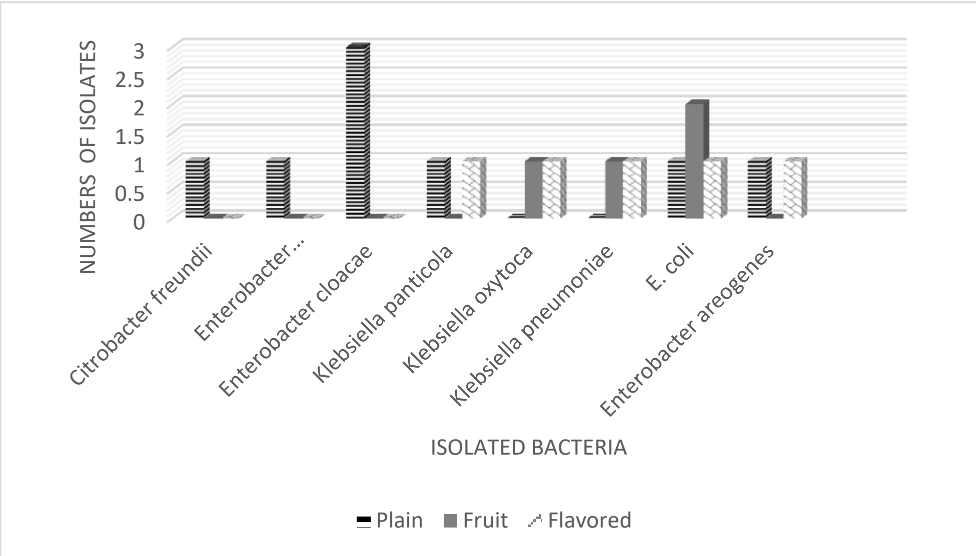

Figure (6): Incidence of isolated Coliforms in examined yoghurt samples

Higher levels of Coliforms (10^6 or more) believed to be necessary for food borne illness to occur **[39].** Certain numbers of *Citrobacter* have been suspected to cause enteric infection **[38].** *Citrobacter freundii* has been found among urinary and other pyogenic infections in humans **[40].** Some strains of *Klebsiella* and *Enterobacter* species had been implicated in acute and chronic diarrhea **[41].** The results tabulated in table (8) revealed that (15%) of examined plain yoghurt samples were contaminated by *Staphylococci*, the level of contamination was ranged from $1.13X10^2$ to $8.30X10^3$ with a mean value of $2.92X10^3$ ± $4.65X10^3$, also (15%) of examined fruit yoghurt samples were contaminated by *Staphylococci*, the level of contamination was ranged from $7.30X10^2$ to $4.30X10^3$ with a mean value of $2.00X10^3$ ±$1.99X10^3$ and the same percentage (15%) of examined flavored yoghurt samples were contaminated by *Staphylococci*, the level of contamination was ranged from $5.20X10^2$ to $6.70X10^3$ with a mean value of $2.59X10^3$ ± $3.55X10^3$.

Table (8): Statistical analytical results of Staphylococci count\ml in examined yoghurt samples.

Examined yoghurt samples	No. of examined samples	Positive samples		Staph count\ml		Mean ± S.D	p. Value
		No.	%	Min.	Max.		
Plain	20	3	15%	$1.13X10^2$	$8.30X10^3$	$2.92X10^3 \pm 4.65X10^3$	0.954
Fruit	20	3	15%	$7.30X10^2$	$4.30X10^3$	$2.00X10^3 \pm 1.99X10^3$	
Flavored	20	3	15%	$5.20X10^2$	$6.70X10^3$	$2.59X10^3 \pm 3.55X10^3$	

The results reported in table (9) reveals that 66.66% of examined plain, fruit and flavored yoghurt samples lies in the range of $1.0X10^2$-$1.0X10^3$. Relatively similar results were obtained by **[1, 4, 6, 16, 17, 21]**, while higher values were reported by **[34].**

Table (9): Frequency distribution of examined yoghurt samples based on their Staphylococci count.

Intervals	Yoghurt samples					
	Plain yoghurt		Fruit yoghurt		Flavored yoghurt	
	No.	%	No.	%	No.	%
$1.0X10^2$-$1.0X10^3$	2	66.6%	2	66.6%	2	66.6%
$1.01X10^3$-$1.0X10^4$	1	33.3%	1	33.3%	1	33.3%
Total	3	100%	3	100%	3	100%

Table (10) and figure (7) show that the isolated Staphylococci, *Staphylococcus aureus* was detected in 40% of positive examined plain yoghurt samples while 60% of contaminated examined samples were by *Staphylococci epidermidis*.

Table (10): Incidence of isolated Staphylococci in examined yoghurt samples.

Isolated strain	Yoghurt samples					
	Plain yoghurt		Fruit yoghurt		Flavored yoghurt	
	Positive samples	%	Positive samples	%	Positive samples	%
Staphylococcus aureus	2	40%	1	25%	1	33.3%
Staphylococcus epidermides	3	60%	3	75%	2	66.6%
Total	5	100%	4	100%	3	100%

But in case of fruit yoghurt only 75% were contaminated by *Staphylcoccus epidermidis* while *staphylococcus aureus* detected in 25% of positive samples, while in examined flavored yoghurt samples 33.3% and 66.6% of samples were contaminated by *Staphylococcus aureus* and *Staphylococcus epidermides*.

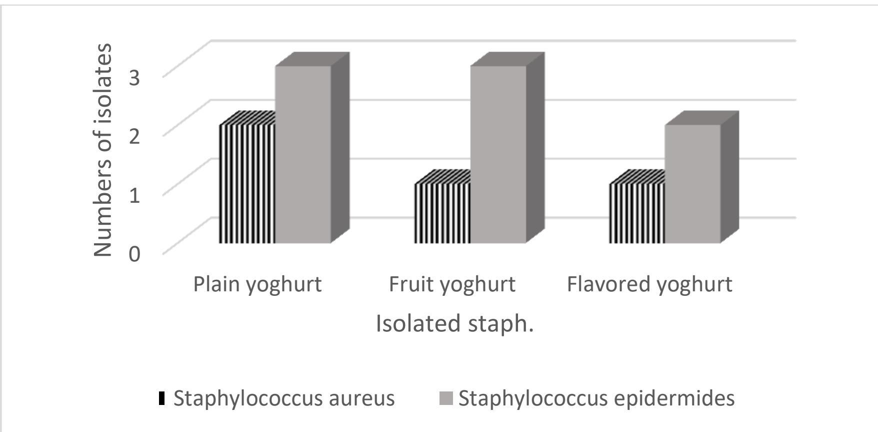

Figure (7): Incidence of isolated Staphylococci in examined yoghurt samples

Staph. aureus may be found in the eyes, throat and the intestinal tract. Therefore, nasal carriers and individuals whose hands and arms were infected with boils and carbuncles are dangerous sources of food poisoning **[43]**. *Staphylococcus aureus* is by far the most important human pathogen among the Staphylococci. Under certain circumstances, *Staph. aureus* may cause a variety of infectious diseases, ranging from relatively benign skin infectious diseases, to life threating systemic illness. Enterotoxin producing Staphylococci are the leading cause of food borne illness throughout the world. *Staph. aureus* possess a public health hazard due to production of thermostable enterotoxin that is responsible for food poisoning.

Leucocidin, Enterotoxin (A to E) and toxic shock syndrome toxin, TSST, all were produced by Staphylococcus aureus, and Enterotoxins are heat stable molecules that are responsible for the clinical feature of Staphylococcal food poisoning. Ingestion of preformed enterotoxins in food, results in vomiting and diarrhea within 2 to 8 hours, sometimes followed by collapse **[42].** Although *Staph. aureus*, the coagulase positive, is the most dangerous, but nowadays, coagulase negative Staphylococci have been recognized as important agents of human disease which include nosocomial and community-acquired urinary infections, bacteremia in compromised hosts, osteomyelitis and post-surgical infections.

Table (11) reported that just 3 plain yoghurt samples were unacceptable with the total bacterial count and Staphylococci, 4 plain yoghurt samples were unaccepted with coliform, while in examined fruit yoghurt samples there were 3 samples unaccepted with total bacterial count and Staphylococci and only 2 samples unacceptable with coliform in compare with Libyan standard.

And in flavored yoghurt samples most of the samples were going with the Libyan standard (total bacteria count, staphylococci, coliform and yeast) which were (17- 17 -18) respectively accepted with the Libyan standard.

Table (11): Comparison between Libyan standard criteria and examined yoghurt samples.

	Standard	Plain samples				Fruit samples				Flavored samples			
		Unacceptable		Acceptable		Unacceptable		Acceptable		Unacceptable		Acceptable	
		No	%	No.	%	No.	%	No.	%	No.	%	No	%
Total Bacterial count	$<10^5$	3	15	17	85	3	15	17	85	3	15	17	85
Staphylococci	Free	3	15	17	85	3	15	17	85	3	15	17	85
Coliforms	Free	4	20	16	80	2	10	18	90	2	10	18	90

REFERENCES

1. Al-Ashmawy, A.M.; El-Sayed, M.; Amer, I.H. and Mansour, M.A. (1991): Hygienic status of plain yoghurt (yoplait). Zag. Vet. J. 19 (3): 639-645.
2. Al-Ashmawy, A.M.; Mohamed, S.M.; Shelaih, M.A. and Hafez, R.S. (1982): Incidence of enteropathogenic E.coli in Egyptian F.M. Zagazig Vet. J. Vol. 4: 297.
3. Al-Hadethi, H.A.; Hammed, D.A. and Al-Kananny, E.K. (1992): Incidence of coliform bacteria in milk products in Mosul city. Iraqi. J. of Vet. Sci. 5 (2): 151-158.
4. Ali, M.M.; Nahed, M.; Wahba; Seham and Farag, A. (2004): Microbiological evaluation of Assuit market yoghurt through the shelf life time in refrigerator. Assuit Vet. Med. J. 50 (101): 64-74.
5. American Public Health Association "A.P.H.A." (1992): Standard methods for the examination of dairy products. 16th Ed., New York.
6. Arnott, D.R.; Duitschaever, C.L. and Bullock, D.H. (1974): Microbiological evaluation of yoghurt produced commercially in Ontario. J. of Milk and Food Techn. 37 (1): 11-13.
7. Bahout, A.A. and El-Shawaf, A.M. (1999): Evluation of some chemical and bacteriological aspects of commercial yoghurt in Sharkia province. J. Vet. Med. Res. 1 (1): 1-9.
8. Bernardeau, M.; Vernoux, J.P.; Henri-Dubernet, S. & Guéguen, M. (2008): Safety assessment of dairy microorganisms: the Lactobacillus genus. International Journal of Food Microbiology, 126, 278-285.
9. Brown-Esters, O.; McNamara, P. & Savaiano, D. (2012): Dietary and biological factors influencing lactose intolerance. International Dairy Journal, 22, 98-103.
10. Cowan, S.T. and Steel, K.J. (1974): Manual for identification for medical bacteria. 2nd Cambrige Uni. Press, England.
11. El-Barbary, M.M. (1999): Occurrence of enteric microorganisms in some dairy products in Sharkia Governorate. M.V.Sc. Thesis Fac. Vet. Med., Zag. Univ.
12. El-Diasty, E.M. and El- Kaseh. R.M. (2008): Microbiological monitoring of raw milk and yoghurt samples collected from El-Beida city. Libya.
13. El-Eisawi, Hasham Nouh Eisa (2000): Prevalence of enteropathogenic Escherichia coli in raw milk and some dairy products with special reference to E.coli O157:H7. M.Sc. Thesis, Depart. Of Hygiene, Control of Milk, its products, Cairo Univ. Fac. Vet. Med. 619: 637.
14. El-Jendy,I.M. (2004): Studies on enterobacteriacae in milk and dairy products. M.V.Sc. Thesis, Fac. Vet. Med., Zag. Univ., Egypt.
15. El-Kholy, A. (1986): Indicator organisms as indices of sanitary quality for milk and some products. Ph. D. Thesis, Fac. Vet. Med. Alex. Univ.
16. El-Nockrashy, S.; Naguib, M.M.; Sabbour, M.M. and Naguib, K. (1978): Occurrence of coagulase positive staphylococci in Egyptian raw milk and dairy products, ice cream and zabady, REs. Bull. Fac. Agric. Ain Shams, Univ. No. 945: 11.
17. El-Shinawy, S.H. (1987): Microbiological studies on fermented milks. Ph.D. Thesis Fac. Vet. Med. Zag. Univ. Egypt.
18. Hafez, N.M. (1984): Incidence and public health importance of coliforms with special reference to enteropathogenic serotypes of E.coli in milk and some dairy products. M.V.Sc. Thesis, Fac. Vet. Med. Cairo Univ.
19. Iwuoha, C.I. & Eke, O.S. (1996): Nigerian indigenous fermented foods: their traditional process operation, inherent problems, improvements and current status. Food Research International, 29, 527-540.
20. Jackie Reynolds, Richland College, BIOL 2420.
21. Kahlon,S.S. and Grover, N.K. (1984): Incidence of Staph. in milk products sampled from ludhiana. Indian. J. of Dairy Sci. 37 (4): 381-383. (Fd. Sci. Technol. Abst. Vol. 18 (1986) No. 4.
22. Lalas, M. and Mantes,A. (1985): Microbiologial quality. Deltia Ethnikes Epitropes Galaktos Ellados. 2 (1): 28-29. Dairy Sci. ABst. 4, 9.
23. Lee, C-H. (1997): Lactic acid fermented foods and their benefits in Asia. Food Control, 8, 259-269.
24. Lopez, C.; Rodriguez, V.; Medina, L.M.; Barrios, M.J. and Jordano, R. (1993): Microbiological quality of French yoghurts commercialized in Spain. Zentralbl Veterinar med B. 40 (9-10): 727-9.
25. Mansour, M.A.; Bahout, A.A.; Al-Abeedy, A.A. and El-Barbary, M.M. (1999): Occurrence of some enteric microorganisms in some dairy products in Sharkia governorate. Beni Suef Vet. Med. J. 9 (3): 387-398.
26. Molska, I.; Nowosielka, R. and Frelik, I. (2003): Changes in microbiological quality of kefir and yoghurt on the warsaw market in the years (1995-2001). Rocz. Panstw. Zakl Hig. 54 (2): 145-52.

27. Motarjemi, Y. (2002): Impact of small scale fermentation technology on food safety in developing countries. *International Journal of Food Microbiology.*

28. Moustafa, M.K.; Ahmed, A.A.H. and Abdel-Hakiem, E.H. (1988): Sanitary condition of commercially available yoghurt in Assuit city. Assuit vet. Med. J. 20 (39): 99-103.
29. Potter, N.A. and Hotchkiss, J.H. (1995): Food Science. 5^{th} ed., Chapman and Hall. London, England.
30. Robinson, R.K. and Khan, P. (1978): Plant foods for man, 2: 113.
31. Rodriguez, A.C.; Pintor, D.; Gonzalez, P.A.; Alvarez, H.R. and Hardisson, D.T.A. (1990): Microorganisms of food hygiene interest in commercial yoghurt in the canary islands. Alimentaria 212, 55-58. Dairy Sci. Abst. 57: 78-92.
32. Ross, R.P.; Morgan, S. and Hill, C. (2002): Preservation and fermentation: past, present and future. *International Journal of Food Microbiology.*
33. Saad, N.M.; Moustafa, M.K. and Ahmed, A.H. (1987): Mirobiological quality; of yoghurt produced in assuit city. Assuit Vet. Med. J. Egypt. 19 (37): 87-91.
34. Salih, A.W.; Abdullah, J.M.; Hameed, A.; Abdul Karim, N. and Abdul, A.D. (1981): Studies on the hygienic quality and nutritive status of market yoghurt in Baghdad (edited by Collins, J.D. and Hannan, J.), 287.
35. Saudi, A.S.; Morgan, S.D.; Hafez, R.S. and El-Essawy (1988): Aspects on the microbial status in fruit yoghurt. Zag. Vet. J. 16 (2): 130-140.
36. Tamime, A.R. and Robinson, R.K. (1985): Yoghurt science and Technology. Copyright Pergamon Press. Ltd.
37. Tankoano, A.; Kabore, D.; Savadogo, A.; Soma, A. and Fanou-Fogny, N. (2016): Evaluation of microbiological quality of raw milk, sour milk and artisanal yoghurt from Ouagadougou, Burkina Faso.
38. Cruickshank, R.; Duguid, J.P.; Marmion, B.P. and Swain, R.H.A. (1975): Medical Microbiology. 12^{th} Ed., Vol. II, Churchill Livingstone Ltd., Edinburgh, London and New York.
39. Doyle, M.P. and Cliver, D.O. (1990): E.coli. In: Ed. Food borne diseases. Academic Press, Inc. London, 209-215.
40. Mackie, T.J. and MacCarteny, J.E. (1962): Handbook of practical bacteriology, 10^{th} Ed. E85. Livingstone Ltd., London.
41. Twedt, R.M. and Boutin, B.K. (1978): Potential public health significance of non-Escherichia coli, coliforms in food. *J. Food. Prot.*
42. Hobbs, B.C. and Roberts, D. (1993): Food poisoning and food hygiene. 6^{th} Ed., Singular publishing group. Inc., San Diego, California.
43. Jay, J.M. (1992): Modern food microbiology. 4^{th} Ed. Van Nostrand, Reinhold, New York.

المواصفات القياسية الليبية للبن الحامض 357/ 2000

المواصفات القياسية الليبية للزبادي المعامل حراريا بعد التخمر 559/ 2005