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EVALUATION OF HYGIENIC QUALITY OF LARGE SCALE MANUFACTURED YOGHURT SOLD IN SHARKIA GOVERNORATE

(With 14 Tables)

By

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تقييم الحالة الصحية للزبادي المصنع علي نطاق واسع والمباع فى محافظة الشرقية

نسمة ابراهيم صالح يسن البياع ، صلاح فتحى احمد عبد العال ، محمد احمد حسن منصور ، ابراهيم حسن عامر

تمثل الألبان المتخمرة أقدم منتجات الألبان. حيث استخدمها الانسان كغذاء لما لها من قيمة غذائية عالية لاحتوائها على المواد الغذائية الموجودة بالألبان بصورة ساعدت على سهولة الهضم وسرعة الامتصاص. وللالبان المتخمرة اهمية بالغة من حيث الناحية العلاجية نظرا لاحتوائها على حمض اللاكتيك الذى له اثر مثبط لنمو البكتريا فى الأمعاء. كما لجأ الكثيرون إلى الألبان المتخمرة كعلاج لبعض حالات أمراض الجهاز الهضمى كالامساك والاضطرابات المعوية. وبالرغم من أن الحموضة العالية نسبيا فى الألبان المتخمرة لها تأثير على حيوية بعض الميكروبات الا أن بعضها كانت سببا فى انتشار بعض الأمراض الوبائية. ونظرا لأهمية الألبان المتخمرة وانتشارها فى مصر كانت لهذه الدراسة أهميتها لتقرير حالتها الصحية وما قد تنقله من ميكروبات ممرضة تسبب الأمراض للمستهلك. وقد أجريت الدراسة على مائة عينة من الزبادي (٥٠ زبادى عادى ، ٢٥ زبادى بالفراولة ، ٢٥ زبادى بالعسل) جمعت من مختلف محلات بيع الالبان والسوبرماركت بمحافظة الشرقية. ولقد تضمنت الدراسة فحص العينات فحصا ظاهريا من حيث الشكل واللون مع فحصها للجودة من خلال تحديد نسبة الحموضة اضافة الى فحصها بكتيريولوجيا لتقرير حالتها الصحية ومدى تلوثها بالميكروبات الممرضة والمسببة لتلفها مما يؤدى الى خسارة اقتصادية كبيرة. وقد اسفرت الدراسة ان النسبة المئوية للفحص الظاهرى لعينات الزبادى العادى والزبادى بالفراولة والزبادى بالعسل والتى تحقق اعلي الدرجات من ٩٣-١٠٠% هي (٤٤% ، ٧٢% ، ٨٨%)على التوالى. بلغ متوسط النسبة المئوية للحموضة فى الزبادى العادى والزبادى بالفراوله والزبادى بالعسل ٠,٨٣% ، ٠,٩٠% ، ١,٠٦% على التوالى. تواجدت ميكروبات الكوليفورم فى عينات الزبادى العادى والزبادى بالفراوله والزبادى بالعسل بنسبة ٤٠% ،

٢٠% ، ٨% على التوالي. وقد كان متوسط عدد تلك الميكروبات فى هـذه العينـات هـو ٤,٦×١٠^{٤} و ٤,٧×١٠^{٣} و ٤,٦×١٠^{٢}على التوالي. تم عزل وتصنيف عتـرات سـتروباكتر فريندای وانتيروباكتر اجلوميرانز وكليبسيلا اوكسى توكا مـن عينـات الزبـادى العـادى والزبادى بالفراولة بنسبة (٢٠% و ١٤% و ١٨%) و (١٢% و ١٦% و ٨%) على التوالى بينما تم عزل عترات ستروباكتر فريندای وانتيروباكتر اجلوميرانز فقط من عينات الزبـادى بالعسل.بنسبة ٨% ، ٤% على التوالى. بلغ متوسط عدد المكورات العنقودية فـى عينـات الزبادى العادى والزبادى بالفراولة والزبادى بالعـسل ١,٧×١٠^{٤} و ٥,٢×١٠^{٢}و ١,٦×١٠^{٣} على التوالي. تم عزل ميكروب المكور العنقودى الذهبى من عينات الزبادى العادى والزبادى بالفراولة والزبادى بالعسل بنسبة ١٢% ، ١٢% ، ٤% على التوالى اما ميكروب المكـور العنقودى من نوع ابيدرميدس فتم عزله من نفس العينات بنسبة ٦% ، ٤% ، ٤%. لم نتمكن من عزل ميكروب السالمونيلا من عينات الزبادى المفحوصة. بلغت النسبة المئوية لليرسينيا فى عينات الزبادى بالفراولة ١٦%، بينما لم يتم عزلها من عينات الزبادى العادى والزبادى بالعسل. تم عزل وتصنيف عترات اليرسينيا سيدوتوبركلوسس ويرسينيا انترميديا ويرسـينيا كريستن سناى من عينات الزبادى بالفراولة بنسبة ٨% ، ٤% ، ٤% على التوالى. هذا وقد تمت مناقشة أهمية الميكروبات المعزولة من كل من الناحيتين الصحية والاقتصادية بالإضافة إلى اقتراح التوصيات الواجب اتباعها لتحسين جودة الألبان الزبادى للحفـاظ علـى صـحة المستهلك من الأخطار الصحية.

SUMMARY

hundred random samples, fifty of natural yoghurt (plain) and 25 each of fruit yoghurt (strawberry) and flavored yoghurt (honey) were collected from different dairy shops and markets in Sharkia Governorate, Egypt. The collected samples were examined physically (sensory evaluation), sanitary (determination of titratable acidity), and bacteriologically. The obtained results revealed that the highest frequency distribution of examined yoghurt samples based on suggested score for sensory evaluation was 44% of examined natural yoghurt samples and lies within the range of 93-100%, while 72% of the examined fruit yoghurt samples and 88% of examined flavored yoghurt lie within the same range. Acidity% in natural and fruit yoghurt samples was 0.83% and 0.90%, respectively, while in case of flavored yoghurt samples was 1.06%. The mean coliform count/g in examined yoghurt samples was $4.6x10^4$ and $4.7x10^3$ for natural and fruit yoghurt, respectively while, coliform organisms in examined flavored yoghurt were $4.6x10^2$/g. The most prevalent coliform species isolated from examined natural and fruit yoghurt samples were *Citrobacter freundii, Enterobacter agglomerans and K. oxytoca* in percentages of (20%, 14% and 18%) and (12%, 16% and 8%), respectively, while in the examined flavored yoghurt samples the most prevalent coliform species were *Citrobacter freundii and*

Enterobacter agglomerans in percentages of 8% and 4%, respectively. The mean staphylococci count/g in examined natural and fruit yoghurt was $1.7x10^4$ and $5.2x10^2$, respectively but in case of flavored yoghurt it was $1.6x10^3$. *Staphylococcus aureus and Staph. epidermidis* were the predominated staphylococci isolated from the examined natural, fruit and flavored yoghurt samples in percentages of (12% and 6%), (12% and 4%) and (4% and 4%), respectively. *Salmonellae* failed to be detected in examined samples. Yersinia spp. could be detected in 16% out of examined fruit yoghurt samples while they couldn't be detected in both examined natural and flavored yoghurt samples. *Yersinia pseudotuberculosis, Y. intermedia and Y. kristensenii* could be isolated from the fruit yoghurt samples in percentages of 8%, 4% and 4%, respectively. The sanitary and public health importance of isolated microorganisms as well as their control measures were discussed to improve the quality of yoghurt and to safe guard the consumers from infection.

Key words: *Hygienic quality, yoghurt, coliforms, staphylococci, yersinia spp., salmonellae.*

INTRODUCTION

Milk and milk products are universally recognized as first class food stuff due to their exceptional richness in high quality animal protein, milk fat, high content of calcium, phosphorous and performed vitamin A and B2. The outstanding nutritive value of these products makes them indispensable in human nutrition. Moreover, such products are good supplement for a deficient diet at all times.

Many claims may be made for the therapeutic value of fermented milk in the diet of people. Ancient physicians prescribed sour milk for dysentery, tuberculosis, liver complaints and inflammation of intestinal tract a host of other maladies. Even though most of the claims for therapeutic value of fermented milks are grossly exaggerated, physicians today prescribe acidophilus milk in the diet of some persons affected with constipation or diarrhoea. In fact, researches have recently proved that antibiotics could be produced by organisms used in milk fermentation as acidophilin, lactocidin, lactoline, nicin and diplococcin. Such antibiotics may inhibit the growth of several food-borne pathogens (Steven, 1969; Goel *et al.,* 1971; Shahani *et al.,* 1974; Abou-Donia *et al.,* 1975).

Natural or plain yoghurt was the traditional type of fermented milks with a sharp acidic taste, while fruit yoghurt was made by the

addition of fruits and sweeting agents to natural yoghurt (Potter and Hotchkiss, 1995). Fruit yoghurt usually have incorporated stabilizers to reduce whey separation during distribution and many of the stabilizers are complex carbohydrates which providing "a bulking agent "for stimulating intestinal peristalsis and avoiding some the risk of colonic malfunction. It also absorbs some of the potentially toxic chemicals that may be formed in the large intestine as a result of bacterial action. These unavailable carbohydrates are 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. (Robinson and Khan, 1978 and Tamime and Robinson, 1985).

Contaminations of milk with coliforms with special reference to faecal coliforms give an indication of either direct or indirect faecal contamination and considered as a mirror for the degree of disregard of numerous hygienic rules during milking (ICMSF, 1986 and Varnam and Evans, 1991). Coliform also appear to be capable of colonizing in the human gut and producing potent enterotoxins in high yield. During the last few years, strains of *Klebsilla, Enterobacter and Citrobacter* have been isolated from stools or the intestinal tract of children and adults in several epidemiological studies of acute and chronic diarrheal diseases (Robert and Brenda, 1979).

The presence of coagulase positive, *Staphylococcus aureus* in a food gave an indication about its contamination from skin, mouth or handling, but inadequately cleaned utensils or equipment may be also a source of contamination. (Newsome, 1988). *Staphylococcus aureus* possess a public health hazard due to production of thermostable enterotoxins that was responsible for food-poisoning. (Wernozy-Rozand *et al.*, 1996).

Salmonellae are world wide and universally recognized as zoonotic agents. Numerous animal reservoirs have been identified, many foods, particularly of animal origin and those subjected to sewage pollution, have been identified as vehicle for transmitting these pathogens to human being (Robert *et al.*, 1996).

Yersinia enterocolitica is one of the human pathogenic species of Yersinia and of major importance in view of food hygiene. Raw milk and milk products have been shown to be a vector of infections in a number of *Yersinia enterocolitica* related food-borne outbreaks. (Moustafa *et al.*, 1983; Odinot *et al.*, 1995). *Yersinia enterocolitica* has been isolated from many animal species, with most isolates being virulent strains for humans. Exception was swine; they were principle resevior for virulent strains, which were often isolated from oral cavity

(tounge and tonsils) of apparently healthy animals. (Anonymous, 1977; Tacket *et al.*, 1984).

In recognition of public health and economic significance of these microorganisms, therefore, the present study was done to investigate the examined yoghurt samples physically, sanitary and bacteriology as well as assessment of the economic and public health significance of isolated microorganisms in relation to yoghurt.

MATERIALS and METHODS

Collection of samples:

One hundred random samples, fifty of natural yoghurt (plain) and 25 each of fruit yoghurt (strawberry) and flavored yoghurt (honey) were collected in their retail packages from different dairy shops and markets in Sharkia Governorate. Collected samples were transferred directly to the laboratory in an insulated ice-box at 4°C with a minimum of delay to be examined physically, sanitary and bacteriologically.

Preparation of the Samples:

On arrival to the laboratory each sample was perfectly mixed and examined physically (sensory evaluation) before being divided into two parts. The first one used for sanitary examination (determination of titratable acidity), while the second one was examined bacteriologically.

A- Physical examination:

Sensory evaluation (Sangwan, 2008): Yoghurt samples were examined for physical properties then graded according to the scores.

Table 1: Sensory evaluation of yoghurt samples.

Attribute	Maximum score
Flavor	45
Body and Texture	30
Appearance	10
Sediment	10
Container	5
Total	100

Table 2: Suggested scores for a defective yoghurt samples.

Attribute	Defect	Degree of defect		
		Slight	Moderate	Pronounced
Flavor	Bitter	38-40	35-37	32-34
	Cheesy	38-40	35-37	32-34
	Flat	40-42	37-39	34-36
	Low-acid	40-42	37-39	34-36
	High-acid	40-42	37-39	34-36
	Yeasty	38-40	35-37	32-34
	Foreign	38-40	35-37	32-34
Body and Texture	Curdy	26-27	23-26	21-22
	Gassly	26-27	23-26	21-22
	Lumpy	27-28	24-26	21-22
	Ropy	27-28	24-26	22-23
	Thin	27-28	24-26	22-23
	Wheyed off	27-27	24-26	22-23
Sediment	Chalky,dull	9	7	5
Appearance	Unsighty,	9	8	7
Container	Soiled	4	3	2

B-Sanitary examination:

Determination of titratable acidity percentage by using standard method (A.P.H.A., 1992).

C-Bacteriological examination:

1- Preparation of serial dilutions (A.P.H.A., 1992).

2- Enumeration and isolation of coliforms (MPN/gm); (A.P.H.A., 1992).

Identification of the isolated coliform organisms (Krieg and Holt, 1984).

3- Enumeration and isolation of staphylococci (A.P.H.A., 1992).

Identification of the isolated Staphylococcus organisms (Cowan and Steel, 1974).

4- Isolation of Salmonella spp. (A.P.H.A., 1992).

5- Isolation of Yersinia spp. (A.P.H.A., 1992).

Identification of the isolated Yersinia spp. (FDA, 1998).

RESULTS

Table 3: Frequency distribution of the examined yoghurt samples based on their sensory evaluation.

Degree of defect	Suggested Scores	Natural yoghurt (Plain) (n=50)		Fruit yoghurt (Strawberry) (n=25)		Flavored yoghurt (Honey) (n=25)	
		No.	%	No.	%	No.	%
No defect	93-100	22	44.0	18	72.0	22	88.0
Slight	86-92	12	24.0	4	16.0	2	8.0
Moderate	76-83	7	14.0	2	8.0	1	4.0
Pronounced	67-73	9	18.0	1	4.0	0	0.0
	Total	50	100.0	25	100.0	25	100.0

n: means the number of examined samples

Table 4: Statistical analytical results of titratable acidity % in the examined yoghurt samples.

Type of samples	Minimum	Maximum	Mean	±S.E.M
Natural yoghurt (Plain) (n=50)	0.66	1.12	0.83	0.012
Fruit yoghurt (Strawberry) (n=25)	0.73	1.24	0.90	0.021
Flavored yoghurt (Honey) (n=25)	0.99	1.32	1.06	0.017

Table 5: Frequency distribution of the examined yoghurt samples based on their titratable acidity %.

Type of samples / Intervals	Natural yoghurt (Plain) (n=50)		Fruit yoghurt (Strawberry) (n=25)		Flavored yoghurt (Honey) (n=25)	
	No.	%	No.	%	No.	%
0.60-0.70	9	18.0	0	0.0	0	0.0
0.71-0.80	18	36.0	5	20.0	0	0.0
0.81-0.90	14	28.0	13	52.0	0	0.0
0.91-1.00	6	12.0	2	8.0	18	72.0
1.01-1.10	2	4.0	2	8.0	3	12.0
1.11-1.20	1	2.0	0	0.0	2	8.0
1.21-1.30	0	0.0	3	12.0	1	4.0
1.31-1.40	0	0.0	0	0.0	1	4.0
Total	50	100.0	25	100.0	25	100.0

Table 6: Statistical analytical results of coliform count/g. in the examined yoghurt samples (MPN/g).

Type of samples	No. of examined samples	Positive samples		Count/g.		
		No.	%	Min.	Max.	Mean
Natural Yoghurt (Plain)	50	20	40.0	$3.0 X10^2$	$7.0X10^5$	$4.6x10^4$
Fruit Yoghurt (Straw berry)	25	5	20.0	$8.0X10^2$	$6.0X10^4$	$4.7x10^3$
Flavored Yoghurt (Honey)	25	2	8.0	$2.0X10^2$	$9.0X10^3$	$4.6x10^2$

Table 7: Frequency distribution of the examined yoghurt samples based on their coliform count/g.

Type of samples / Intervals	Natural yoghurt (Plain) (n=50)		Fruit yoghurt (Strawberry) (n=25)		Flavored yoghurt (Honey) (n=25)	
	No.	%	No.	%	No.	%
10^{2-}	13	65.0	4	80.0	1	50.0
10^3-	4	20.0	0	0.0	1	50.0
10^4-	1	5.0	1	20.0	0	0.0
10^5-	2	10.0	0	0.0	0	0.0
Total	20	100.0	5	100.0	2	100.0

Table 8: Incidence of isolated coliforms in the examined yoghurt samples.

Type of samples / Isolated organisms	Natural yoghurt (Plain) (n=50)		Fruit yoghurt (Strawberry) (n=25)		Flavored yoghurt (Honey) (n=25)	
	No.	%	No.	%	No.	%
Citrobacter freundii	10	20.0	3	12.0	2	8.0
Enterobacter agglomerans	7	14.0	4	16.0	1	4.0
Klebsiella oxytoca	9	18.0	2	8.0	0	0.0

Table 9: Statistical analytical results of staphylococci count/g. in the examined yoghurt samples.

Type of samples	No. of examined samples	Positive samples		Count/g.		
		No.	%	Min.	Max.	Mean
Natural yoghurt (plain)	50	9	18	$2.0x10^2$	$4.0x10^5$	$1.7x10^4$
Fruit yoghurt (Straw berry)	25	4	16	$4.0x10^2$	$7.0x10^3$	$5.2x10^2$
Flavored yoghurt (Honey)	25	2	8	$2.0x10^2$	$3.0x10^3$	$1.6x10^3$

Table 10: Coagulase production of the isolated staphylococci strain in examined yoghurt samples.

Coagulase	Natural yoghurt (Plain) (n=50)		Fruit yoghurt (Strawberry) (n=25)		Flavored yoghurt (Honey) (n=25)	
	No.	%	No.	%	No.	%
+++	6	12.0	3	12.0	1	4.0
++	2	4.0	1	4.0	0	0.0
+	1	2.0	0	0.0	1	4.0

+++ means strong positive coagulase
++ means positive coagulase
+ means weekly positive coagulase

Table 11: Incidence of the isolated staphylococci in the examined yoghurt samples.

Type of samples / Isolated organism	Natural yoghurt (Plain) (n=50)		Fruit yoghurt (Straw berry) (n=25)		Flavored yoghurt (Honey) (n=25)	
	No.	%	No.	%	No.	%
Staphylococcus aureus	6	12.0	3	12.0	1	4.0
Staphylococcus epidermidis	3	6.0	1	4.0	1	4.0

Table 12: Incidence of Yersinia spp. in the examined yoghurt samples.

product	No. of examined samples	Positive samples	
		No.	%
Natural yoghurt (Plain)	50	0	0.0
Fruit yoghurt (Strawberry)	25	4	16.0
Flavored yoghurt (Honey)	25	0	0.0
Total	100	4	4.0

Table 13: Incidence of different types of Yersinia spp. in the examined fruit yoghurt samples.

Type of samples / Isolated organisms	Fruit yoghurt (Straw berry) (n=25)	
	No.	%
Yersinia pseudo tuberculosis	2	8.0
Yersinia intermedia	1	4.0
Yersinia kristensenii	1	4.0

Table 14: Correlation between titratable acidity, coliforms, staphylococci and Yersinia spp. in the examined yoghurt samples.

Type of samples	Organisms	Pearson correlation between isolated M.Os and acidity	Sig. (2-tailed)	Sig.
Natural yoghurt (plain) (n=50)	Coliforms	0.240-	0.094	N.S.
	Staphylococci	0.155-	0.284	N.S.
Fruit yoghurt (Straw berry) (n=25)	Coliforms	0.330	0.107	N.S.
	Staphylococci	0.161	0.441	N.S.
	Yersinia spp	0.102	0.074	N.S.
Flavored yoghurt (Honey) (n=25)	Coliforms	0.570	0.003	**H.S.
	Staphylococci	0.080	0.702	N.S.

**H.S.: Highly significant ($P < 0.01$)
N.S.: Non significant

DISCUSSION

Table 3 revealed that the highest frequency distribution of examined yoghurt samples based on their suggested score for sensory evaluation was 44% in examined natural yoghurt samples and lies within the range of 93-100%, while it was 72% for examined fruit yoghurt samples and 88% for examined flavored yoghurt which lies within the previously mentioned range. Sensory criteria are essential parameters that constitute the "eating quality" of dairy products which cann't be easily measured either chemically or physically. All primary classic senses: sight, smell, taste, touch and sound should be used in the sensory evaluation of yoghurt. Sight is used for evaluation of many factors as style, cleanliness of package, exterior attractiveness of finished product, package closure, body and texture, color and overall appearance and quality defects. The flavor sense of yoghurt samples detects the normal and impact (defective) flavor. Quality of yoghurt samples can be described as a value related to flavor, color and texture. It also includes imperceptible traits such as aesthetic value and safety, evaluation of raw material and final products standards, the design of dairy plant, process line layout and the design storage and distribution of yoghurt. It also concerned with packaging, storage and distribution of yoghurt samples (Al-Ashmawy *et al.*, 1991).

The results summarized in Table 4 showed that the titratable acidity % in examined natural yoghurt samples was ranged from 0.66 to 1.12% with an average of 0.83, while in examined fruit yoghurt samples it was ranged from 0.73-1.24% with a mean value of 0.90 ± 0.021 and 0.99 to 1.32% with a mean value of 1.06 ± 0.017 for examined flavored yoghurt samples. The highest frequency distribution of examined natural yoghurt samples based on their titratable acidity % was 36.0% which lies within the range of 0.71-0.80, while in case of fruit yoghurt samples was 52.0% and lies within the range of 0.81-0.90 but in case of examined flavored yoghurt samples it was 72.0% and lies within the range of 0.91-1.0 (Table 5).

Nearly similar data were obtained by Ayoub (1986); Moustafa *et al.* (1988); Al-Ashmawy *et al.* (1991); Abd El-Fatah (2007), while higher values were reported by El-Shinawy (1987); Ayoub (1991)

Titratable acidity% of yoghurt samples has a greater importance where it is used for assessing the keeping quality. Higher acidity of yoghurt samples may be attributed to contamination either by lactic acid producing microorganisms or pathogenic microorganisms which ferment

lactose and elevate the acidity. It is rendering the yoghurt samples unmarketable due to off-taste and unfit for human consumption due to pathogens (A.P.H.A., 1992).

The therapeutic value of yoghurt is due to it's acidity which lead to inhibiting or inactivating the most pathogens such as Salmonella spp. and coliforms. The inhibition of potential pathogens is reinforced by the production of antibiotic substances produced by lactic acid-producing bacteria (Rubin 1985; Prakash and Kulkarni, 1986).

The result tabulated in Table 6 showed that the total coliform count (MPN/g.) of examined natural yoghurt was ranged from $3.0x\ 10^2$ to $7.0x\ 10^5$ with an average of $4.6x\ 10^4$, while in those of fruit type it ranged from $8.0x\ 10^2$ to $6.0x\ 10^4$ with a mean value of $4.7x\ 10^3$ but it ranged from $2.0x10^2$ to $9.0x\ 10^3$ with a mean of $4.6x10^2$ in case of examined flavored yoghurt samples. These finding were inagreement with those reported by Aboul-Khire *et al.* (1985); Saad *et al.* (1987); Moustafa *et al.* (1988); Al-Hadethi *et al.* (1992); El-Barbary (1999); Mansour *et al.* (1999); Abd El-Fatah (2007), while higher results were obtained by Ayoub (1986); Farid *et al.* (1992); El-Badry (1998). But lower results were recorded by Saudi *et al.* (1988); Al-Ashmawy *et al.* (1991).

The results listed in Table 7 revealed that the highest frequency distribution of examined yoghurt based on their coliform counts was 65% in examined natural yoghurt and lies within the range of 10^2-10^3, while it was 80% and 50% in examined fruit and flavored yoghurt samples, respectively which lies within the same range.

The result reported in Table 8 showed that *Citrobacter freundii, Enterobacter agglomerans and Klebsiella oxytoca* could be isolated from the examined natural yoghurt samples in percentages of 20%, 14% and 18%, respectively, while in examined fruit yoghurt samples were 12%, 16% and 8%, respectively. Only Citrobacter freundii and Enterobacter agglomerans were isolated from examined flavored yoghurt samples in percentages of 8% and 4%.

Slightly higher findings were reported by Ayoub (1986). The result obtained by Pintor *et al.* (1989); El-Badry (1998); Abd El-Fatah (2007) showed that *E. coli* failed to be detected in examined plain and fruit yoghurt samples, but it could be isolated by Brazal Garcia *et al.* (1986); Ahmed (1989); Bahout and El-Shawaf (1999).

It's evident from the last few years that some strains of Klebsiella, Enterobacter and Citrobacter had been isolated from stools and the intestinal content of both children and adults in several epidemiological studies of acute and chronic disturbances (Twedt and Boutin, 1979).

Certain numbers of Citrobacter had been suspected to cause enteric infection (Cruickshank *et al.*, 1975), while *Citrobacter freundii* had been found among urinary and other pyogenic infections in humans (Mackie and MacCarteny, 1962).

The results tabulated in Table 9 revealed that (18%) of examined natural yoghurt samples were contaminated by staphylococci, the level of contamination was ranged from $2.0x10^2$ to $4.0x10^5$ with a mean value of $1.7x\ 10^4$, while (16%) out of examined fruit yoghurt samples were ranged from $4.0x\ 10^2$ to $7.0x\ 10^3$ with a mean value of $5.2x10^2$. Only (8.0%) of examined flavored yoghurt samples were contaminated by staphylococci, as the level of contamination was ranged from $2.0x\ 10^2$ to $3.0x\ 10^3$ with an average of $1.6x\ 10^3$.

Relatively similar results were obtained by El-Shinawy (1987), Al-Ashmawy *et al.* (1991); Ali *et al.* (2004), while higher values were reported by El-Badry (1998).

The results presented in Table 10 showed coagulase production of isolated staphylococci strains in examined yoghurt samples 12% of natural yoghurt samples were strongly coagulase positive and 4% were positive coagulase and 2% were weakly positive coagulase, while in examined fruit yoghurt samples 12% and 4% were strongly positive and positive coagulase, respectively, but in case of flavored yoghurt samples 4 % were strongly coagulase positive and the same percentages were weakly positive coagulase.

According to identification of isolated staphylococci Table 11 showed that *Staphylococcus aureus* was detected in 6(12%) out of examined natural yoghurt samples, while (6%) out of them were contaminated by *Staphylococcus epidermidis*, while in case of fruit and flavored yoghurt samples (12% and 4%) and (4% and 4%) were contaminated by *Staphylococcus aureus and Staphylococcus epidermidis*, respectively.

These findings were inagreement with that reported by Saleem *et al.* (1989) and coincided with those obtained by El-Bessery (2001) who found that all examined yoghurt samples were free from *Staphylococcus aureus*.

Human being normally harbours *Staphylococcus aureus* as the main reservoir is the nasal cavity and from this source, organisms find their way to skin and into wounds either directly or indirectly. In addition, *Staphylococcus aureus* may be found in eyes, throat and intestinal tract. Therefore, nasal carriers and individuals whose hands and arms were infected with boils and carbuncles are dangerous sources of food-poisoning (Jay, 1992).

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.

Enterotoxins producing staphylococci are the leading cause of food borne illness throughout the world. *Staph. aureus* posses a public health hazard due to production of thermostable enterotoxins that is responsible for food-poisoning. The growth of *Staphylococcus aureus* in food is a potential public health hazard, since many strains of *Staphylococcus aureus* produce enterotoxins which cause food-poisoning if ingested (Pazakova *et al.*, 1997).

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 nasocomital and community-acquired urinary infections, bacteremia in compromised hosts, osteomyleitis and post-surgical infections.

Salmonella failed to be detected in all examined samples. The findings substantiated what had been reported by Aboul-khier *et al.* (1985); Amer *et al.* (1985); Rodriguez *et al.* (1990); Mufandaedza *et al.* (2006); Abd El-Fatah (2007). On the contrary Wang *et al.* (2004) could detect Salmonella in (3%) out of examined yoghurt samples. These results may be attributed to that yoghurt culture had an inhibitory effect on salmonellae within the range of (92.5-99.8%) (Lukasova *et al.*, 1990).

The results recorded in Table 12 revealed that yersinia failed to be isolated from all examined natural and flavored yoghurt samples while 4(16%) out of 25 examined fruit yoghurt samples were contaminated with yersinia.

Table 13 showed that the isolated strains of Yersinia spp. were *Yersinia pseudotuberculosis, Y. intermedia* and *Y. kristensenii* in percentages of 8%, 4% and 4% of examined fruit yoghurt samples respectively. *Yersinia enterocolitica* failed to be detected in all examined natural, flavored and fruit yoghurt samples. *Y. enterocolitica* could be isolated by El-Prince and Sabreen (1998); El-Barbary (1999), but it failed to be detected by El-Kholy (1992). Umoh *et al.* (1984) could detect *Y. enterocolitica* in 2% out of examined yoghurt samples. This bacterium could contaminate yoghurt through raw milk used without sufficient heating or through contaminated equipment used for its production.

Y. enterocolitica has the distinction of surviving and multiplying in food held at refrigeration temperature, therefore milk and its products contaminated initially with even low level of this bacterium may serve not only as a vehicle, but also as a medium for its proliferation (Stern *et al.*,

1980). Milk and its products had been incriminated in several outbreaks of yersiniosis due to the psychrotrophic nature of Y. enterocolitica which was accompanied by increasing use of refrigeration in food preservation. A highly publicized food associated outbreak of yersiniosis among school children occurred in Oneida, New York, due to consumption of contaminated chocolate milk (Black *et al.*, 1978).

Table 14 showed that the relation between acidity and isolated microorganisms. PH of the yoghurt prevents growth of coliform and act as inhibitor factors of the isolated microorganisms. These findings substantiated what had been reported by Mohammed and Younis (1990); Pesic (1991); Altaf *et al.* (1995); Hsin and Chou (2001); Mufandaedza *et al.* (2006).

From the previously mentioned date, we observed that efficient heat-treatment of milk used in manufacture of yoghurt caused a pronounced reduction in the bacterial load of yoghurt and in turn, keeping quality were usually improved and the shelf-life may be expanded to twenty one days of storage periods (Dagher and Ali, 1985; Mohanan *et al.*, 1985). Although, plastic cups were not satisfactory and may be responsible for poor quality of yoghurt and its post-pasteurization contamination (Saudi *et al.*, 1989).

CONCLUSION

The assessment of the results obtained allow to conclude that most of fermented milk in Sharkia Governorate don't satisfy the consumer's demand in obtaining such products in good sanitary condition and retaining as far as possible their nutritive value. Information given by the results of bacteriological examination reported here-in points out that the sanitary measures adopted during production and handling of this product is neglected in most cases as coliforms existed in some samples of yoghurt which are supposed to be heat treated before being manufactured.

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