COMPARATIVE STUDIES ON THE MICROBIAL LOAD OF FLAVOURED AND UNFLAVOURED DRINKING YOGHURT

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ABSTRACT

To assess the accuracy and helpfulness of plain and flavoured drinking yoghurt, one hundred and fifty random samples (thirty each) of plain, strawberry, mango, banana and orange were collected from different supermarkets in Cairo and Giza Governorates, Egypt. The examined samples were analyzed to assess and compare the microbiological quality of plain and flavoured drinking yoghurt samples before their expiry or sell-by date. The data obtained revealed that, aerobic spore former count were determined in different types of examined plain, strawberry, mango, banana and orange samples with a mean values of $6.4\times10^3\pm1.1\times10^2$, $1.3\times10^5\pm2.1\times10^4$, $3.2\times10^6\pm4.3\times10^5$, $6.9\times10^5\pm7.2\times10^4$ and $4.2\times10^6\pm6.6\times10^5$, respectively. Mould and yeast counts were detected in 30, 100, 100, 100 and 90% of the examined samples. *Coliforms, Enterococci* and, *Staphylococci* were detected in different kinds of examined flavoured drinking yoghurt. *Pseudomonas* and *Aeromonas* as well as *Bacillus cereus* were present in the examined samples with percentages ranged from 23.3 to 66.7% and 0 to 36.6%, respectively. *Salmonella* and *Yersinia* were not detected in all examined samples. The survival of *Listeria monocytogenes* in drinking yoghurt stored at refrigerator for 30 days was investigated. The public health significance and economical importance of different microbial groups as well as recommended hygienic measures were discussed.

Keywords: drinking yoghurt, flavoured yoghurt, yeasts, moulds, *Salmonella, Listeria monocytogenes.*.

INTRODUCTION

In recent years, there has been a large increase in the popularity of fermented milk products. The consumers search for new and unique fermented milks to maintain the healthy eating habits (Adhikari, *et al*., 2000, Pereg, *et al*., 2005).

Drinking yoghurt is delicious cultured stirred yoghurt recently prevails in the Egyptian markets. It is one of the fermented milk products which consumed by large segments of the population either as a part of diet or as a refreshing beverage. It is nutritiously balanced food containing almost all the nutrients present in milk as well as valuable therapeutic properties (Bartram *et al*., 1994, Yuceer *et al*., 2001, Lubbers *et al*., 2004).

The preparation of this product involves stirring of the coagulum after fermentation, diluted with fruit juices, fruit syrup, sugar and other flavouring materials to obtain drinkable properties followed by aseptic packaging and then cooled (Varnam & Sutherland, 1994, Harte *et al.*, 2003).

Due to the fact that drinking yoghurt is purchased by the consumer on the basis of flavour and ingredients, there are several different flavours used in their manufacture which may be plain (natural) or in a wide variety of flavours as strawberry, apricot, apple, banana or mango. Generally, these flavours are added to drinking yoghurt after pasteurization and fermentation have been completed. Therefore, microbial hazard may be of considerable importance to drinking yoghurt industry (Beal, *et al*., 1999, Kozlowska *et al*., 2002, Martin *et al*., 2004).

Plain and flavoured fermented milks are associated with several human health benefits. In addition to being palatable and nutritious, the starter culture bacteria help to maintain a well-balanced microflora (Gabriela, *et al*., 1998, De Moreno & Perdigon, 2004). This positive influence on the gut may contribute to decrease diarrhea, increase lactose tolerance in susceptible

individuals, decrease incidence of colon cancer, prevent DNA damage induced by the carcinogens, improve the immune response and inhibit serum cholesterol level (Gilliland, 1998, Wollowski, *et al*., 1999, Ott *et al*., 2000, Kozlowska *et al*., 2002, Seppo, *et al*., 2003, Moreno, 2005).

It was believed that, low pH and low storage temperature of drinking fermented milk may protect from pathogenic bacteria but some pathogens as well as moulds and yeasts may get entry to the product either before or post processing leading to economical and public health hazards. Yeasts find good medium and suitable environment for growth and multiplication with consequent alteration in its quality (Garbutt, 1998, WHO, 2000). Some yeasts ferment lactose into carbon dioxide and ethanol leading to blown cartons, off flavour and odour. The major source of contamination is the added fruit purees which is often contaminated by unsatisfactory handling procedures. In addition, presence of these organisms indicating shortage in the process of pasteurization or post pasteurization contamination (Kurmann *et al*., 2000, Al-Kadamany *et al*., 2002, Kora, *et al*., 2004).

In contrast to the extensive bibliography available on yoghurt and other fermented milks, there is an almost lack of microbial evaluation of flavoured and unflavoured drinking yoghurt. Nowadays, the increase demand of Egyptian markets for drinking yoghurt for children and elderly has made its microbiological quality of primary concern. Therefore, this work was planned to evaluate the effect of different additives (flavours) on actual microbiological hazard of flavoured drinking yoghurt and to examine the ability of *Listeria monocytogenes* to survive in plain and flavoured drinking yoghurt.

MATERIALS AND METHODS

Materials

One hundred and fifty random samples (thirty each) of flavoured mix with strawbery, mango, banana, orange and unflavoured drinking yoghurt stored in refrigerator, in its retail containers were collected from different dairy shops and supermarkets from Cairo and Giza governorates. The samples were collected at the first or second day of production (fresh) samples. All samples have a shelf life time of 14 days when refrigerated at 4ºC as recommended by the producing companies. The collected samples were transported to the laboratory in an insulating icebox with a minimum of delay to be immediately examined for microbial load (Messer *et al*., 1992).

Methods

Samples were prepared as the technique described by Messer *et al*. (1992).

Enumeration of different types of microorganisms:

Aerobic spore formers counts was carried out using the technique recommended by Marshall (1992).

Yeast and mould counts were applied according to Lodder (1990). Macroscopic and microscopic identification of the isolated moulds according to Samson *et al*., (1995) and Pitt & Hocking (1997) while identification of yeasts strains according to the method applied by Elzataar, *et al*. (1990) and Rama *et al*. (1998).

Coliforms count was carried out using the most probable number (MPN) technique as described by Hitchins *et al*. (1992). *Enterococci*, *Staphylococci*, *Pseudomonas* and *Aeromonas* counts were undertaken according to MSF (1996), Robinson (1990) and Colins *et al*. (1995), respectively.

Isolation and identification of *Salmonella* (Flowers *et al*., 1992), Yersinia entercolitica (Donald & George, 1992) and *Bacillus cereus* (Granum, 1997) were also investigated.

Survival of *Listeria monocytogenes*:

Bacterial culture: *Listeria monocytogenes* ATCC 1037 serotype 4b was used in this study. A stock culture of the organism was obtained from Institute for Microbiology (Hannover University, Germany) and maintained in vials. Inoculum from the vial was transferred into tryptic soy broth supplemented with yeast extract (TSBYE) and stored at 4°C.Fresh culture was grown on TSBYE at 37°C for 18-24 hr. Serial dilutions were prepared with buffered peptone water (0.1%) and bacterial count were enumerated on modified Oxford agar media. Twenty five grams of the plain and strawberry flavoured drinking yoghurt were aseptically transferred into stomacher bags and kept at 4°C for 30 days. Uninoculated control samples were plated on Oxford agar media. Test samples were inoculated by 6.6×10^{6}CFU/ml. Samples were mixed to ensure the proper mixing of the samples. The pH of the samples was measured with ORION pH meter (model 520, Orion Research, Inc, Bevery, Mass). Samples were ex-

amined every 5 days for a total storage time of 30 days at 4°C. The technique adopted according to Tipparaju *et al*. (2004).

Statistical analysis

The ANOVA test (Analysis of Variance) was applied at 95% to point out if there were significant differences between the different microbiological counts by Duncan's multiple range test (SPSS, 1993).

RESULTS AND DISCUSSION

Results presented in Table (1) showed that *Aerobic spore formers* were present in plain and flavoured drinking yoghurt samples in a percentages of 20, 96.7, 83.3, 100 and 60% with a mean values of $6.4\times10^3\pm1.1\times10^2$, $1.3\times10^5\pm2.1\times10^4$, $3.2\times10^6\pm4.3\times10^5$, $6.9\times10^5\pm7.2\times10^4$ and $4.2\times10^6\pm6.6\times10^5$ CFU/ml for plain, mango, strawberry, banana and orange drinking yoghurt samples, respectively. There was significant difference between the plain and different kinds of flavoured samples ($P< 0.05$). The higher occurrence of aerobic spore formers in drinking yoghurt may be due to their heat resistance to pasteurization. They produce heat resistant proteolytic and lipolytic enzymes which may cause spoilage in the refrigerated products (Varnam & Sutherland, 1994, Armesto & Alastair, 1997).

The data given in Table (2) illustrate that yeasts and moulds were detected in 30, 100, 100, 100 and 90% of the examined drinking yoghurt samples with a mean values of $2.4\times10^3\pm1.1\times10^2$, $6.1\times10^7\pm 8.1\times10^6$, $3.3\times10^6\pm 4.2\times10^4$, $7.3\times10^6\pm5.9\times10^5$, $3.5\times10^7\pm4.8\times10^5$ CFU /ml for plain, mango, strawberry, apricot and orange samples, respectively. There was significant difference between the plain and different kinds of flavoured samples ($P< 0.05$) while there was no significant difference between mango, strawberry, banana and orange samples. Yeast and mould counts should not exceed 10 CFU/ml according to Egyptian Standard (Egyptian Standards, 2005). All samples of flavoured drinking yoghurt were heavily contaminated with moulds and yeasts. The high mould and yeast counts may be attributed to inadequate hygienic measures in production or the use of bad quality flavoured materials (Varnam & Sutherland, 1994, Graciela, *et al*., 2001). Results presented in Table (3) revealed that the most common mould species isolated from drinking yoghurt samples are belonging to genera : *Penicillium* (33.3, 66.7, 80, 63.3 and 77.8%) which represented by *P. spinulosum* (16.6, 36.7, 33.3, 43.3 and 59.3%), *P. expansum* (10, 20, 26.7, 16.7 and 18.5%) and *P. digitatum* (6.6, 10, 20, 3.3 and 0%), *Aspergillus* (23.3, 56.7, 40, 53.3 and 48.1%) which represented by *A. flavus* (10, 25, 23.3, 30, 29.6%), *A. niger* (10, 10, 13.3, 20, 18.5) and *A. ochracious* (3.3, 15, 3.4, 3.3 and 0%), *Bysochlamys* which represented by *B. fulva* (16.6, 40, 30, 23.3 and

Table 1: Total aerobic spore former count in examined drinking yoghurt samples (n=30) CFU/ml

Type	Positive samples		Min.	Max.	Mean
	No.	%			
Plain	6	20	10	2.2×10^5	$6.4\times10^3\pm1.1\times10^{2a}$
Mango	29	96.7	100	3.0×10^7	$1.3\times10^5\pm2.1\times10^{4b}$
Strawberry	25	83.3	90	8.0×10^7	$3.2\times10^6\pm4.3\times10^{5b}$
Banana	30	100	90	6.5×10^7	$6.9\times10^5\pm7.2\times10^{4b}$
Orange	18	60	10	1.9×10^8	$4.2\times10^6\pm6.6\times10^{5b}$

CFU: colony forming units

Means of different superscript (a and b) considered significantly different at 95%.

Table 2: Total yeast and mould counts in examined drinking yoghurt samples (n=30)

Type	Positive samples		Min.	Max.	Mean
	No.	%			
Plain	10	30	100	4.6×10^5	$2.4\times10^3\pm1.1\times10^{2a}$
Mango	30	100	3.0×10^2	1.2×10^9	$6.1\times10^7\pm8.1\times10^{6b}$
Strawberry	30	100	2.1×10^2	9.7×10^8	$3.3\times10^6\pm4.2\times10^{4b}$
Banana	30	100	1.6×10^2	8.0×10^8	$7.3\times10^6\pm5.9\times10^{5b}$
Orange	27	90	5.0×10^2	9.9×10^9	$3.5\times10^7\pm4.8\times10^{5b}$

Means of different superscript (a and b) considered significantly different at 95%.

Table 3: Incidence of isolated moulds in examined drinking yoghurt samples

Species	Plain		Mango		Strawberry		Banana		Orange	
	No.	%	No.	%	No.	%	No.	%	No.	%
Penicillium	10	33.3	20	66.7	24	80	19	63.3	21	77.8
P. spinulosum	5	16.6	11	36.7	10	33.3	13	43.3	16	59.3
P. expansum	3	10	6	20.0	8	26.7	5	16.7	5	18.5
P. digitatum	2	6.6	3	10.0	6	20.0	1	3.30	0	0
Aspergillus	7	23.3	15	56.7	12	40	16	53.3	13	48.1
A. Flavus	3	10	10	25	7	23.3	9	30	8	29.6
A. niger	3	10	4	10	4	13.3	6	20	5	18.5
A. ochracious	1	3.3	1	15	1	3.4	1	3.3	0	0
Bysochlamys fulva	5	16.6	10	40	9	30	7	23.3	0	0
Euepenicillium labidosum	0	0	9	30	6	20	1	3.3	0	0
Talaromyces flavus	0	0	8	20	4	13.3	0	0	8	29.6

0%), *Euepenicillium* which represented by *E. labidosum* (0, 30, 20, 3.3 and 0%) and *Talaromyces* which represented by *T. flavus* (0, 20, 13.3, 0 and 29.6%) in examined plain, mango, strawberry, apricot and orange samples, respectively. Results presented in Table (4) showed that the isolated yeasts from the examined drinking yoghurt samples could be identified as genera *Candida* (30, 73.3, 63.3, 50 and 37%) which represented by *C. albicans* (13.3, 53.3, 33.3, 23.3 and 18.5%), *C. lipoliticum* (10, 10, 20, 13.3 and 11.1), *C. curvataq* (6.6, 6.7, 6.7, 10 and 7.4%) and *C. tenius* (0, 3.3, 3.3, 3.3 and 0), *Torulopsis* (0, 60, 40, 43.3 and 29.6) represented by *T. versatilis* (0, 33.3, 20, 40 and 22.2%) , *T. ernobii* (0, 26.7, 20, 3.3 and 7.4%) and *Saccharomyces* (20, 30, 23.3, 16.6 and 22.2%) which represented by *S. cervisae* (13.3, 16.7, 13.3, 13.3 and 11.1%) and *S. farinosum* (6.7, 13.3, 10, 3.3 and 11.1) in examined plain, mango, strawberry, banana and orange drinking yoghurt samples, respectively. The presence of *Bysochlamys, Euepenicillium, Talaromyces* and *Saccharomyces* in flavoured drinking yoghurt may be due to their frequent presence in flavouring materials (fruit juices and concentrates) as well as their high resistance to heat treatment (Gourama & Bullerman, 1995, Robert, 1997, Li & Li, 1998). Therefore the producers should be aware with those flavouring materials. As moulds are widely distributed as environmental contaminants of air, water and soil, they are responsible for spoilage of drinking yoghurt which causes economical losses. The public health importance of mould has been emphasized as certain types of moulds produce mycotoxins which implicated in liver cancer (Chapman, 2003). The high count of moulds and yeasts in all examined samples may be due to any of the following reasons: high count in

Table 4: Incidence of yeast species in examined samples

Species	Plain		Mango		Strawberry		Banana		Orange	
	No.	%	No.	%	No.	%	No.	%	No.	%
Candida	9	30	22	73.3	19	63.3	15	50	10	37.0
C. albican	4	13.3	16	53.3	10	33.3	7	23.3	5	18.5
C. lipoliticum	3	10	3	10.0	6	20.0	4	13.3	3	11.1
C. curvata	2	6.6	2	6.7	2	6.7	3	10.0	2	7.4
C. tenius	0	0	1	3.3	1	3.3	1	3.3	0	0
Torulopsis	0	0	18	60.0	12	40	13	43.3	8	29.6
T.versatilis	0	0	10	33.3	6	20	12	40	6	22.2
T. ernobii	0	0	8	26.7	6	20	1	3.3	2	7.4
Saccharomyces	6	20	9	30.0	7	23.3	5	16.6	6	22.2
S. cervisae	4	13.3	5	16.7	4	13.3	4	13.3	3	11.1
S. farinosum	2	6.7	4	13.3	3	10.0	1	3.3	3	11.1

raw materials, ineffective processing methods, ineffective sanitizing methods or faulty storage of the products. Therefore sanitary control measures should be adapted to dairy processing plants by application of HACCP system on processing, packaging, storage and distribution of such products (Spreer & Mixa, 1998, Ray, 2004).

The data recorded in Table (5) revealed that *coliforms* count were presented in percentages of 13.3, 50, 86.7, 76.7 and 56.7% with an average of $1.9\times10^3\pm2\times10^2$, $5.0\times10^5\pm2.9\times10^4$, $4.7\times10^6\pm8.5\times10^5$, $1.0\times10^6\pm1.7\times10^5$ and $9.9\times10^6\pm4.8\times10^5$ CFU/ml for plain, mango, strawberry, banana and orange drinking yoghurt samples, respectively. There was significant difference between the plain and different kinds of flavoured samples ($P<0.05$). *Coliform organism*s may be implicated in food illness among the consumers. The additives were the most important source of contamination with *Coliforms*. In general, it may be used as indicator for processing sanitation. Moreover up to 30% of people in industrialized countries and millions of people in developing countries suffer from food borne illness (WHO, 2000).

Regarding the data presented in Table (6) *Enterococci* was existed in a percentages of 0, 60, 50, 63.3 and 50% of the examined samples with a mean values of 0, $2.2\times10^4\pm0.33\times10^2$, $4.7\times10^4\pm9.9\times10^3$, $8.6\times10^4\pm0.4\times10^2$ and $5.2\times10^4\pm2.5\times10^2$ CFU/ml for plain, mango, strawberry, banana and orange drinking yoghurt samples, respectively. There was significant difference between the plain and different kinds of flavoured samples ($P<0.05$). The *Enterococci* resist heat treatment and can grow at wide range of temperature. They are normal inhabitants of the alimentary tract of man and animals, from which such organisms can find its way into milk and drinking yoghurt. Therefore these organisms are useful indicators of the possible presence of enteric pathogens. Furthermore, may help in assessing the standard of hygiene in dairy factories (Rodriguez *et al*., 2003).

Results demonstrated in Table (7) revealed that mean values of *Staphylococci* count in different kinds of drinking yoghurt were $1.5\times10^2\pm0.28\times10^2$, $2.7\times10^3\pm0.9\times10^2$, $6.3\times10^3\pm1.1\times10^2$, $4.4\times10^3\pm0.1\times10^2$ and $5.0\times10^3\pm2.5\times10^2$ CFU /ml with percentages of 26.6, 53.3, 63.3, 56.6 and 60.0%, respectively. For the examined mango, strawberry, banana and orange drinking yoghurt samples respectively. There was significant difference between the plain and different kinds of flavoured samples ($P<0.05$). Yoghurt may be subjected to contamination by different pathogens as *Staphylococcus aureus* which may get access before, during, or even after processing rendering the product unsafe for

Table 5: Total *Coliform* count in examined drinking yoghurt samples (n =30)

Type	Positive samples		Min.	Max.	Mean
	No.	%			
Plain	4	13.3	10	2.4×10^4	$1.9\times10^3\pm2\times10^{2a}$
Mango	15	50	10	3.2×10^6	$5.0\times10^5\pm2.9\times10^{4b}$
Strawberry	26	86.7	1.0×10^2	9.1×10^7	$4.7\times10^6\pm8.5\times10^{5b}$
Banana	23	76.7	6.1×10^2	2.0×10^7	$1.0\times10^6\pm1.7\times10^{5b}$
Orange	17	56.7	1.9×10^2	7.0×10^6	$9.9\times10^6\pm4.8\times10^{5b}$

Means of different superscript (a and b) considered significantly different at 95%.

Table 6: Statistical analytical results of *Entercocci* counts in examined stirred yoghurt samples (n=30)

Type	Positive samples		Min.	Max.	Mean
	No.	%			
Plain	0	0	0	0	0^b
Mango	18	60	100	2.1×10^5	$2.2\times0^4\pm0.33\times10^{2a}$
Strawberry	15	50	20	6.3×10^5	$4.7\times0^4\pm9.9\times10^{3a}$
Banana	19	63.3	10	2.4×10^5	$8.6\times0^4\pm0.4\times10^{2a}$
Orange	15	50	10	1.9×10^5	$5.2\times0^4\pm2.5\times10^{2a}$

Means of different superscript (a and b) considered significantly different at 95%.

Table 7: Statistical analytical results of *Staphylococci* count in examined samples (n=30)

Type	Positive samples		Min.	Max.	Mean
	No.	%			
Plain	8	26.6	20	5×10^4	$1.5\times10^2\pm0.28\times10^{2a}$
Mango	16	53.3	20	4.0×10^5	$2.7\times10^3\pm0.9\times10^{2b}$
Strawberry	19	63.3	10	8.9×10^4	$6.3\times10^3\pm1.1\times10^{2b}$
Banana	17	56.6	30	3.2×10^5	$4.4\times10^3\pm0.1\times10^{2b}$
Orange	18	60	10	1.1×10^4	$5.0\times10^3\pm2.5\times10^{2b}$

Means of different superscript (a and b) considered significantly different at 95%.

human consumption. The growth of *Staphylococcus aureus* in drinking yoghurt presents a potential public health hazard since many strains of *Staphylococcus aureus* produce entertoxins that cause food poisoning. The most common symptoms are nausea, vomiting and diarrhea. However in severe cases they may be accompanied by acute abdominal cramps. Symptoms usually occur 2-6 hours after ingestion of the contaminated food (Zuniga *et al*., 1999).

Inspection of data presented in Table (8) revealed that *Pseudomonas* and *Aeromonas* could be detected in plain and flavoured drinking yoghurt samples in a percentages of 23.3, 50**,** 66.7**,** 33.3 and 40% with a mean values of $2\times10^2\pm0.1\times10^2$, $1.1\times10^3\pm0.7\times10^2$, $2.4\times10^3\pm1.2\times10^2$, $1.0\times10^3\pm 0.01\times10^2$ and $3.0\times10^3\pm1.7\times10^2$ CFU/ ml of the examined plain, mango, strawberry, banana and orange samples, respectively. There was significant difference between the plain and different kinds of flavoured samples ($P<0.05$). Nearly similar finding were reported by Salminen, (1994) and Kraft (1995). *Pseudomonas* species are widely distributed in nature, they has been found in external environmental conditions surrounding dairy plant such as water, soil, sewage and air. These organisms represent the most common psychrotrophs that contaminated drinking yoghurt and cause a variety of defects including fruity, rancid, bitter and putrid flavour as well as color defects. With the extensive use of refrigerated storage of drinking yoghurt, the significance of *Pseudomonas* species in the spoilage of drinking yoghurt has increased dramatically due to production of proteinase, lipase, phospholipase C and glycosidase enzymes strongly damaging milk fat protein membrane which reflect on the quality of the finished products (Jayarao & Yung, 1999). *Pseudomonas* species can be eliminated by pasteurization or UHT treatment, but their enzymes are able to resist heat treatment used for processing of raw milk and have been implicated in spoilage of ultra heat treated milk and other dairy products (Lira & Nielsen, 1998, McKay, *et al*., 2000).

Salmonella and *Yersinea* species failed to be detected in all examined samples of drinking yoghurt. *Bacillus cereus* could be detected in flavoured drinking yoghurt samples in a percentages of 0, 33.3, 30, 36.6 and 23.3% with an average of 0, $5.1\times10^4\pm1.3\times10^2$ and $1.1\times10^4\pm 0.01\times10^3$, $1\times10^4\pm2.2\times10^3$, $2.4\times10^5\pm1.1\times10^4$ in examined plain, mango, strawberry, banana and orange samples, respectively (Table 9). Presence of *Bacillus cereus* in examined flavoured drinking yoghurt is not only of concern as a public health hazard, but also as a cause of eco-

Table 8: Statistical analytical results of *Pseudomonas* and *Aeromonas* counts in examined samples (n=30)

Type	Positive samples		Min.	Max.	Mean
	No.	%			
Plain	7	23.3	10	1.0×10^3	$2\times10^2\pm 0.1\times10^{2a}$
Mango	15	50	100	3.0×10^4	$1.1\times10^3\pm0.7\times10^{2b}$
Strawberry	20	66.7	100	6.8×10^4	$2.4\times10^3\pm1.2\times10^{2b}$
Banana	10	33.3	50	1.2×10^4	$1.0\times10^3\pm0.01\times10^{2b}$
Orange	12	40	60	6.6×10^4	$3.0\times10^3\pm1.7\times10^{2b}$

Means of different superscript (a and b) considered significantly different at 95%.

Table 9: Statistical analytical results of *Bacillus cereus* count in examined samples (n=30)

Type	Positive samples		Min.	Max.	Mean
	No.	%			
Plain	0	0	0	0	0^{b}
Mango	10	33.3	100	1.0×10^{5}	$5.1\times10^{4}\pm1.3\times10^{3a}$
Strawberry	9	30	10	2.2×10^{5}	$1.1\times10^{4}\pm0.01\times10^{3a}$
Banana	11	36.6	10	3.9×10^{5}	$1.0\times10^{4}\pm2.2\times10^{3a}$
Orange	7	23.3	10	1.9×10^{3}	$2.4\times10^{5}\pm1.1\times10^{4a}$

Means of different superscript (a and b) considered significantly different at 95%.

nomic losses as off flavour, bitter taste and unpleasant odour (Salminen, 1994, Giffel, *et al*., 1996). *Bacillus cereus* is a food poisoning microorganisms widely distributed in nature. Its ability to form spores ensures survival of the organism through all stages of yoghurt processing. The organism has been well documented as being able to produce two distinct food poisoning. The emetic type is mainly characterized by acute attack of nausea and vomiting within 1-5 hours after food consumption while the diarrhoeagenic type is characterized by abdominal pain and diarrhea within 8-16 hours incubation and lasts 12-24 hours. Although the diseases are characterized by low mortality rate and short duration, the frequency of outbreak and severity of the symptoms make them as an important food borne hazard. Relatively high number of *B. cereus* need to be present in food to cause food illness. Food implicated with the syndrome outbreaks had counts ranging from 1×10^{3} to 5×10^{10} CFU /g with an average of 1×10^{5} CFU/g (Rossland *et al*., 2005).

Results concerning the survival of *Listeria monocytogenes* in plain and flavoured strawberry yoghurt stored at 4C inoculated at 6.6×10^{5} CFU/ml are summarized in Table (10). The achieved results revealed that *Listeria monocytogenes* was slightly decreased in count in plain yoghurt from 6.6 $x10^{5}$ to 2.5×10^{5} CFU/ml after 30 days of cold storage and pH was reduced from 4.2 to 3.0 while in flavoured strawberry yoghurt *Listeria monocytogenes* was decreased from 6.6×10^{5} to 2.6×10^{5} CFU/ml with pH 4.25 to 3.11 during cold storage. There was no change in the surviving populations of *Listeria monocytogenes* in both plain and flavoured drinking yoghurt. The population of *Listeria monocytogenes* remained unchanged for up to 10 days. There was reduction in viable populations after 10 days and up to 30 days of storage averaged 4.1×10^{5} CFU/ml. This finding show that *Listeria monocytogenes* can survive at pH as low as 3.1. During cold storage plain and strawberry flavoured yoghurt exhibited the same decline in surviving populations. Nearly similar findings were reported by Zuniga *et al*. (1995), Benkerroum *et al*. (2002) and Barrantes *et al*. (2004). The pH of the samples was changed from 4.62 and 4.25 to 3 and 3.11 for both plain and flavoured drinking yoghurt, respectively. The decrease of pH in drinking yoghurt can be explained by the persistant metabolic activity of lactic acid bacteria during cooling (Berrocal *et al*., 2005). Acid resistance and

Table 10: Survival of *Listeria monocytogenes* in plain and flavoured yoghurt inoculated at $6.6x10^{5}$ CFU/ml and stored at 4°C for 30 days

Storage time/day	Plain		Flavoured with strawberry	
	pH	CFU/ml	pH	CFU/ml
0	4.26	6.60×10^{5}	4.25	6.60×10^{5}
5	4.12	6.60×10^{5}	4.13	6.60×10^{5}
10	4.00	6.50×10^{5}	4.00	6.50×10^{5}
15	3.92	4.93×10^{5}	3.95	4.81×10^{5}
20	3.81	3.52×10^{5}	3.51	3.45×10^{5}
25	3.60	3.88×10^{5}	3.40	3.30×10^{5}
30	3.00	2.50×10^{5}	3.11	2.60×10^{5}

acid tolerance bacteria are important virulence determinants that contribute to the survival and pathogenicity of infectious food borne pathogens to cause disease. Acid resistance increases the portion of the population that survives gsastric infectivity once the pathogens attaches to the intestinal tract (Peterson *et al*., 1989, Jayaro & Yung, 1999). Although there have been no reports of the isolation of *Listeria monocytogenes* from drinking yoghurt, the possibility of contamination exists. A potential health hazard could arise if sufficient number of *Listeria monocytogenes* contaminate drinking yoghurt after heat treatment under unhygienic measures or improper sanitary methods during production and handling.

CONCLUSION

One can realize from the results achieved that flavoured drinking yoghurt still harbor undesirable organisms that affect its quality and utility. Therefore, to improve the quality of the products good hygienic measures should be adopted during milk storage, added ingredients (fruit mixes, flavours, stabilizers and emulsifiers), periodical inspection of the dairy plants as well as the control of the final product during production, packaging and distribution.

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دراسة مقارنة على الحمل الميكروبي لمشروب اللبن الزبادي المصري المُطَعم وغير المُطَعم

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لتحقيق الدقة في الاختيار الخاص باللبن الزبادي المطعم وغير المطعم فقد تم تجميع عدد ١٥٠ عينة من الزبادي الساده والمطعم بأي من الفراولة، المانجو، الموز، البرتقال من أسواق محافظات القاهرة والجيزة بجمهورية مصر العربية. وتوصلت النتائج المتحصل عليها في هذه الدراسة إلى أن الجراثيم الهوائية في عينات الزبادي الساده والمطعم بالفراولة، المانجو، الموز، البرتقال تتراوح بين ٦,٤×١٠^{٣}±١,١×١٠^{٢}، ١,٣×١٠^{٥}± ٢,١×١٠^{٤}، ٣,٢×١٠^{٦}± ٤,٣×١٠^{٥}، ٦,٩×١٠^{٥}±٧,٢×١٠^{٤}، ٤,٢×١٠^{٦}±٦,٦×١٠^{٥} وحدة تكوين مستعمرة/١مل على الترتيب. بينما ظهرت الفطريات والخمائر بنسبة مئوية ٣٠، ١٠٠، ١٠٠، ١٠٠، ٩٠% على الترتيب من العينات المختبرة.

كما لوحظ أن الـ *Coliform*، *Enterococci*، *Staphylococci* تتواجد بالأنواع المختلفة في الزبادي المطعم بينما تراوحت النسبة المئوية لكل من الـ *Aeromonas*، *Psudomonas* بالإضافة إلى الـ *B. cereus* ما بين ٢٣,٣-٦٦,٧%، صفر-٣٦,٦% على الترتيب.

وقد اختفت كل من *Salmonell*، *Yersinia* في كل العينات المختبرة. كما تم اختبار مدى بقاء الـ *Listeria monocytogenes* في عينات الزبادي المطعم والمخزنة في الثلاجة لمدة ٣٠ يوماً. وقد نوقشت النواحي المتعلقة بالصحة العامة والأهمية الاقتصادية للمجاميع الميكروبية من حيث معايير السلامة الغذائية الموصى بها.