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A COMPARATIVE STUDY ON THE QUALITY OF PLAIN AND PROBIOTIC YOGHURT

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ABSTRACT

A total of 40 random samples of plain and probiotic yoghurt (20 of each) were collected from different markets in Kaliobia governorate to evaluate their quality. The sensory characteristic score of probiotic yoghurt (91.69± 7. 86) was significantly better than plain one (77.91 ± 8.16). The chemical examination indicated that the average values of pH and titratable acidity were 3.63 ± 0.07 & 0.91 ± 0.07 for plain yoghurt and 4.29 ± 0.05 & 0.72 ± 0.06 for probiotic yoghurt. On the other hand, the bacterial counts in the examined plain and probiotic yoghurt samples were $1.35 \times 10^4 \pm 0.22 \times 10^4$ and $2.12 \times 10^3 \pm 0.39 \times 10^3$ /g for aerobic spore formers count, $4.78 \times 10^2 \pm 0.61 \times 10^2$ and $1.31 \times 10^2 \pm 0.17 \times 10^2$ /g for B.cereus count, $2.01 \times 10^3 \pm 0.34 \times 10^3$ and $4.73 \times 10^2 \pm 0.52 \times 10^2$ /g for staphylococci count and $7.24 \times 10^2 \pm 1.83 \times 10^2$ and $2.58 \times 10^2 \pm 0.41 \times 10^2$ /g for coliform, respectively. Furthermore, B. cereus, B. circulans, B.coagulans, B.licheniformis, B.macerans, B.stearothermophilus and B. subtilis, S. aureus and S. epidermidis as well as micrococci were isolated from both plain and probiotic yoghurt with varying percentages. However, Enteropathogenic Escherichia coli (EEC) were isolated from 5% of plain yoghurt samples and serologically identified as O_{26} : K_{60} (B_6) and O_{128}: K_{67} (B_{12}). In contrast, all examined samples of probiotic yoghurt were free from E.E.coli. According to sensory, chemical and bacteriological results, probiotic yoghurt appeared to be better and more safe for human consumption as compared with plain one.

INTRODUCTION

Yoghurt is the best known of all-cultured milk products and the most popular almost allover the world. A high-quality yoghurt with the required flavour, aroma, viscosity and appearance can be adopted by carefully choose of milk, adequate milk standardization, use of good quality stabilizers, efficient heat treatment of yoghurt mix, use of active non-contaminated starter culture, adequate incubation with good storage conditions as well as good hygienic measures during processing, storage and marketing of yoghurt ***(Tamime & Robinson, 1985 and Tetra Pak, 1995).***

The typical starter culture for plain yoghurt are *Streptococcus salivarius subsp. thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus* which added in a ratio of (1:1) to produce an ideal titratable

acidity around 0.65- 0.70% following by cooling at 5°C to be fit for consumption up to 2 weeks ***(ES, 1990 and Jay et al., 2005)***.

Bifidobacteria, *Lactobacillus acidophilus* and some enterococci species are the most potential probiotics used. They can grow in association with other typical yoghurt culture for their successful growth and doing the best role ***(Dave and shah, 1998 and Fooks et al., 1999).*** Foods containing probiotic are known as "Functional Foods" that have live active probiotic microorganisms which produce their healthy benefits over its normal nutritional value and being similar in appearance to conventional food patterns ***(FAO/WHO, 2001; Kwak and Jukes, 2000 and Saxelin et al., 2003).***

Healthy effects attributed to consumption of probiotic food are; alleviation of lactose maldigestion, reduction of serum cholesterol level, prevention and treatment of some intestinal and urogenital infections and alleviation of constipation as well as increasing the immune response ***(Cathy, 1995 a & b; Gardiner et al., 2002; Hui, 1993 and WHO, 1995)***.

Owing to the great healthy benefits as well as the nutritional value of plain and probity yoghurt, the evaluation of their quality at market level is essential. The evaluation was planned out to include sensory, chemical and bacteriological examination during their sale.

MATERIAL AND METHODS

Fourty samples of plain and probiotic yoghurt (20 of each) were randomly collected from various markets in Kaliobya governorate to compare between their sensory, chemical and bacteriological quality.

1. Sensory evaluation:

The sensory evaluation of yoghurt samples was adopted by well trained 9 panelists from Food Control Department, Faculty of Veterinary Medicine, Benha University. The sensory scores were carried out as that recommended by ***Tamime and Robinson (1985)*** .

2. Chemical examination:-

2.1. Determination of pH *(pearson, 1981)*:-

The pH values were measured by using pH meter ***(C.D. 2026, Fischer model, Germany).***

2.2. Determination of Titratable acidity:-

Titratable acidity of yoghurt samples was carried out according to ***Kosikowski and Mistry (1997).***

3. Bacteriological examination:-

3.1. Preparation of the samples:-

Yoghurt containers were opened aseptically and 10g of each sample were homogenated with 90 ml sterile saline where tenth fold serial dilutions were prepared ***(BSI, 1984).***

3.2. Enumeration and identification of aerobic spore formers:-

Aerobic spore formers count was essayed according to ***Harrigan and Macane (1976)*** by surface plating on Dextrose Tryptone agar medium. Suspected colonies were picked up and subcultured on nutrient agar slopes for further identification.

3.3 Enumeration and identification of *Bacillus cereus*:-

The method adopted by ***Holbrook and Anderson (1980)*** was performed by using *B. cereus* selective agar medium.

3.4. Enumeration and identification of *Staphylococcus aureus*:-

Staphylococcus aureus count was determined by the technique recommended by ***ICMSF (1986)*** using Baired Parker agar medium.

3.5. Enumeration of coliforms (MPN):-

Three replicate MacConkey broth tubes were applied for enumeration of coliform bacteria according to the method adopted by ***APHA (1992).***

3.6. Screening of *E. coli*:-

The technique recommended by ***APHA (1992)*** was carried out using MacConkey broth and Eosin Methylene Blue **(EMB).** The suspected green metallic colonies were identified biochemically and seriologically according to ***Krieg and Holt (1984).*** Antisera used for typing of *E-coli* were coli test sera Poly I, coli test sera Poly II and Bacto *E.coli* antisera.

4. Statistical analysis:-

The obtained results were statistically evaluated by student t. test according to ***Feldman et al. (2003).***

RESULTS

Table (1) Mean values of sensory characteristics of the examined samples of plain and probiotic yoghurt (n=20).

Type of samples Sensory Character	Final Score	Plain yoghurt Mean ± S.E	Probiotic yoghurt Mean ± S.E
Flavor	45	36.21 ± 4.15	42.30 ± 5.25
Body & Texture	30	23.97 ± 3.07	28.12 ± 2.79
Acidity	10	6.13 ± 0.83	9.04 ± 1.15
Appearance	10	7.72 ± 0.90	8.28 ± 0.81
Container & Closure	5	3.88 ± 0.49	3.95 ± 0.57
Overall score	100	77.91 ± 8.16	91.69 ± 7.86

Table (2) statistical analytical results of acidity indices of the examined samples of plain and probiotic yoghurt (n=20).

Type of samples	Plain yoghurt			Probiotic yoghurt		
Acidity indices	**Min**	**Max**	**Mean± S.E**	**Min**	**Max**	**Mean ± S.E**
pH	3.35	4.42	3.63± 0.07	3.84	4.71	4.29 ± 0.05*
Titratable acidity %	0.61	1.40	0.91 ± 0.07	0.55	1.03	0.72 ± 0.06**

*Significant differences ($p < 0.05$) ** high significant differences ($p< 0.01$)

Table (3) Bacteriological aspects of the examined samples of plain and probiotic yoghurt (n=20).

Type of samples / Bacterial group	Plain yoghurt					Probiotic yoghurt				
	positive samples		Min	Max	Mean±S.E	positive samples		Min	Max	Mean ± S.E
	No	%				No	%			
Aerobic sporeformers count	12	60	$4X10^2$	$3.2X10^4$	$1.35X10^4 \pm 0.22X\ 10^4$	7	35	$1X10^2$	$7X10^3$	$2.12X10^3 \pm 0.39X10^{3*}$
B.cereus count	8	40	2X10	$1.5X\ 10^3$	$4.78X10^2 \pm 0.61X10^2$	3	15	1X10	$9X10^2$	$1.31X10^2 \pm 0.17X\ 10^2$
Staphylococci count	14	70	$1X10^2$	$4.8X\ 10^3$	$2.01X10^3 \pm 0.34X\ 10^3$	6	30	$1X10^2$	$1.1X10^3$	$4.73X10^2 \pm 0.52X\ 10^2$
Coliform count (MPN)	9	45	4X10	$1.1X\ 10^3$	$7.24X\ 10^2 \pm 1.83X\ 10^2$	4	20	1X10	$7X10^2$	$2.58X10^2 \pm 0.41X\ 10^2$

* Significant differences ($p <0.05$)

Table (4) Incidence of aerobic spore forming bacteria in the examined samples of plain and probiotic yoghurt (n=20)

Type of samples / Identified bacteria	Plain yoghurt		Probiotic yoghurt	
	No	%	No	%
B.cereus	8	40	3	15
B.circulans	-	-	2	10
B.coagulans	2	10	-	-
B.licheniformis	7	35	3	5
B.macerans	1	5	-	-
B.stearothemophilus	3	15	-	-
B.subtilis	4	20	1	5

Table (5) Incidence of Gram positive staphylococci in the examined samples of plain and probiotic yoghurt (n=20)

Type of samples / Gram + ve cocci	Plain yoghurt		Probiotic yoghurt	
	No	%	No	%
Staph.aureus	3	15	-	-
Staph.epidermidis	7	35	2	10
Micrococci	12	60	5	25

Table (6) Incidence and serotyping of Enterpathogenic *E.coli* in the examined samples of plain and probiotic yoghurt (n=20).

Type of samples / E.coli serotypes	Plain yoghurt		Probiotic yoghurt		Strain characteristic
	No	%	No	%	
O_{26} : K_{60} (B_6)	1	5	-	-	EHEC
O_{128} : K_{67} (B_{12})	1	5	-	-	ETEC
Total	2	10	-	-	

EHEC= Enterohaemorhagic *E.coli* ETEC= Enterotoxigenic *E.coli*

DISCUSSION

Quality can be judged by subjective and objective tests. The subjective tests include; odour, taste and texture. While, objective tests include; chemical, physical and the microbial aspects ***(Hayes, 1992).***

Table (1) pointed out that the overall mean scores for sensory evaluation of the examined plain and probiotic yoghurt samples were 77.91± 8.16 and 91.69 ± 7.86, respectively.

The current results come in accordance with those reported by ***Mohamed (1990)*** and ***EL-Shibiny et al. (2005)*** who mentioned that both plain and probiotic yoghurts were very acceptable with good sensory characteristics prevalid for 10 days when stored in refrigerator.

A good yoghurt should have a clean mild acid, walnutty to delicate flavour and smooth homogenous body texture with no free whey ***(Savello& Dargan 1995 and Kosikowski & Mistry, 1997).*** In addition, the yoghurt container should be clean, sound, free from any leakers and tightly closed by well sealed lid. All ingredients, expirey date and nutritional value should be labeled clearly on it ***(Vaclavik and Christian, 1998).***

In this respect, all examined samples of probiotic yoghurt had higher sensory characteristic scores as compared with those of plain yoghurt. In other words, probiotic strains was significantly improved the flavour, texture as well as acidification of yoghurt to be acceptable for consumer.

Inspection of table (2) indicated that the pH ranged from 3.35 to 4.42 with a mean value of 3.63 ±0.07 for plain yoghurt and 3.84 to 4.71 with a mean value of 4.29 ± 0.05 for probiotic yoghurt.

Nearly similar pH values were recorded by ***Abdel All (1997).*** While, higher results were obtained by ***Neamah (2006).***

The pH 4.73 is considered the minimum pH value that sustained successful probiotic microbial numbers ***(EL-Shibiny et al., 2005).***

The ideal pH value should be 3.5- 4.5 at the end of fermentation period at which the yoghurt curd formed ***(Kosikowski and Mistry, 1997).*** As a result, Yoghurt acts as safe food where many spoilage and pathogenic microorganisms can't grow under such condition ***(ICMSF, 1986 and Zambou et al, 2004).***

The titratable acidity ranged from 0.61 to 1.40 with an average of 0.91 ± 0.07 for plain yoghurt and 0.55 to 1.03 with an average of 0.72 ± 0.06 for probiotic yoghurt samples (table, 2).

The titratable acidity of yoghurt acts as indicator for the activity of starter culture in which the ideal curd is formed and should be followed by controlled cooling to avoid excess acidity ***(Robinson & Tamime, 1993)***.

In general, both pH and titratable acidity are of great concern to judge the yoghurt quality. Therefore, selection criteria of ideal starter culture of fermented milks that produce suitable acidification with acceptable flavour and texture for consumer is wanted ***(Dako et al., 1995 and Lee et al., 1990).***

Contributing to bacteriological examination, the results in table(3) declared that 60% and 35% of examined plain and probiotic yoghurt samples were contaminated with aerobic sporeformers with average counts of $1.35 \times 10^4 \pm 0.22 \times 10^4$/g and $2.12 \times 10^3 \pm 0.39 \times 10^3$/g, respectively.

The mean values of *B. cereus* counts were $4.78 \times 10^2 \pm 0.61 \times 10^2$/g and $1.31 \times 10^2 \pm 0.17 \times 10^2$ for plain and probiotic yoghurt samples, respectively.

Lactic acid bacteria (LAB) showed marked inhibition against spore forming bacteria as the pH decreased. They can inhibit the growth of *B. cereus* and obviously depressed its spore germination ***(Driessen & Stadhouders, 1982 and Sultan, et al., 1988).***

In England, *B. cereus* was found to be the most frequently spore former that responsible for spoilage of most dairy products ***(Davies, 1975).***

The mean coliform counts were $7.24 \times 10^2 \pm 1.83 \times 10^2$/g for plain yoghurt and $2.58 \times 10^2 \pm 0.41 \times 10^2$/g for probiotic yoghurt samples (table, 3).

High counts of coliform give an indication about the unsatisfactory sanitary conditions under which the yoghurt was manufactured and stored ***(El-Etriby et al., 1997; Hosny, 2002 and Neamah, 2006).***

In this respect coliforms couldn't survive the normal yoghurt acidity due to the inhibitory effect of LAB starter culture ***(Mohmed & Younis 1990).***

Incidence of aerobic spore forming bacteria isolated from the examined samples of plain and probiotic yoghurt is shown in table (4). Accurately, *B. cereus* (40%), *B. coagulans (10%), B. licheniformis*

(35%), B. macerans (5%), B. stearothermophilus (15%) and B. subtilis (20%) were isolated from the examined plain yoghurt samples. However, *B.cereus B. circulans, B.licheniformis and B. subtilis* were isolated from 15, 10, 5 and 5% of the examined samples of probiotic yoghurt.

These findings indicate that probiotic strains had great destructive effects on aerobic spore forming bacteria either qualitatively or quantitatively.

Presence of heat resistant Bacillus species may attributed to their introduction into milk supplies from various sources including water, soil, tanks, pumps, pipelines and/or processing equipments ***(Meer et al., 1991).***Some strains of Bacillus species especially *B. cereus* was recognized as food poisoning pathogen and can produce diarrhoegenic toxin ***(Griffiths, 1990 and Eley, 1996).***

Results given in table (5**)** proved that *S.epidermidis* (35% & 10%) and micrococci (60% & 25%) were isolated from examined samples of plain and probiotic yoghurt, respectively.

In contrast, *S. aureus* was isolated only from 15% of examined plain yoghurt samples. While, all probiotic yoghurt samples were free from such organism.

Accurately, 70% and 30% of examined plain and probiotic yoghurt samples were contaminated by staphylococci with average counts of $2.01 \times 10^3 \pm 0.34 \times 10^3$/g and $4.73 \times 10^2 \pm 0.52 \times 10^2$/g, respectively (table 3).

Similar results were recorded by ***EL-Shafei et al. (2002) and Dabiza et al. (2005).***

Lactic acid bacteria metabolites (organic acids, hydrogen peroxide, diacetly, bacteriocins and bactericidal protein) have antimicrobial activity against food borne pathogens especially *S. aureus* and *B.cereus* as well as they can control some food spoilage organisms ***(Holzapfel et al., 1995 ; Stiles, 1996; Sharaf et al., 1997; Bayàzyt and Yýlsay, 2000;Du Toit et al., 2000 and Navarro et al., 2000).***

Fermented dairy products which fortified with bifidobacteria have more metabolites with high concentration of lactic and acetic acids leading to longer keeping quality ***(Gobbetti et al., 1998 and Abou Dawood, 2002).***

Seriological identification of Enteropathogenic *E.coli* "EPEC" isolated from the examined plain yoghurt samples were one strain **(**5%) of O_{26} : K_{60} (B_6) and one strain (5%) of O_{128} : K_{67} (B_{12}). While, probiotic yoghurt samples were free from Enteropathogenic *E. coil* as shown in table (6).

Escherichia coli O_{26}*:* K_{60} serovar is belonged to Enterohemorrhagic *E.coli* "EHEC" that associated mainly with bloody diarrhea in human ***(Levine, 1987).*** While, O_{128} : K_{67} is referred to Enterotoxigenic *E.coli* "ETEC" which produce heat labile and/or heat stable toxins and may exist in feaces of human carriers up to several months ***(Cliver, 1990).***

It is of great concern to mention that presence of probiotic strains in the yoghurt leads to complete destruction of all strains of *E.coli* resulting in a safety product for the consumers.

Generally, all examined samples of plain yoghurt were highly contaminated with most bacterial groups than probiotic ones. This evidence may explain the role of probiotic strains which produce a wide range of antimicrobial metabolites against certain spoilage bacteria to gain access in yoghurt.

Finally, the present study allow to conclude that the probiotic yoghurt is preferable than plain one where the probiotic stains have the ability to improve the sensory, chemical and bacteriological profiles of yoghurt in addition to its known health benefits for human being.

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