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Antibacterial Activity of Probiotic Yoghurt and Soy-Yoghurt against *Escherichia coli* and *Staphylococcus aureus*

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Abstract

Introduction: Biopreservation systems in food are becoming increasingly interesting for the industry and consumer.

Methods: Yoghurt milk and soymilk samples were inoculated separately with *E.coli* or *S. aureus* immediately after adding the starter (bifidobacteria and/or yoghurt culture) to investigate the antimicrobial activity of probiotic yoghurt and soy-yoghurt.

Results: Probiotic yoghurt containing *Bifidobacterium lactis* (Bb-12) and *Bifidobacterium longum* (Bb-46) exhibited a slight pH drop compared with plain yoghurt (without bifidobacteria) during the refrigerated storage period. Plain yoghurt and probiotic yoghurt containing Bb-12 and Bb-46 inoculated with or without test organisms showed a significant ($P<0.05$) increase in lactic and acetic acids than the probiotic soy-yoghurt containing Bb-12 and Bb-46 which produces not only lactic and acetic acids but also formic acid. From the initial count of *E.coli*, % decreases were 96.00, 99.43, 99.14, 97.14 and 98.43%, for the plain yoghurt, probiotic yoghurt containing Bb-12 & Bb-46 and soy-yoghurt containing Bb-12 and Bb-46, respectively. E. coli counts were disappeared in probiotic yoghurt, soy- yoghurt and plain yoghurt after 2, 3 and 5 days of storage, respectively .The decrease percentage for the plain yoghurt, probiotic yoghurt containing Bb-12 and Bb-46 and soy-yoghurt containing Bb-12 & Bb-46 were 85.62, 93.36, 95.58, 93.36 and 95.58 from the initial inoculum level, respectively. The growth of *S.aureus* was not detected in the probiotic yoghurt containing Bb- l 2 and Bb-46 after the 10th day of storage. Low numbers of *S.aureus* survived in the plain yoghurt and probiotic soy-yoghurt Bb- 12 and Bb-46, after 15 days of cold storage.

Conclusion: According to this data probiotic yoghurt, Soy-yoghurt and their antibacterial metabolites can be used to control pathogenic microorganisms

Keywords: Antibacterial activity; *E. coli*; *S.aureus*; yoghurt; Soy-yoghurt; pH; Organic acids

Introduction

The interaction between food and health is a very complex one. Accordingly, the food industry has unique opportunities to develop products that are not only nutritional in the traditional sense, but which have additional activity that can lead to an improved state of health and well-being and/or reduction in risk disease «functional foods» Probiotics have been defined as «live microbial feed supplements that have beneficial effects on the host by improving its intestinal microbial balance» [1]. Bifidobacteria are known to exhibit inhibitory effects on many pathogenic organisms both in vivo and in vitro, including Salmonella, Shigella, Colstridium, Bacillus cereus, *Staphylococcus aureus*, *Candida albicans*, *Listeria monocytogenes*, *Escherichia coli* and *Campylobacter Jejuni* [2-4]. Recent studies on probiotics showed that the fermented products of probiotics possess strong anti-bactericidal effects against foodborne pathogens [5]. Because bifidobacteria has been associated with health-promoting effects, there has been an increasing in incorporating this microbial group into dairy and dairy like foods or supplementing dairy foods with these organisms. The ultimate intent of this strategy is to provide the gastrointestinal tract of humans with viable populations of bifidobacteria.

Fermented milks containing bifidobacteria are made either using pure strains or in combination with other lactic acid bacteria [6] recently, soy-yoghurt has been prepared with the fermentation of lactic acid bacteria [7] or bifidobacteria in soymilk [8].

Although, numerous studies have been focused on the antibacterial activity of yoghurt, still relatively little is known about the potentially beneficial roles of yoghurt and soy-yoghurt containing bifidobacteria with regards to their potential role in inhibiting food-borne pathogens. Therefore, this study was designed to determine the inhibitory activity of probiotic yoghurt and soy-yoghurt against *Escherihia coli* and *Staphylococcus aureus* during the refrigerated storage.

Materials and Methods

Preparation of yoghurt from buffalo milk

Low-fat buffalo milk 1.5% (w/w) was inoculated with a 3% (v/v) liquid culture of *Lactobacillus delbrueckii* subsp. bulgaricus and *Streptococcus salivarius* subsp. thermophilus (Chr. Hansen Laboratories, Copenhagen, Denmark), and then divided into three portions. No bifidobacteria were added to the first portion (plain yoghurt). To the second portion, a 0.07% (w/v) standardized freeze-dried culture of *Bifidobacterium lactis Bb-12* was added. To the third portion, a 0.07% (w/v) standardised freeze-dried culture of *B. longum Bb-46* was

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added. The two strains were obtained from Chr. Hansen Laboratories (Copenhagen, Denmark).

Preparation of soy- yoghurt

Fresh and non-beany-flavor soy milk was prepared according to Tanteeratarm [9] and divided into two portions for preparation of soy yoghurts Bb-12 and Bb-46 according the method described by Abd El-Gawad [8]. The manufacture of soy yoghurts Bb-12 and Bb-46 involved the addition of gelatin at a level of 1% (w/w) to the soy milk, heating at 95°C for 5 min, and cooling to 37°C. One portion was inoculated at a level of 0.07% (w/v) with a freeze-dried *B.lactis Bb-12* the other portion was inoculated at a level of 0.07% (w/v) with a freeze-dried *B.Longum Bb-46*.

The Pathogenic microorganisms

E. coli and *S. aureus* (as a liquid culture cultivated in nutrient broth were obtained from Egyptian Microbial Culture Collection (EMCC) at Microbiological Resources Center (MIRCEN) Faculty of Agriculture, Ain Shams University, Cairo, Egypt and Dairy Microbiology Department, National Research Center, Giza, Egypt, respectively. These two test organisms were added separately to pasteurized yoghurt milk and soy-milk at 45°C immediately after adding the starter cultures. After incubation all samples (yoghurt & soy yoghurt) were stored at 4 ± 1°C.

Analytical methods

Counts of yoghurt cultures and Bifidobacterial: Bacterial counts of *S. salivarius subsp. thermophilus* and *L. delbrueckii subsp. bulgaricus* in yoghurt were determined according to Lee [10] in which the yoghurt sample was added to Lee's agar and incubated at 43°C for 3 days. Bifidobacteria were enumerated in soy yoghurt, which did not count in yoghurt cultures, by a poured plate method using Lactobacilli MRS-agar medium as described by [11] Bifidobacteria were enumerated in yoghurt containing Bb-12 or Bb-46 according to the method of [12] in which a mixture of antibiotics, including 2 g paromomycin sulfate, 0·3 g nalidixic acid, and 60 g lithium chloride, was dissolved in 1 litre distilled water, filter-sterilised (0.2 mm) and stored at 4°C until use. The antibiotic mixture (5 ml) was added to 100 ml MRS-agar medium. L-Cysteine-HCl 0.5% (Sigma Chemical Co., St Louis, MO, USA) was also added to decrease the redox potential of the medium. Plates were incubated at 37°C for 48 h anaerobically.

***E. coli* count:** The *E.coli* count was estimated by platting suitable dilution on Macconkey Agar medium (Oxid) as recommended by the [13] the plates were incubated at 37°C for 24 h.

***Staphylococcus aureus* count**: Determination of *S.aureus* was carried out using mannitol salt agar medium as described in [14].

pH value: It was measured by using Orion pH-meter, model 501 at 20°C.

Determination of organic acids by HPLC: Organic acids in yoghurt and soy-yoghurt were determined using HPLC according to the method, of [15] with slightly modification as follows;

Extraction of organic acids: 10 g of sample was centrifuged at 10000 rpm for 25 min and the supernatant was filtrated through a 0.2 μm Millipore membrane filter then 1-3 ml was collected in a vial for injection by auto-injector into HPLC.

Chromatographic separation: HPLC Hewllet Packared (series 1050) equipped with autosampling injector, solvent degasser, ultra violet (UV) detector set at 210 nm and quaternary HP pump (series 1050), Hewllet Packared software. The column temperature was maintained at 35°C. An isocratic separation was carried out with 0.01 N H_2SO_4 as a mobile phase at flow rate of 1 ml/min. the organic acids standard (lactic, acetic and formic acid) from Fluka Co, were dissolved in a mobile phase and injected into HPLC

Statistical analysis: The results were analysis statistically using one way analysis of variance (version 16.0 SPSS, USA). When there was statistically significant difference post hoc analysis was applied. The statistical significance of the data was determined using *P* values less than 0.05.

Results and Discussion

Viability of bifidobacteria and yoghurt starter cultures

Changes in the population of bifidobacteria in probiotic yoghurt and soy yoghurt inoculated with and without test organisms during refrigerated storage period are shown in Table 1. There was a sharp decline in all treatments (probiotic yoghurt and soy-yoghurt) during the refrigerated storage period. Bifidobacterial population in the probiotic yoghurt containing Bb-12 & Bb-46 and probiotic soy-yoghurt made with Bb-12 and Bb-46, decreased by 98.56, 92.00, 96.43 and 98.86%, without inoculation with test organisms at the end of refrigerated storage period, respectively. In case of the treatments inoculated with E. coil the corresponding decrease ratios were 74.22, 99.36, 48.13 and 62.72%, respectively, whereas for the treatments inoculated with *S.aureus* the population of bifidobacteria decrease by 82.50, 97.96, 84.19 and 99.00%, respectively. The decline of bifidobacterial population during storage may be due to the decrease of pH value and accordingly increase of acidity as well as their ability to produce organic acids [16]. Maintaining viability of bifidobacteria has been a challenge to the dairy processors because the organism requires low oxidation reduction potential for growth and is sensitive to low pH [17]. Another study [18] showed that 14 out of 17 strains lost their viability in fermented milk in the first week of storage. Also it is reported that the presence of yoghurt culture adversely affected the growth of bifidobacteria irrespective of their species [11]. Klaver et al. [18] reported the survival of only three out of nine bifidobacterial strains in the pH range of 3.7 to 4.3. In the studies by Adhikari, Shin, Medina [15,17,19], the population of bifidobacteria with yoghurt starter culture decreased during refrigerated storage period.

On the other hand, Shin et al. [17] and El-Sayed et al. [20] found that the soymilk fermented by bifidobacteria were rapidly reduced the survival of bifidobacteria during the refrigerated storage period. It could be seen from the data in Table 1, that the bifidobacterial population was higher in probiotic soy-yoghurt Bb-12 and Bb-46 inoculated with or without test organisms than the corresponding probiotic yoghurt Bb-12 and Bb-46 treatments, over the refrigerated storage period. The increasing of bifidobacterial counts in probiotic soy-yoghurt Bb-12 and Bb-46 compared with probiotic yoghurt Bb-12 and Bb-46 may be due to the presence of oligosaccharides(stachyose and raffinose) in soymilk, which was approach as growth factors for several species of bifidobacteria [8,21]. Although the bifidobacterial level in all probiotic treatments were variable in products investigated, they were always above 10^6 cfu / ml until the end of refrigerated storage period (15 days), which is recommended dose to receive the health benefits of these organisms [6,17].

Table 1 showed the viability of yoghurt culture (*Lactobacillus delbrueckii* spp *bulgaricus* and *Streptococcus salivarius spp thermophilus*)

Treatments	(a) Count of Bifidobacteria(cfu x10^8 /ml) *											
	Storage period (days)											
	0**			5			10			15		
	Without inoculation	Inoculated with *E. col*	Inoculated with *S. aureus*	Without inoculation	Inoculated with *E. col/*	Inoculated with *S. aureus*	Without inoculation	Inoculated with *E. col*	Inoculated with *S. aureus*	Without inoculation	Inoculated with *E. coil*	Inocul ated with *S. aureus*
Yoghurt Bb-12	2.09	1.28	2.40	0.91	1.20	2.67	0.28	0.50	1.03	0.03	0.33	0.42
Yoghurt Bb-46	2.50	3.15	2.21	0.35	1.70	3.34	0.24	0.23	0.11	0.20	0.02	0.045
Soy-yoghurt Bb-12	30.80	4.24	6.64	14.30	*3.64*	*3.05*	2.14	2.33	2.33	1.10	2.20	1.05
Soy-yoghurt Bb-46	88.00	5.58	6.00	13.75	3.18	4.25	1.79	1.73	1.73	1.00	2.08	0.06

Treatments	(b) Count of yoghurt culture (cfu x 10^8 /ml) *											
	Storage period (days)											
	0**			5			10			15		
	Without inoculation	Inoculated with *E. coli*	Inoculated with *S. aureus*	Without inoculation	Inoculated with *E. coli*	Inoculated with *S. aureus*	Without inoculation	Inoculated with *E. coli*	Inoculated with *S. aureus*	Without Inoculation	Inoculated with *E. coli*	Inoculated with *S. aureus*
Plain yoghurt	810.0	342.5	650.0	520.0	88.2	107.0	41.2	15.0	103.2	28.0	5.0	75.0
Yoghurt Bb-12	714.5	408.2	300.0	82.5	71.3	73.8	62.0	77.4	41.2	21.0	15.7	31.0
Yoghurt Bb-46	748.0	704.5	200.0	77.8	45.5	117.6	11.4	53.8	48.4	10.0	18.9	20.0

* mean of three replicates
**Direct after coagulation

Table 1: The viable count of bifidobacteria (a) and yoghurt culture (b) in experimental probiotic yoghurt and soy-yoghurt inoculated with *Escherichiacoli and Staphylococcus aureus* during refrigerated storage period.

Treatment	pH-values*											
	Without Inoculation				**Inoculated with *E.coli***				**inoculation with *S.aureus***			
	Storage period (days)											
	0	**5**	**10**	**15**	**0**	**5**	**10**	**15**	**0**	**5**	**10**	**15**
Plain Yoghurt	4.47 ± 0.02	4.14 ± 0.03	3.95 ± 0.01	4.03 ± 0.03	4.35 ± 0.02	4.10 ± 0.02	4.00 ± 0.02	4.03 ± 0.02	4.35 ± 0.02	4.09 ± 0.02	4.01 ± 0.01	4.03 ± 0.02
Yoghurt Bb-12	4.34 ± 0.03	4.07 ± 0.04	3.98 ± 0.02	3.98 ± 0.01	4.25 ± 0.03	4.04 ± 0.02	3.87 ± 0.02	3.97 ± 0.02	4.31 ± 0.04	4.03 ± 0.03	3.83 ± 0.02	3.99 ± 0.02
Yoghurt Bb - 46	4.34 ± 0.03	4.08 ± 0.01	3.95 ± 0.02	3.95 ± 0.02	4.32 ± 0.02	4.07 ± 0.02	3.90 ± 0.02	4.01 ± 0.00	4.28 ± 0.03	4.07 ± 0.03	3.99 ± 0.01	3.99 ± 0.01
Soy yoghurt Bb-12	4.90 ± 0.03	4.84 ± 0.04	4.79 ± 0.01	4.71 ± 0.03	4.97 ± 0.02	4.79 ± 0.02	4.72 ± 0.01	4.76 ± 0.02	4.54 ± 0.03	4.62 ± 0.01	4.61 ± 0.02	4.63 ± 0.03
Soy yoghurt -Bb- 46	4.46 ± 0.02	4.41 ± 0.02	4.34 ± 0.01	4.37 ± 0.02	4.50 ± 0.02	4.37 ± 0.01	4.36 ± 0.03	4.05 ± 0.59	4.35 ± 0.03	4.37 ± 0.03	4.36 ± 0.03	4.35 ± 0.03

*** Means values (± SD; n=3)**

Table 2: Changes in pH –values of probiotic yogurt and soy-yogurt inoculated with *Escherichia coli* and *Staphylococcus aureus* during refrigerated storage period.

in plain and probiotic yoghurt Bb-12 & Bb-46 inoculated with or without test organisms, during refrigerated storage period. The initial number of yoghurt culture was 810.0, 714.5 and 748×10^8 cfu /ml in plain yoghurt, yoghurt Bb-12 and yoghurt Bb-46, respectively. The decrease in the yoghurt culture population in all of these treatments may be attributed to the decrease in pH (Tables 1 and 2). Though, these considerable declines, it could be observed that the yoghurt culture population was maintained above 10^8 cfu/ml until the end of refrigerated storage period (Table 1).

pH-values

Changes in pH-values of probiotic yoghurt and soy-yoghurt inoculated by *E.coli* and *S.aureus* during refrigerated storage period are shown in Table 2. The pH-values of the most of experimental treatments decrease and consequently the titratable acidity increase during the refrigerated storage period (Data not shown). The decrease in pH-values observed in the treatments may be due to metabolic activity of bifidobacteria and yoghurt starter culture as well as test organisms. As shown in Table 2 the probiotic yoghurt containing Bb-12 and Bb-46 inoculated with or without test organisms exhibited a slight pH drop direct after the incubation time and during the refrigerated storage period compared with plain yoghurt. This may be attributed to the added acidity, which produced by bifidobacteria in the probiotic products.

Also, it could be seen from the same Table that pH-values in the probiotic soy-yoghurt made with Bifidobactertum Bb-12 and Bb-46 inoculated with and without test organisms were higher than the corresponding values in the probiotic yoghurt inoculated with and without test organisms over the refrigerated storage period. This may be due to the absence (not added) of yoghurt culture in probiotic soy-yoghurt.

Lactic, acetic and formic acids contents of probiotic yoghurt and soy-yoghurt inoculated with test organisms

Changes in the values of lactic, acetic and formic acids contents of probiotic yoghurt and soy-yoghurt inoculated by *E.coli* and *S.aureus* during refrigerated storage period are illustrated in Table 3a-c respectively. It could be noticed that probiotic yoghurt containing Bb-12 and Bb-46 inoculated with or without test organisms showed significantly (P>0.05) increase in lactic and acetic acids than the corresponding: values in plain yoghurt direct after the incubation time, and these increasing continued for acetic acid only over the refrigerated storage period. This finding confirmed the high ability of bifidobacteria and yoghurt starter cultures (*Lactobacillis, dulbrueekii*

(a)Lactic acid

Treatment	Without Inoculation			Inoculated with *E.coli*			Inoculated with *S.aureus*		
	Storage period (week)								
	0	1	2	0	1	2	0	1	2
Plain Yoghurt	170 ± 6.0^{rst}	510 ± 2.0^{ghij}	571 ± 3.6^{bcd}	538 ± 7.2^{defg}	535 ± 6.08^{efgh}	614 ± 7.2^{a}	421 ± 4.6^{lm}	498 ± 10.6^{ij}	568 ± 5.6^{bcde}
Yoghurt Bb-12	556 ± 6.0^{cdef}	481 ± 6.2^{jk}	260 ± 10^{o}	414 ± 3.5^{m}	503 ± 8.9hij	592 ± 5.6^{ab}	435 ± 6.4^{lm}	523 ± 8.5^{fghi}	586 ± 8.0^{abc}
Yogurt - Bb - 46	426 ± 5.3^{lm}	507 ± 5.3^{ghij}	606 ± 5.57^{a}	455 ± 755^{kl}	509 ± 6.2^{ghij}	584 ± 5.3^{abc}	440 ± 5.3^{lm}	529 ± 8.7^{fghi}	533 ± 8.7^{fgh}
Soy yoghurt -Bb-12	137 ± 4.6^{t}	151 ± 3.0^{st}	152 ± 3.6^{st}	159 ± 4.4^{lm}	139 ± 6.1^{t}	157 ± 3.6^{t}	145 ± 3.6^{t}	147 ± 3.6^{t}	156 ± 4.4t
Soy yoghurt -Bb- 46	181 ± 3.6^{rs}	278 ± 4.4^{no}	314 ± 2.0^{op}	198 ± 4.0^{qr}	440 ± 6.6^{lm}	300 ± 3.6^{n}	250 ± 8.7^{op}	196.67 ± 3.8^{qr}	216 ± 3.5^{pq}

(b)Acetic acid

Treatment	Without Inoculation			Inoculated with *E.coli*			Inoculated with *S.aureus*		
	Storage period (week)								
	0	1	2	0	1	2	0	1	2
Plain Yoghurt	188 ± 6.0^{o}	205 ± 3.0^{n}	222 ± 5.0^{m}	281 ± 9.0^{j}	291 ± 3.0^{t}	104 ± 3.0^{tu}	263 ± 6.0^{k}	158 ± 4.0^{p}	74 ± 3.0^{bc}
Yoghurt Bb-12	590 ± 4.0^{a}	397 ± 5.0^{f}	429 ± 7.0^{e}	478 ± 5.0^{c}	451 ± 4.0^{d}	485 ± 7.0^{b}	350 ± 4.0^{h}	375 ± 4.0^{g}	239 ± 5.0^{l}
Yoghurt - Bb - 46	438 ± 10^{de}	430 ± 5.0^{e}	474 ± 7.0^{bc}	468 ± 4.0^{c}	445 ± 7.0^{de}	481 ± 3.0^{bc}	317 ± 6.0^{i}	246 ± 6.0^{l}	218 ± 6.0^{mn}
Soy yoghurt -Bb-12	78 ± 7.0^{vw}	32 ± 6.0^{xy}	85 ± 4.0^{vw}	130 ± 3^{qrs}	47 ± 4.0^{x}	1.00 ± 0.0^{z}	142 ± 7^{pq}	115 ± 3^{t}	2.00 ± 0.0^{z}
Soy yoghurt -Bb- 46	108 ± 5.0^{tu}	93 ± 2.0^{uv}	23 ± 2.0^{yz}	119 ± 4.0^{rst}	106 ± 2.0^{tu}	12 ± 1.0^{z}	283 ± 4.0^{j}	135 ± 6.0^{qr}	113 ± 2.0^{t}

(c)Formic acid

Treatment	Without Inoculation			Inoculated with *E.coli*			Inoculated with *S.aureus*		
	Storage period (week)								
	0	1	2	0	1	2	0	1	2
Soy yogurt -Bb-12	29 ± 3.0^{gh}	27 ± 2.0^{ghi}	39 ± 4.0^{def}	33 ± 2.0^{fg}	37 ± 3.0^{ef}	63 ± 2.0^{b}	28 ± 2.0^{ghi}	44 ± 3.0^{cde}	88 ± 2.0^{a}
Soy yogurt -Bb- 46	22 ± 2.0^{hij}	43 ± 3.0^{cde}	62 ± 2.0^{b}	17 ± 1.0^{j}	28 ± 2.0^{ghi}	65 ± 3.0^{b}	46 ± 3.0^{cd}	51 ± 3.0^{c}	21 ± 3.0^{ij}

$^{a-z}$ Means values (± SD; n=3) with unlike subscripts letters were significantly different (p□0.05).

Table 3: Changes in lactic (a), acetic (b) and formic acids (c) content of probiotic yogurt and soy-yogurt inoculated with *Escherichia coli* and *Staphylococcus aureus* during refrigerated storage period (mg/100 g).

sub sp. bulgaricus and *Streptococcus salivarius sub sp, thermophilus*) to produce such organic acids. Furthermore it could be observed from the data in the same Tables that inoculation with test organisms (*E. coli* and *S.aureus*) had little effect on the production of these organic acids by bifidobacteria and yoghurt culture.

On the other hand and as shown in Table 3, the probiotic soy-yoghurt made with Bb-12 and Bb-46 inoculated with or without test organisms showed significantly decrease in lactic and acetic acids than the plain yoghurt and probiotic yoghurt Bb-12 and Bb-46 direct after incubation time and after the refrigerated stroge period. These differences may be due to the presence of yoghurt culture with bifidobacteria in the probiotic yoghurt. It was of considerable interest that the probiotic soy-yoghurt Bb-12 and Bb-46 and inoculated with or without test organisms produces not only lactic and acetic acids but also formic acid, and when these acids are ranked in order of decreasing activity (as function of concentration mg/100 g), they form the series: lactic> acetic> formic. This finding is in agreement with that reported by [22] who found the lactic, acetic and formic acids contents of bifidobacteria-fermented soymilk were 225.7, 187.1 and 20.7 μmol/g respectively. In contrast, Adhikari et al. [15] decided that the bifidobacteria with yogurt cultures (*L.delbrueckii ssp bulgaricus* and *Streptococcus salivarus sub sp. thermophilus*) produce lactic and acetic acid only.

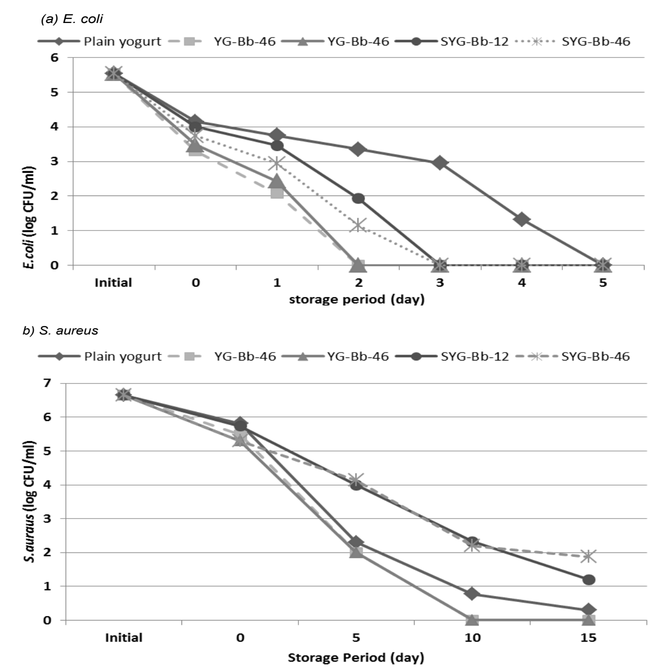

Figure 1 (a, b): Effect of probiotic yoghurt and soy-yoghurt on the growth and survival rate of *Escherichia. coli Staphylococcus aureus* during storage period.

Effect of probiotic yoghurt and soy-yoghurt on the growth and survival rate of *E. coli:*

The growth and survival patterns of *E.coli* as affected by probiotic yoghurt and soy-yoghurt during refrigerated storage period are shown in Figure 1a. It could be seen that the counts of *E.coli* in all treatments decreased during the cold storage period. Direct after incubation time, the decrease percentage was 96 for the plain yoghurt from the initiate level (3.5×10^5 cfu/ml), whereas in the probiotic yoghurt containing Bb-12 and Bb-46 and soy-yoghurt made with Bb-12 and Bb-46, the % decrease was 99.43, 99.14, 97.14 and 98.43 respectively. While after one day of storage the corresponding decrease were 98.40, 99.96, 99.17, 99.17 and 99.75% for the plain yoghurt, probiotic yoghurt Bb-12 and Bb-46 and probiotic soy-yoghurt Bb-12 and Bb-46, respectively. Interestingly, while *E.coli* had disappeared after 5 days m plain yoghurt, it could not be detected in the probiotic yoghurt and soy-yoghurt after 2 and 3 days of storage, respectively (Figure 1a).

However, It could be seen from Table 3 that the acetic acid in probiotic yoghurt Bb-12 and Bb-46 higher than in plain yoghurt, probiotic soy-yoghurt Bb-12 and Bb-46, direct after coagulation (0 week), one and two weeks. These data also showed that the plain yoghurt produce lactic acid more than probiotic yoghurt Bb-12 and Bb-46. In contrast, probiotic soy-yoghurt Bb-12 and Bb-46 produced lactic acid less than plain and probiotic yoghurts. Buchanan and Gibbons [23], studied the effect of HCl, malic acid, citric acid, acetic acid and lactic acid on the activity of 9 strains of *E. coli* and they found that the all of these strains more sensitive to lactic acid than acetic acid. In addition, the minimum pH at which all the *E.coli* strains were able to grow in adjusted tryptic soy broth (TSB) was 5.5 for acetic acid. Acetic acid was a more effective inhibitor than HCl at an equivalent pH. The higher pKa of acetic acid (4.75) compared with other organic and mineral acids are responsible for the observed greater efficacy of this acid against *E. coli* [24]. Oh et al. [25] studied the effect of organic acids on the survival of *E. coli* and they found the minimum inhibitory pH of acetic acid and lactic acid was 5.0 and 4.0, respectively.

Cherrington et al. [26] reported that the antimicrobial effect of organic acids has been attributed to undissociated acid molecules that interfere with cellular metabolism or a decrease in biological activity as a result of pH changes of the cells environment. On the other hand, the inhibition mechanisms of bifidobacteria on *E. coli* not only depend on organic acids but also may be on antibacterial substances [27].

Hussein and Kebary [28] found that the immobilized cells of *Bifidobacterium bifidum* and *Bifidobacterium infant*s are able to produce antimicrobial in yoghurt agents, which inhibited *E.coli*. The present results showed that the probiotic yoghurt and soy-yoghurt containing bifidobacteria suppressed the *E.coli* population more effectively than non-probiotic yoghurt (plain yoghurt).

Effect of probiotic yoghurt and soy-yoghurt on the growth and survival rate of *Staphylococcus aureus*

The growth and survival patterns of *S.aureus* as affected by probiotic yoghurt and soy-yoghurt during refrigerated storage period are illustrated in Figure 1b. It could be observed that the counts of *S.aureus* in all treatments decreased during the cold storage period. Direct after the incubation time, the percentage decrease for the plain yoghurt was 85.62% from the initial inoculation number (4.5×10^6 cfu/ml), whereas the % decrease in the probiotic yoghurt containing bifidobacterium Bb-12 and Bb-46 was 93.36% and 95.58%, respectively. For the probiotic soy-yogurt made with Bifidobacterium Bb-12 and Bb-46, the decrease percentage was 87.83% and 95.58% after same period, respectively. The present results indicated that while *S.aureus* growth in the probiotic yoghurt containing Bb-12 and Bb-46 was not detected after the 10^{th} day of storage, low numbers of *S .aureus* were survived in the plain yoghurt as well as the probiotic soy-yoghurt made by Bb-12 and Bb-46 after 15 days of cold storage (Figure 1b).

As seen from Figure 1b, this markedly inhibitory effect of probiotic yoghurt containing Bb-12 and Bb-46 may be due to their ability to produce acetic acid much more than plain yoghurt and probiotic soy-yoghurt Bb-12 and Bb-46, direct after coagulation and during refrigerated storage period. Minor and Marth [29] reported that the acetic acid inactivated 99.99% of the *S. aureus* at pH 4.4 and this finding was in agreement with our results. Notermans and Heuelman [30] found that growth of *S.aureus* occurred at pH 4.6 but not at pH 4.3. This finding may be explained that *S.aureus* still detected in probiotic soy-yoghurt Bb-12 and Bb-46 during refrigerated storage period, where they have a pH-values still higher than 4.3 after 15 day (Table 2 and Figure 1b). Dahiya and Speck [31] found that the *Lactobacillus delbruecku* spp *bulgaricus* and *Lactobacillus delbrueckii spp Lactis* inhibited the growth of *S. aureus*. They proposed that inhibition of *S. aureus* resulted from the formation of hydrogen peroxide by certain Lactobacilli. Gilliland and Speck [32] found that lactic streptococci in milk inhibited Salmonella and *S. aureus*, where its inhibition levels were 88.2-93.4% for Salmonella and 98.1-98.9% for *S. aureus*. The authors showed also that inhibition was due partially to organic acids production and partially to small molecular weight compounds in whey.

It is evident from the present results that the metabolites are significantly effective. In addition, The pH – values were positively correlated with the viability of *E. coli* and *S. aureus* in the probiotic yoghurt and soy- yoghurt containing Bb-12 & Bb-46 (r .421, r .324; $p<0.05$, respectively). This can be explained from the fact that the metabolites produced by the probiotics include bioactive products such as organic acid, hydrogen peroxide (H_2O_2) and bacteriocins [33]. It is reported by Cheikhyoussef et al. [34] that the principal metabolites of probiotic bacteria are acetic acid and lactic acid in ratio 3:2 and these acids are responsible for the consequent drop in pH and may be sufficient to antagonize many pathogenic bacteria belonging to both Gram-positive and Gram-negative bacteria.

Conclusion

From the previous results, we can concluded, that the probiotic products containing bifidobacteria caused antigonistic effects against foodborne pathogenic bacteria such as *E.coli* and *S. aureus* and the use of probiotic bacteria like bifidobacteria in the production of yoghurt and soy yoghurt restricts or prohibits the growth of these pathogenic bacteria. Our results suggest that these probiotic bacteria could be used as a nature biopreservatives in different food products.

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