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A Research about Viable *Lactobacillus bulgaricus* and *Streptococcus thermophilus* Numbers in the Market Yoghurts

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Abstract: The industrial production of yoghurt is increasingly developed in the world. Yoghurt is a fermented milk product obtained from fermentation of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* strains. In Turkey, yoghurt is produced by the two ways; one of them is a traditional method without using starter culture in small dairy plants and the second production method by using industrial starter culture in modern plants. In this study yoghurt samples were collected from the local markets which were produced traditional process and produced in modern plants by addition of starter cultures, their viable *L. bulgaricus* and *S. thermophilus* bacteria numbers, coliform, *Escherichia coli*, yeast and mould counts, pH values were determined and compared each others. Yoghurts have pH values between 3.95-4.23, viable *S. thermophilus* and *L. bulgaricus* numbers were determined between 10^7-10^8 cfu/g for yoghurts producing with starter culture, 10^5-10^6 cfu/g and 10^6-10^7 cfu/g for yoghurts producing with traditional methods, respectively. Coliforms, *E. coli*, yeast and mould counts have at low numbers for all yoghurt samples. As the result, yoghurts which are produced by starter cultures have high numbers of yoghurt bacteria means that yoghurts produced by using starter cultures have higher therapeutic and/or antimicrobial properties beside of their organoleptic characteristics. Importance of yoghurt production by using starter cultures should be known and advantages of using starter cultures in fermentation products should be stated.

Key words: Yoghurt bacteria · market yoghurts · yoghurt microbial quality

INTRODUCTION

Traditionally, yoghurt is manufactured using *Streptococcus thermophilus* and *L. delbrueckii* ssp. *bulgaricus* as starter cultures. These organisms are claimed to offer some health benefits however, they are not natural inhabitants of the intestine. Therefore, for yoghurt to be considered as a probiotic product. *L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus* are at a daily dose of 10^9 cfu and several authors have indicated that a minimal concentration of 10^{-6} cfu/g of a product is required for a probiotic effect [1-4].

The traditional cultures used in making yoghurt (i.e., *L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus*) contain substantial quantities of b-D-galactosidase and so both yoghurt and probiotic yoghurt are tolerated well by lactose malabsorbers. The administration of probiotic preparation reduced the frequency of diarrhoea. A similar study conducted with Finnish tourists using a lyophilized preparation of *Lactobacillus* GG also showed a reduction in the occurrence of traveller's diarrhoea [5].

Yoghurt is reported to have hypocholesterolaemic and antitumour effects and consumption of large quantities of cultured yoghurt lowered serum cholesterol levels in human volunteers. *S. thermophilus* TMC 1543 in a single blind parallel study, subjects consuming fermented milk (200mL/ day) showed significant increases of high density lipoprotein cholesterol, compared with the pre-intervention levels after eight weeks supplementation. The levels of triglycerides were also reduced significantly in subjects receiving the fermented milk [6]. Realizing health benefits, probiotic bacteria must be viable and available in a high concentration typically 10^6 cfu g/1 of a product [7-9]. It has been postulated that this is due to a factor produced by, or enhanced by, the action of starter culture during yoghurt fermentation [10, 11]. *L. bulgaricus* 291, *S. thermophilus* F4, V3; *B. longum* BB536 clear evidence for DNA-protective effects of *lactobacilli*

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used for yoghurt production against DNA damage caused by HCAs in organs which are targets of tumor induction by 1-methyl-3-nitro-1-nitrosoguanidine, 1,2-dimethylhydrazine, N-methyl-N-nitrosourea and azoxymethane and also reduce chemically induced-DNA migration and pre-carcinogenic lesions in colon cells of male Fischer 344 rats. *L. bulgaricus*, *S. thermophilus* ingestion of viable probiotics led to anticarcinogenic effects, through detoxification of genotoxins in the gut of rats. *L. bulgaricus* and *S. thermophilus* possibly influenced metabolic, immunologic and protective functions in the colon [2].

It is questionable whether such products can provide yoghurt is considered the most important carrier of probiotic bacteria. However, a number of factors affect the loss of viability of probiotic organisms in yoghurt, including acidity of products, acid produced during refrigerated storage (also known as post-acidification), level of oxygen in products and oxygen permeation through the package, sensitivity to antimicrobial substances produced by starter bacteria, lysogenic character of bacteria and lack of nutrients in milk [7, 12].

The nutritional and therapeutic effects of yoghurt are well known and mainly attributed to fermentative changes in the milk and/or the metabolic effects of the yoghurt microflora. In this study some yoghurt samples were collected from the markets, their viable lactic acid bacteria, coliforms, *E. coli*, yeast and mould counts analysed and their pH values were determined and compared each others.

MATERIALS AND METHODS

In the methods, whole set yoghurt samples (two samples for each) were taken (before 10 days shelf-life ending date) from ordinary markets in Balikesir region. A yoghurt sample of 10 g was decimally diluted in sterile peptone water (0.1%) and dilution plated over the media. *S. thermophilus* were determined on M17 agar medium (supplemented with %5 sterile 10% w/v lactose) at 45°C /48 h with duplicate plates under microaerophilic conditions, *L. bulgaricus* were counted on MRS agar medium duplicate plates at 45°C/48 h which pH of agar medium was adjusted to 5.2 [11]. Coliforms were determined by the MPN method for a three tube series using Mac Conkey Broth incubated at 35°C/48 h and *E. coli* were counted on Violet Red Bile Agar (VRBA) (Merck)at 37°C/18-24 h, yeast and mould enumarated on Oxytetracycline Glucose Yeast Extract agar at 22°C/ 5-7 days [13].

RESULTS and DISCUSSION

L. bulgaricus, *S. thermophilus*, yeast, mould, coliforms, *E. coli* average numbers and pH values in traditional and industrial yoghurt samples are seen in Table 1.

In Noni *et al*. [3], research *L. bulgaricus* and *S. thermophilus* bacteria counts were between 10^7-10^8 cfu/g for 10 days yoghurt samples but in sweetened yoghurts *L. bulgaricus* viable count was 10^4-10^5 cfu/g. Venir *et al*. [14] found in fresh yoghurts *S. thermophilus* count between 10^4-10^8 cfu/ g and 10^6-10^7 cfu/ g for *L. bulgaricus*. In this study lactic acid bacteria count was similar that results. Husson-Kao *et al*. [15] determined lysogenic character of *S. thermophilus* is important on bacteria viability during storage period of yoghurts. Vinderola *et al*. [16] reported that high fat content yoghurts are more inhibitory for probiotic bacteria.

In Birollo *et al*. [4] study; coliforms, yeast and mould were also not detected in any of samples. They also found numbers of *S. thermophilus* are higher than the *L. bulgaricus* during storage and at the end of the storage initial cell counts reaching 5×10^5 cfu/g. In this research yeast and mould were detected in some of the traditional yoghurt samples. It was may be due to the unhygienic enviroment conditions during the processings. As seen in Table 1. *L. bulgaricus* are always higher than the *S. thermophilus* during storage for all kinds of yoghurt samples. In Birollo *et al*.[4] study pH ranges were between 3.8-4.0 for yoghurt samples. In this research pH values were similar for two kinds of yoghurt samples.

The beneficial effects of the regular ingestion of yoghurt on the consumers's health have always been related to the presence of a high concentration of viable lactic acid bacteria in the product. In several countries have established minimum values of lactic acid bacteria for yoghurts and/or fermented milks during shelf life. These values range from 1×10^6 to 5×10^8 cfu/g [17].

The use of industrial starters with low proportion of *L. bulgaricus* allows the production of yoghurt with a reduced acidity and with lesser risks of post acidification. Actual temperature of storage in markets is important for bacteria viability in yoghurts. Industrial standards recommends for yoghurts a conservation temperature not higher than 8°C. Generally skimmed milk yoghurts showed higher viable numbers than whole yoghurts [4]. High concentration of sugars in sweetened yoghurts might effected bacilli viability [16].

Table 1: *L. bulgaricus*, *S. thermophilus*, yeast, mould, coliforms, *E. coli* average numbers and pH values in traditional and industrial yoghurt samples

	L. bulgaricus	*S.thermophilus*	Yeast	Mould	Coliforms	*E. coli*	pH
Samples (Production with traditional method)							
T1	1.2×10^6	2.2×10^5	4.4×10^2	< 10	< 3	0	4.07
T2	9.7×10^6	1.6×10^5	3.0×10^2	< 10	<3	0	3.95
T3	1.5×10^6	7.3×10^6	4.2×10^3	4×10	<3	0	4.23
T4	3.4×10^6	6.5×10^6	< 10	<10	<3	0	4.12
T5	1.1×10^7	4.2×10^6	3.7×10^2	3.2×10	<3	0	4.43
T6	4.3×10^5	4.7×10^5	<10	<10	<3	0	3.98
T7	3.4×10^7	2.3×10^5	<10	<10	<3	0	4.56
T8	4.5×10^6	2.1×10^6	<10	<10	<3	0	4.49
T9	3.3×10^7	6.9×10^5	<10	<10	<3	0	4.35
T10	5.7×10^7	7.8×10^5	<10	<10	<3	0	4.4
T11	4.6×10^6	4.0×10^5	4.3×10	<10	<3	0	4.21
T12	2.1×10^6	3.2×10^6	6.1×10^2	2.3×10	<3	0	4.19
T13	3.2×10^7	2.5×10^7	<10	<10	<3	0	4.54
T14	4.1×10^5	9.1×10^4	3.7× 10	4.3×10	<3	0	3.97
T15	3.4×10^7	5.6×10^6	<10	<10	<3	0	4.67
Samples (Production with industrial culture)							
S1	4.5×10^6	5.9×10^7	<10	<10	<3	0	4.10
S2	3.2×10^7	6.3×10^8	<10	<10	<3	0	4.20
S3	5.1×10^6	5.3×10^7	<10	<10	<3	0	4.14
S4	4.6×10^6	9.7×10^7	<10	<10	<3	0	4.20
S5	1.2×10^8	6.8×10^7	<10	<10	<3	0	4.15
S6	6.8×10^6	4.3×10^7	<10	<10	<3	0	3.98
S7	2.3×10^7	5.6×10^7	<10	<10	<3	0	3.78
S8	1.5×10^6	4.2×10^7	<10	<10	<3	0	3.67
S9	7.9×10^8	5.8×10^8	<10	<10	<3	0	4.19
S10	4.8×10^8	3.9×10^8	<10	<10	<3	0	4.23
S11	5.3×10^8	6.2×10^8	<10	<10	<3	0	4.20
S12	9.0×10^7	4.5×10^7	<10	<10	<3	0	4.18
S13	4.2×10^6	6.7×10^7	<10	<10	<3	0	3.89
S14	3.8×10^8	8.1×10^7	<10	<10	<3	0	4.16
S15	2.7×10^7	2.8×10^7	<10	<10	<3	0	3.97

This work demonstrated that industrial yoghurt production with starter cultures increases the viable yoghurt microflora and bioavailability of the product.

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