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Research Paper

Traditional and probiotic yoghurts in a comparative study

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ABSTRACT

The present study was designed to evaluate traditional starter cultures for the preparation of yoghurt. The yoghurt prepared from these starter cultures was compared for various sensory, chemical and microbial characteristics with yoghurt prepared from probiotics. A total of 40 random samples of traditional and probiotic yoghurt (20 of each) were collected from different markets in Menofyia Governorate to evaluate their quality. The sensory characteristic score of probiotic yoghurt (91.05 ± 7. 00) was significantly better than the traditional one (84.25 ± 8.60). The chemical examination indicated that the average titratable acidity was 0.90 ± 0.1 for traditional yoghurt and 0.75 ± 0.2 for probiotic yoghurt. On the other hand, the bacterial counts in the examined traditional and probiotic yoghurt samples were 7.85 x10^3 ± 0.16 x10^3 and 6.5 x10± 0.16 x10 cfu/gm for *Staphylococcus aureus* count; 1.97 x10^3 ± 1.10 x10^3 and 7.5 x10 ± 2.12 x 10 cfu/gm for psychrotrophes and 1.37 x10^3 ± 0.16 x10^3 and 1.4 x10^2 ± 2.12 x 10^2 cfu/gm for coliform, respectively. In addition, yeast and mould were detected in both traditional and probiotic yoghurt with varying counts. In contrast, *E. coli* could be detected in traditional yoghurtonly. Serologically identification revealed that O_{124}:K_{72} (EPEC) and O_{26}:K_{60}(EHEC) were the most serotype isolated from traditional yoghurt. On the other hand, all examined samples of yoghurt were free from aerobic spore formers. According to sensory, chemical and microbiological results, probiotic yoghurt appeared to be better and safer for human consumption as compared with traditional one.

Key words: Starter cultures, probiotics, yoghurt, quality.

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INTRODUCTION

Yoghurt is a very popular dairy product in Egypt. Its production and consumption is growing continuously due to its health benefits beside its high nutritive value. It is nutritionally rich in protein, calcium, riboflavin, vitamin B6 and B12 (Karagul et al., 2004; Ashraf andShah, 2011). Yoghurt is produced through the fermentation of milk lactose by *Streptococcus thermophiles* and *Lactobacillus delbrueckii* subspp. *bulgaricus* (Tamime and Robinson, 2007).

A number of dairy products are marketed as containing probiotic bacteria. However, the most widely encountered one is yoghurt. The number of probiotic organisms in a probiotic product should meet the suggested minimum of >10^6cfu/ ml, which is the recommended minimum daily intake (Akin et al., 2007). The consumption of yoghurt with probiotic organisms such as *Lactobacillus* and *Bifidobacterium* enhance the function of gastrointestinal tract and immune system, and help in the digestion of lactose in patients suffering from lactose intolerance, as well as decrease the prevalence of allergy in susceptible individuals (Parvez et al., 2006; Ayman and Omer, 2009).

Yoghurt shelf life is based on the physical or organoleptic, chemical and microbiological characters that could be decreased, if there are any changes in these characters during storage (Salvador and Fiszman, 2004).

The quality of yoghurt in local markets varies from producer to another. A practical approach towards the quality of yoghurt is to evaluate the different samples of yoghurt sold in local markets. Moreover, research in this field of quality evaluation of market yoghurt is the basic need to create awareness among common people about the existing situation and protect the consumers` health and rights. Therefore, this study was designed to evaluate and compare between market yoghurts in relation to their starter cultures for organoleptic, chemical and microbiological properties.

MATERIALS AND METHODS

Collection of samples

Forty random commercially produced yoghurt samples were obtained from retail outlets in Menofyia Governorate during the period from February to July 2017. The samples included 20 traditional yoghurt samples and 20 probiotic yoghurt samples. Collected samples were transferred immediately to the laboratory in an ice box and analyzed for organoleptic, chemical and microbiological characteristics.

Organoleptic examination

The organoleptic quality of yoghurt was evaluated for flavor (50 points), body and texture (40 points), and appearance (10 points) according to score card suggested by Keating and Randwhite (1990)

Chemical analysis

Titratable acidity

It was determined using the direct titration method according to the method described by BSI (2010).

Microbiological examination

Preparation of serial dilutions

Yoghurt samples were thoroughly mixed before being examined after opening of the yoghurt cup using sterile forceps to remove the lid under complete aseptic condition. Serial dilution was prepared; 1 ml of each thoroughly mixed yoghurt sample was added to 9 ml sterile distilled water to make ten-fold serial dilution, from which decimal dilutions were prepared and used in the following examinations (Prescott et al., 2004).

Total coliform count and E. coli

MacConkey agar and Eosin Methylene Blue (Oxoid, UK) were used for enumeration of Coliform and *E. coli*, respectively in the examined samples and incubated at 37ºC for 48 h (Adams and Mosa, 1990)

Psychrotrophs and aerobic spore formers

Plate count agar was used to enumerate psychrotrophs at 7ºC for 7-10 days and Dextrose Trypton Agar medium (DTA) was used for aerobic spore formers at 37ºC for 24-48 h (APHA, 1992).

Total yeast and mould count

From the already prepared serial dilutions, 1 ml was transferred into duplicated Petri dishes and thoroughly mixed with sabouraud dextrose agar medium supplemented with chloramphenicol and chlortetracycline (100 mg of each) as described by IDF(1990). The plates were incubated at 25°C for 5-7 days.

Staphylococcus aureus count

It was done according to FDA (2001).

Serotyping

The confirmed *E. coli* isolated strains were serologically identified using standard polyvalent and monovalent *E. coli* antisera in Central Laboratories of Ministry of Public Health-Bacteriology Department –Cairo.

Statistical analysis

Statistical comparisons were done using one-way analysis of variance (ANOVA). The results were considered significantly different with $P < 0.05$ as described by Clarke and Kempson (1997).

RESULTS AND DISCUSSION

The research was conducted to develop prepared yoghurt with acceptable organoleptic, chemical and microbiological quality. Two different yoghurts with different starter cultures (traditional and probiotic) were used in this experiment.

Organoleptic parameters

Organoleptic characteristic of traditional yoghurt (*S. thermophiles* and *L. bulgaricus*) was compared with probiotic yoghurt, which is mainly incorporating with *Bifidobacteria* species, in Egypt fo r average flavor, body

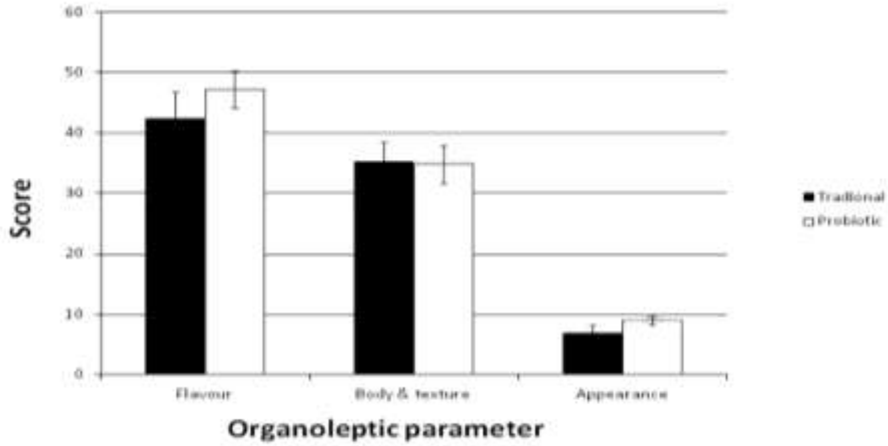

Figure 1: Organoleptic characteristics of examined yoghurt samples.

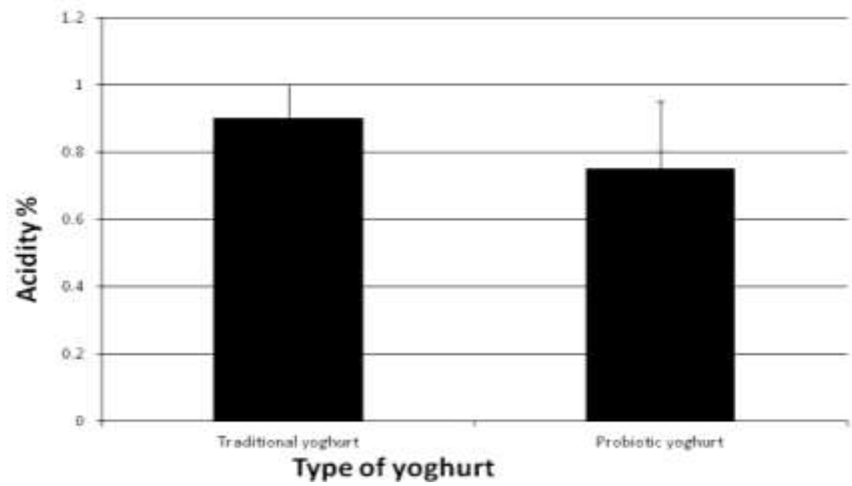

Figure 2: Titratable acidity % of examined yoghurt samples.

and texture and appearance by a team of judges. The results of the organoleptic tests are presented in Figure 1. Statistical analysis showed that there was significant difference ($P < 0.05$) among the flavor and appearance score of different types of yoghurt.

The highest flavor score (47.2 ± 3.0) was recorded in the case of bifidus yoghurt. On the other hand, 42.45 ± 4.3 was seen in the case of traditional yoghurt (Figure 1). Similar results were also reported by Ibrahim (2008). El-Shibiny et al. (2005) reported that bifidus yoghurt, made from *B. bifidum*, had almost similar flavor to standard yoghurt and both were very acceptable with good sensory characteristics, which prevailed for 10 days of storage. The smell and taste of yoghurt are largely dependent on not only the condition of raw materials used, especially the milk, but also the type of starter cultures used (Uddin et al., 2013).

The body and consistency scores of traditional and bifidus yoghurt samples were 35.3 ± 3.1 and 34.85 ± 3.2, respectively (Figure 1). There was no significant difference for body and texture scores of different types yoghurt. Body of bifidus yoghurt was softer than that in traditional one. This is may be due to high acid production in traditional yoghurt than bifidus yoghurt. Furthermore, Pette and Lolkema (1950) attributed *S. thermophilus* to improve the body of yoghurt by affecting the viscosity characteristics of milk cultures of *L. bulgaricus*.

The overall mean score was a measure of the most accepted product in terms of the sensory characteristics, and the increasing order of acceptability of the products by the panelists was traditional yoghurt<bifidus yoghurt.

Chemical parameter

Total acidity is an important indicator of quality measures of prepared yoghurt. The results obtained are shown in Figure 2. The acidity of bifidus yoghurt was lower than

Table 1: Bacteriological and mycological aspects of examined yoghurt samples.

Yoghurt samples	Average coliform count (cfu/gm)	Average psychrotrophic count (cfu/gm)	Yeast and mould count (cfu/gm)
Tradional yoghurt	$1.37\ x10^3 \pm 0.16x10^{3a}$	$1.97\ x10^3 \pm 1.10\ x10^{3\ a}$	$4.86x10^3 \pm 2.12x10^{3a}$
Probiotic yoghurt	$1.4\ x10^2 \pm 2.12x10^{2b}$	$7.5\ x10 \pm 2.12\ x\ 10^b$	$1.24\ x10^2 \pm 2.12x10^{2b}$

The values indicated were the mean of three trials ± SD (Standard deviation).
[ab]Values in the same column having different superscripts differ significantly (P < 0.05).

Table 2: The mean count of some pathogenic bacteria in examined yoghurt samples.

Yoghurt samples	*E. coli* count (cfu/gm)	*S. aureus* count (cfu/gm)	Aerobic spore formers count (cfu/gm)
Tradional yoghurt	$1.22\ x10^3 \pm 0.16\ x10^{3a}$	$7.85\ x10^3 \pm 0.16\ x10^{3a}$	ND
Probiotic yoghurt	ND*	$6.5\ x10 \pm 0.16\ x10^b$	ND

* ND: not detected
The values indicated were the mean of three trials ± SD (Standard deviation).
[ab]Values in the same column having different superscripts differ significantly (P < 0.05).

Table 3: Incidence of identified *E. coli* serotypes according to strain character in traditional yoghurt.

Strain character	Serotype	%*
EPEC	$O_{124}:K_{72}$	55.6
EHEC	$O_{26}:K_{60}$	44.4

* The percentage was in relation to the number of isolates.

traditional one (Figure 2). This is in agreement with the results reported by Ibrahim (2008). This is may be attributed to the fact that *Bifidobacteria* is characterized by slower rate of production of lactic acid than *S. thermophiles* and *L. bulgaricus* in traditional yoghurt (Samona et al.,1996). However, all kinds of yoghurt are organoleptically acceptable.

Statistical analysis showed that the differences of acidity percentage between traditional and probiotic yoghurts were significant (P < 0.05).

Comparison of microbiological characteristics of yoghurts

Microbiological characteristics are indicators of safety, quality and shelf life of the prepared yoghurt. Total coliform count, total psychrotrphes count, aerobic spore former, *S. aureus*, *E. coli* and yeast and moulds counts of the examined yoghurt samples were determined and shown in Tables 1 and 2. The Microbial loads showed significant differences between two types of yoghurt ($p < 0.05$). The highest coliform count of $1.37\ x10^3 \pm 0.16x10^3$ cfu/ml and the highest yeast and moulds counts of $4.86 \times 10^3 \pm 2.12 \times 10^3$ cfu/ml were recorded for traditional yoghurt (Table 1) as compared with $1.4\ x10^2 \pm 2.12 \times 10^2$ and $1.24 \times 10^2 \pm 2.12 \times 10^2$ cfu/ml in bifidus yoghurt, respectively. In the same context, bifidus yoghurt had lower psychrotrophes count with $7.5 \times 10 \pm 2.12 \times 10$ cfu/gm than in traditional yoghurt (Table, 1). These results are not in agreement with EOSQ (2005) that stated, yoghurt should not contain more than 10 cfu/gm coliforms or yeast cells.

Regarding pathogenic bacteria, it was found that *E. coli* count could not be detected in bifidus yoghurt as compared with $1.22\ x10^3 \pm 0.16\ x10^3$ cfu/gm in traditional yoghurt (Table 2). *E. coli* has been reported in many researches as acid tolerance organism, as Halawa and AbouZeid (2000) found that *E. coli* persisted for up to 25 days in manufactured yoghurt. It seems that the survival of *E. coli* in both low temperature and pH confirmed the implication of acidic food on some outbreaks due to *E. coli* infection (Sharp et al., 1995). As shown in this study, bifidus yoghurt can restrict E. *coli* growth, and has been shown to overcome the severe outbreaks of *E. coli* infection.

These results came in agreement with those of Elbarbary (2014) who recorded that the count of *E. coli* decreased gradually in bifidus yoghurt on the 3rd day of storage about 3.00 Log. Then the reduction in bacterial population continued till disappeared completely on the 10th day of storage. Similar results were reported by Amer et al. (2010); Abdel-Aziz (2011).

As regard Serotyping, the identified isolates (9 isolates) in the current study were enteropathogenic (EPEC) and enterohemorrahagic *E. coli* (EHEC). Of the type the *E. coli* isolates found, $O_{124}:K_{72}$ (EPEC) and $O_{26}:K_{60}$ (EHEC) were the most identified serotype with a percentage of 55.6 (5 isolates) and 44.4 (4 isolates), respectively (Table 3). In the same context, Ibrahim (2008) isolated *E. coli* from traditional yoghurt and serologically identified as O_{26}: K_{60} and O_{128}: K_{67}. Raw milk may consider as one of the sources of these serotypes in other dairy products. Farhan et al. (2014) were able to isolate O_{124}:K_{72} (9.52%) from raw milk. However, 14.29 and 9.67% O_{26}:K_{60} have been isolated from raw milk and Kareish cheese, respectively. This indicated that *Bifidobacteria* has a powerful antibacterial activity against these serotypes.

Enteropathogenic *E. coli* (EPEC) have been implicated in food and waterborne human illnesses, especially as an important agent of infantile diarrhea in developing countries (Ramos, 1996). EHEC is the most important recently emerged group of food-bornepathogens. It can cause severe gastrointestinal disease, including fatal infections and is being detected more frequently worldwide. EHEC strains not only produce potent cytotoxins (verotoxins) but have also acquired the ability to adhere to the intestinal mucosa in an intimate fashion (Fagan et al., 1999).

The data on the incidence of *S. aureus* in cold storage traditional and bifidus yoghurt are shown in Table 2. The count of *S. aureus* was 7.85 $x10^3 \pm 0.16$ $x10^3$and 6.5 x10± 0.16 x10 cfu/gm in traditional and bifidus yoghurts, respectively. This indicated that *Bifidobacteria* have powerful antibacterial activity against *S. aureus*. This is in agreement with the findings of Elbarbary (2014) who reported that the population decreased gradually during cold storage of bifidus yoghurt and disappeared at the 7th day of storage as compared with the control in which the bacteria was still viable until 15th day of storage. Moreover, probiotics have potential applications as biopreservatives in the food industry (O'Sullivan et al., 2002). They inhibit the growth of some food spoilage and food-borne pathogens, such as *Listeria monocytogenes*, *S. aureus*, *E. coli* and *Clostridium botulinum* (Cintas et al., 1997). At the same time, aerobic spore formers could not be detected in both types of yoghurt.

The differences in microbial loads and other quality parameters can be attributed to differences in type of starter cultures used in the production process. It has been reported that the quality of yoghurt in local market varies from one producer to another and that poor raw material, unhygienic practices and type of starter culture can lead to a product of poor quality (Younus et al., 2002). This is almost similar to the results of in this study due to the application of different starter culture during manufacturing of yoghurt.

In general, probiotic yoghurt with *Bifidobacteria* species was superior to the control yoghurt and this superiority has been shown from the inhibitory activity of *Bifidobacteria* against *E. coli* and *S. aureus*, coliform and yeast and moulds as compared with that of yoghurt culture bacteria. This is may be due to the antimicrobial agents produced by *Bifidobacteria*, such as organic acids; mainly acetic and lactic acids (Bruno and Shah, 2002) and bacteriocins (Murad et al., 2000; Shah, 2001). To date, some bacteriocins such as bifidin 1 (Cheikhyoussef et al., 2010), bifidocin B (Yildirim and Jhonson, 1998; Yildirim et al., 1999) and bacteriocin-like inhibitory substances (BLIS) (Collado et al., 2005; Cheikhyoussef et al., 2009) have been reported to be produced by *Bifidobacteria*, and have an inhibitory activities against wide range of Gram-positive and Gram-negative bacteria.

CONCLUSION

Yoghurt prepared using *Bifidobacteria* is the best in all quality aspects as compared with traditional one. Application of probiotics is useful for yoghurt industries to produce new variety of functional and high quality yoghurts.

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