

APPLICATION OF LABORATORY PRODUCED EPS IN THE MANUFACTURE OF SET YOGHURT AND FERMENTED BUTTER MILK

H. A. Murad² ; M.E. Moussa²; R.I. Refae¹ and Sahar H. Mohamed²

1- Agric. Microbiology Dept., Faculty. Of Agriculture, Cairo University, Giza.

2- Dairy Sci. Dept., National Res. Center, Dokki, Giza.

(Received: Sep., 10 , 2007)

ABSTRACT: *Exopolysaccharides (EPS) and their producing strains were used separately for improving properties of yoghurt and fermented butter milk. Different concentrations of EPS ranged from 0.1 to 0.5% (w/v) were used for preparation of yoghurt prepared by inoculating cow milk with 2% of an active mixed culture (1:1) of Streptococcus thermophilus and Lactobacillus dlbreuckii ssp bulgaricus . Butter milk was fermented at 30 °C using three local isolated-EPS producing Leuconostoc mesenteroides strains in association with non EPS-producing cultures including, Lactococcus lactis ssp diacetylactis and Laetococcus lactis ssp cremoris. The viscosity of yoghurt was improved upon using 0.5% EPS. It was also improved in the fermented butter milk containing EPS-producing cultures Leuconostoc mesenteroides NRC-12 and L.rhamnosus. No alterations of pH values and the total solids of yoghurt and fermented butter milk was detected as a result of addition of EPS or EPS-producing cultures. The yoghurt texture was improved and syneresis was reduced as the EPS concentration was elevated to 0.3% or more. The viable counts were increased upon using 0.3% EPS or more for Streptococcus thermophilus and Lactobacillus dlbreuckli ssp bulgaricus upon using 0.4% EPS. No marked effected was detected in the levels of acetaldhyde or diacetyl could be attributed to supplementation with EPS producing cultures.*

Key words: *yoghurt, fermented buttermilk, exopolysacchrides.*

INTRODUCTION

Lactic acid bacteria (LAB) have an indispensable technological role in food processing, especially in dairy industry. They are often added as milk fermentation starter cultures and occur widely as indigenous contaminants in raw milk (EL-Soda et al., 2003). Some strains of LAB are able to synthesize exopolysaccharides; EPS (Ricciardi and Clementi, 2000). In general, LAB have a food-grade status and EPS produced by these bacteria can be considered as food-grade additives and become an alternative to chemical, plant or animal additives as a source of stabilizing, thickening, gelling or

water-binding agents (De Vuyst and Degeest, 1999). However, appearance and physical characteristics are important quality parameters of yoghurt and fermented buttermilk. Good quality yoghurt should be thick and smooth with no signs of syneresis. Set yoghurt with a high level of syneresis on the surface may be regarded as a low quality product, even though this is a natural phenomenon (Amatayakul *et al.*, 2006). Conventionally, syneresis is reduced by using stabilizers or increasing the total solids of yoghurt mix around 14% (w/w) with dry dairy ingredients (Tamime and Deeth, 1980). Moreover there has been an increasing trend in the use of starter cultures that are able to produce exopolysaccharides. These EPS are homopolysaccharides or heteropolysaccharides. The use of EPS-producing LAB strains may improve the rheological properties of fermented milk. The gel structure and viscosity of the products are affected by the gel formation conditions, as well as the amount and the type of the EPS produced. Hammelehle *et al.* (1998) showed that fast warming rates (20 -50°C) during acidification increased the firmness and storage modulus, and decreased the syneresis of a milk gel. Skim milk fermented by ropy EPS-producing strains exhibited similar rheological properties and had greater viscosity than skim milk fermented by non-ropy strains (Schellhaass and Morris, 1985). In addition to the viscosifying effect of the polysaccharides, the interactions between the EPS and the milk proteins, e.g. caseins, also play a role. The microorganisms and / or the EPS that they produce may affect the protein aggregation, thereby affecting the physical properties of the milk gel (Van Marle and Zoon, 1995). However, the rheological properties of stirred yoghurt were affected by the type of EPS-producing strains used, suggesting an effect due to the interaction between the polymer and milk proteins (Marshall and Rawson, 1999). The objective of the present study was to investigate the effect of laboratory-produced EPS and EPS-producing strains of LAB on the chemical, microbiological and rheological properties of yoghurt and fermented buttermilk.

MATERIALS AND METHODS

Milk

Fresh whole cow milk used in this study was obtained from the Faculty of Agriculture, Cairo University, Giza.

Buttermilk

Fresh buttermilk was obtained from animal production institute, Ministry of Agriculture, Egypt.

Yoghurt starter culture

Streptococcus thermophilus and *Lactobacillus delbrueckii* ssp. *bulgaricus* were obtained from Chr. Hansen Laboratory, Copenhagen, Denmark.

Buttermilk starter culture

Leuconostoc mesenteroides B-512f was obtained from Microbial Properties Research Unit, National Center for Agricultural Utilization Res., USDA, Peoria, Illinois and *L.rhamnosus* NRRL B-442 was obtained from Microbial Genomic and Bioprocess USDA, ARE , USA.

Lactococcus lactis ssp. *diacetylactis* and *Lactococcus lactis* ssp. *cremoris* were obtained from Department of Dairy sciences, National Research Centre, Egypt. Three isolated (10, 12 and 16) from *Leuconostoc mesenteroides*(Refae et al., 2006) are used.

Yoghurt manufacturing using purified EPS as stabilizer

Whole cow milk was heat treated at 85°C for 30 min and cooled to 42°C according to Azzat (2001). Yoghurt starter culture was prepared using 2% (v/v) of active mixed cultures (1:1) of *Streptococcus thermophilus* and *Lactobacillus dlebreuckii* ssp. *bulgaricus* as inoculums. EPS were added to the milk during pasteurization process in different concentrations 0.1, 0.2, 0.3, 0.4 or 0.5% (as dry weight).

The control was whole milk inoculated with mixed starter cultures only. The samples were transferred into 40 ml plastic cups and incubated at 42°C for 2 to 4 h until coagulation, after which the cups were stored at 7°C for 15 days.

Seven treatments of yoghurt were prepared:

- 1- Mixture of *Streptococcus thermophilus* and *Lactobacillus dlebreuckii* ssp. *bulgaricus* 2% (v/v) as control
- 2- Yoghurt prepared with 2% of strain *Lactobacillus rhamnosus* and 2% (v/v) of *Streptococcus thermophilus* and *Lactobacillus dlebreuckii* ssp. *bulgaricus*.
- 3- Yoghurt prepared with 0.1% EPS and 2% (v/v) of *Streptococcus thermophilus* and *Lactobacillus delbreuckii* ssp. *bulgaricus*.
- 4- Yoghurt prepared with 0.2% EPS and 2% (v/v) of *Streptococcus thermophilus* and *Lactobacillus dlebreuckii* ssp. *bulgaricus*.
- 5- Yoghurt prepared with 0.3% EPS and 2% (v/v) *Streptococcus thermophilus* and *Lactobacillus delbreuckii* ssp. *bulgaricus* .
- 6- Yoghurt prepared with 0.4% EPS and 2% (v/v) of *Streptococcus thermophilus* and *Lactobacillus dlebreuckii* ssp. *bulgaricus*.

7- Yoghurt prepared with 0.5% EPS and 2% (v/v) of *Streptococcus thermophilus* and *Lactobacillus dlebreuckli* ssp. *bulgaricus*.

Fermented buttermilk manufacturing using isolated strains.

Fresh buttermilk was fortified with 0.1% sodium citrate, heat treated to 85°C for 30 min and then cooled to 30°C according to Levata-Jovanovic and Sandine (1997). The buttermilk was divided into 5 equal portions and different starter cultures were added to each portion as follows:

Treatment (1): *Leuconostoc mesenteroides* B-512f (3%), *Lactococcus lactis* ssp. *diacetylactis* (1%) and *Lactococcus lactis* ssp. *cremoris* (1%), served as a control.

Treatment (2): the same as in treatment (1) with addition of *Leuconostoc mesenteroides* 3% (isolate no-10), instead of *Leuconostoc mesenteroides* B-512f.

Treatment (3): the same as in treatment (1) with addition of *Leuconostoc mesenteroides* 3% (isolate no-12), instead of *Leuconostoc mesenteroides* B-512f.

Treatment (4): the same as in treatment (1) with addition of *Leuconostoc mesenteroides* 3% (isolate no-16), instead of *Leuconostoc mesenteroides* B-512f.

Treatment (5): the same as in treatment (1) with addition (2%) of *Lactobacillus rhamnosus* NRRL B-445, instead of *Leuconostoc mesenteroides* B-512f.

All portions were incubated at 25°C overnight. The fermented buttermilk was cooled and gently stirred to break the curd and stored at 7°C for 15 days. The yoghurt and fermented buttermilk samples were taken at 0, 3, 5, 7, 10 and 15 days of storage for viscosity assay, bacteriological analysis, pH determination and organoleptic observation.

pH determination

The pH was determined using pH-meter (Model 5.1, Portugal)

Total solid content

Total solids for permeate and buttermilk were determined as described by AOAC (1990).

Determination of diacetyl and acetaldehyde

The determination of diacetyl and acetaldehyde were measured according to Lees and Jago (1970). Carbonyl semicarbazones were formed by the addition of 1 ml of a solution of the carbonyl compound (approximately 1 mM) to 1 ml of 6.7 mM semicarbazide. The tubes were sealed with parafilm and allowed to stand for 90 min before the addition of distilled water to make a total volume of 10 ml. Absorption spectra were determined against a

semicarbazide blank, prepared as above without the addition of carbonyl compounds. Diacetyl was measured at wavelength 270 nm and acetaldehyde was measured at wavelength 224 nm

Synersis:

Synersis was determined by measuring the volume of separated whey according (Sahar 2000) .

Viscosity measurement

Viscosity of yoghurt and fermented buttermilk were measured using Brookfield viscometer (DV-II+ spindle 14, Germany).

Sensory evaluation

The resultant fermented buttermilk and yoghurt samples were organoleptically scored by ten member's panel of sensory judges from Dairy Science and Technology Dep., National Research Center. Yoghurt was sensory evaluated according to Keating and White (1990) using a scheme of 10 points for appearance, 40 points for body and texture and 50 points for flavor. Buttermilk was sensory evaluated according to El-Shafei (2003) using a scheme of 40 points for flavor, 20 points for acidity, 10 points for color and 30 points for consistency.

Microbiological analysis of fermented buttermilk and yoghurt

One yoghurt or fermented buttermilk cup was taken from either treatment after complete coagulation at 0, 3, 5, 7, 10 and 15 days of refrigeration for analysis. One gram of yoghurt or buttermilk sample was diluted with 9 ml of saline and mixed uniformly. Subsequent serial dilutions were prepared and viable numbers were enumerated using pouring plate technique. For yoghurt, the counts of LAB were enumerated on MRS agar adjusted to pH 5.8 at 37°C for 48h in anaerobic incubator. Total bacterial counts were enumerated on nutrient agar at 37°C for 48h. Molds and yeasts were enumerated on malt extract agar acidified to pH 3.5 at 25°C for 5 days (APHA, 1992). For fermented buttermilk, viable cell counts of *Leuconostoc mesenteroides* were determined on MRS agar containing 30 µg/ml of vancomycin after incubation at 30°C for 24 to 36 hr. *Lactococcus* strains were counted on M17 agar after aerobic incubation at 30°C for 48 h (Levata-Jovanovic and Sandine, 1997). *Lactobacillus rhamnosus* was determined on a selective medium *Lactobacillus casei* (LC) agar following 3-4 days of incubation at 27°C (Ravula and Shah, 1998) under anaerobic conditions.

Statistical analysis

Statistical analysis was conducted using the least significant difference according to Gomes and Gomes (1984). Correlations among variable parameters were calculated by correlation coefficients (*r*).

RESULTS AND DISCUSSION

Appearance and physical characteristics are important quality parameters of yoghurt and fermented buttermilk. Good quality of yoghurt should be thick and smooth (with no signs of syneresis). Set yoghurt with a high level of syneresis on the surface may be regarded as a low quality product, even though this is a natural phenomenon.

Set yoghurt

The effect of addition of EPS (0.1, 0.2, 0.3, 0.4 and 0.5%) on the chemical (Table, 1) and microbiological (Table, 2) properties of yoghurt compared with control was presented.

Chemical properties

Data given in Table (1) showed that using EPS (Treatments, 1, 2, 3, 4 and 5) had no effect on the total solids content of all fresh treatments compared to control yoghurt, whereas they decreased in all treatments during cold storage period. This might be due to fermentation of lactose, and hydrolysis of protein and fat with the formation of volatile substances (Tamime and Deeth, 1980 and Abd El Salam *et al.*, 1996). The effect of addition of different levels of EPS (0.1, 0.2, 0.3, 0.4 and 0.5%) on pH values of yoghurt samples as compared with control during cold storage period is presented in Table (1). It could notice that there were no differences in pH values between control and yoghurt treatments during cold storage period. These results confirm those of Abd El Salam *et al.* (1996) who found that the kind of stabilizer had no effect on the development of acidity during the yoghurt storage. The pH of yoghurt samples decreased during storage period. This might be attributed to the continuation of metabolic activity of starter culture (Backer and Pahan 1989). Tamime and Robinson (1985) reported that the catabolism of lactose by *Streptococcus thermophilus* and *Lactobacillus bulgaricus* mainly results in lactic acid. These results coincided with those found by Abd El -Mageed (1987), Barrantes *et al.* (1994) and Farahat (1999).

Microbiological properties

The viable counts of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* in yoghurt made with different concentrations of EPS (0.1, 0.2, 0.3, 0.4 and 0.5%) during 15 days of cold storage are presented in Table (2). The majority of treatments showed appropriate growth of

Streptococcus thermophilus compared with the control. On the other hand, treatments 1 and 2 showed lower viable counts of *Streptococcus thermophilus*. This might be explained by that EPS may affect the viable

Table (1): Changes in pH and Total solids of yoghurt during storage period

| Yoghurt treatments | Storage period (days) | | | | | | | | | | | |
|--------------------|-----------------------|---------------------|---------------------|---------------------|---------------------|---------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|
| | 0 | 3 | 5 | 7 | 10 | 15 | 0 | 3 | 5 | 7 | 10 | 15 |
| | pH | | | | | | Total solids % | | | | | |
| Control | 4.77 cb ±.12 | 4.65 def ±.09 | 4.55 g-i ±.03 | 4.53 g-j ±.04 | 4.50 h-k ±.03 | 4.43 j-q ±.01 | 13.60 abc ±.02 | 13.56 a-e ±.01 | 13.50 d-g ±.01 | 13.50 d-g ±.03 | 13.40 hij ±.06 | 13.35 jkl ±.02 |
| 1 | 4.77 cb ±.04 | 4.57 e-h ±.01 | 4.47 h-m ±.02 | 4.45 o±.05 | 4.40 k-r ±.03 | 4.39 k-q ±.02 | 13.61 ab ±.02 | 13.60 abc ±.01 | 13.54 b-e ±.02 | 13.53 c-f ±.01 | 13.40 hij ±.03 | 13.38 ijk ±.02 |
| 2 | 4.81 b±.02 | 4.61 d-g ±.03 | 4.57 e-h ±.01 | 4.49 h-l ±.01 | 4.40 k-r ±.02 | 4.38 l-r ±.01 | 13.50 d-g ±.03 | 13.60 abc ±.01 | 13.50 d-g ±.03 | 13.40 hij ±.02 | 13.34 jkl ±.03 | 13.30 lmn ±.02 |
| 3 | 4.97 a±.03 | 4.70 cd ±.02 | 4.55 g-i ±.05 | 4.45 i-o ±.02 | 4.35 n-r ±.01 | 4.31 r ±.02 | 13.49 efg ±.01 | 13.40 hij ±.02 | 13.30 lmn ±.02 | 13.23 no ±.03 | 13.20 o ±.01 | 13.20 o ±.02 |
| 4 | 4.66 cb ±.02 | 4.48 h-m ±.01 | 4.46 h-n ±.02 | 4.40 k-r ±.02 | 4.37 m-r ±.02 | 4.35 n-r ±.02 | 13.50 d-g ±.01 | 13.50 d- g±.03 | 13.45 ghiz 01 | 13.41 hij ±.02 | 13.40 hij±.0 3 | 13.35 jkl ±.01 |
| 5 | 4.80 | 4.47 h-m ±.05 | 4.38 r±.02 | 4.35 n-r ±.01 | 4.32 qr ±.03 | 4.33 pqr ±.01 | 13.62 a±.02 | 13.60 abc ±.01 | 13.60 abct. 04 | 13.57 a-d ±.04 | 13.50 d-g ±.02 | 13.46 fgh ±.01 |
| 6 | 4.97 a ±.02 | 4.67 e-h | 4.54 e-h ±.02 | 4.48 h-m ±.03 | 4.44 i-p ±.03 | 4.34 o-r ±.02 | 13.60 abc ±.02 | 13.50 d-g ±.02 | 13.40 hij ±.03 | 13.31 klm ±.01 | 13.30 lmn ±.01 | 13.25 mno ±.02 |

* Treatment 1 (0.1 % EPS), treatment 2 (0.2 % EPS), treatment 3 (0.3 % EPS), treatment 4 (0.4 % EPS), treatment 5 (0.5 % EPS) and treatment 6 (instead of EPS *Lb. rhamnosus* was used). Means with same letters are not significantly different ($p < 0.05$). Values are the means \pm standard error of triplicate measurements.

count of this bacterium by stimulating the growth (Marshall, 1993; Marshall and Tamime, 1997; Shah, 1997; Zimer and Gibson, 1998; Shah, 2000). Addition of 0.4% EPS gave the highest viable counts of *Streptococcus thermophilus* compared with control and other treatments. Amatayakul et al. (2006) reported that viable counts of *Streptococcus thermophilus* in yoghurt made with capsular starter cultures increased slightly during storage and declined thereafter. Also, the viable counts of *Lactobacillus delbrueckii* ssp. *bulgaricus* (Table, 2) increased by increasing the EPS concentration

compared with control and 0.4% EPS (Treatment, 4) gave the highest viable counts of *Lactobacillus delbrueckii* ssp. *bulgaricus*. This might be attributed to the effect of EPS on stimulation the growth of *Lactobacillus delbrueckii* ssp. *bulgaricus*. The viable counts of *Lactobacillus rhamnosus* (Treatment, 6) slightly decreased by increasing the cold storage period and *Lactobacillus rhamnosus* influenced the viable counts of *Streptococcus thermophilus* by increasing the viable counts to some extent compared with the control. While *Lactobacillus rhamnosus* had no effect on the viable counts of *Lactobacillus delbrueckii* ssp. *bulgaricus*. This might suggests the presence of the protective effect of EPS on the employed strain *Streptococcus thermophilus*. Such protective effects were not observed for *Lactobacillus delbrueckii* ssp. *bulgaricus*. This contradicts with Amatayakul et al. (2006) who reported that the use of ropy EPS-producing starter cultures in yoghurt had protective effect on *Lactobacillus delbrueckii* ssp. *bulgaricus* and no such protective effects were observed for *Streptococcus thermophilus*. Moulds and yeasts were not detected in all treatments either at zero time and during cold storage period.

Rheological properties

Synersis

In yoghurt, syneresis is considered to be a defect. In this respect, the methods of controlling syneresis may involve the heating of milk (Harwalkar and Kalab, 1986), the use of thickening agents such as xanthan gum or carrageenan (Kalab et al., 1975; Abd El-Salam et al., 1996), the use of ropy bacterial cultures or fortification of yoghurt milk with milk proteins including whey proteins (Tamime and Deeth, 1980; Tamime et al., 1984).

It is appeared from data presented in Table (3) that the yoghurt made with different EPS concentrations (Treatments, 1, 2 and 6) had a lower syneresis (Wheying-off) than control. While in case of treatments 3, 4 and 5 no syneresis observed at zero time and during the cold storage period. This could be attributed to the high water binding capacity of the added EPS (Walzem et al., 2002; Cerning, 1990; De Vuyst and Degeest, 1999)

Application of laboratory produced EPS in the manufacture

Table (2): The viability of LAB in yoghurt during storage period.

| Storage period (days) | Control | | Treatment 1 (0.1 % EPS) | | Treatment 2 (0.2 % EPS) | | Treatment 3 (0.3 % EPS) | | Treatment 4 (0.4 % EPS) | | Treatment 5 (0.5 % EPS) | | Treatment 6 (- EPS) | | |
|-----------------------|-------------------|----------------|-------------------------|----------------|-------------------------|----------------|-------------------------|----------------|-------------------------|----------------|-------------------------|----------------|---------------------|----------------|--------------|
| | Log c.f.u/ml | | Log c.f.u/ml | | Log c.f.u/ml | | Log c.f.u/ml | | Log c.f.u/ml | | Log c.f.u/ml | | Log c.f.u/ml | | |
| | Str. Thermophilus | Lb. bulgaricus | Str. thermophilus | Lb. bulgaricus | Str. thermophilus | Lb. bulgaricus | Str. thermophilus | Lb. bulgaricus | Str. thermophilus | Lb. bulgaricus | Str. thermophilus | Lb. bulgaricus | Str. thermophilus | Lb. bulgaricus | L. rhamnosus |
| 0 | 9.45 | 9.30 | 8.56 | 9.38 | 9.04 | 9.04 | 9.82 | 9.41 | 9.30 | 9.28 | 9.48 | 9.41 | 9.43 | 9.41 | 9.65 |
| 3 | 9.30 | 9.48 | 9.69 | 8.52 | 9.30 | 8.95 | 10.18 | 9.70 | 9.70 | 9.77 | 9.70 | 9.66 | 9.52 | 9.38 | 9.60 |
| 5 | 9.51 | 9.30 | 8.20 | 7.78 | 8.60 | 8.48 | 9.76 | 9.32 | 9.82 | 9.70 | 9.48 | 9.15 | 9.26 | 9.18 | 9.20 |
| 7 | 9.32 | 9.20 | 7.90 | 6.78 | 7.20 | 6.0 | 8.90 | 6.78 | 9.84 | 9.48 | 9.32 | 9.60 | 9.18 | 9.00 | 9.0 |
| 10 | 8.76 | 8.48 | 7.20 | 6.30 | 5.70 | 4.70 | 8.83 | 8.41 | 9.58 | 8.70 | 9.30 | 9.08 | 9.08 | 7.78 | 8.79 |
| 15 | 8.68 | 8.43 | 5.78 | 4.90 | 5.48 | 4.30 | 8.48 | 8.32 | 8.58 | 8.28 | 8.85 | 8.48 | 8.48 | 7.48 | 8.60 |

Table (3): The syneresis of yoghurt treatments during storage period.

| *Yoghurt treatments | Storage period (days) | | | | | |
|---------------------|-----------------------|--------------|---------------|---------------|---------------|--------------|
| | 0 | 3 | 5 | 7 | 10 | 15 |
| | Syneresis (ml/40g) | | | | | |
| Control | 0.5a ±.12 | 0.5a ±.12 | 0.6a ±.06 | 0.6a ±.12 | 0.65a ±.09 | 0.6a ±.06 |
| 1 | 0d±0 | 0d±0 | 0.2bc ±.06 | 0.2bc ±.06 | 0.3b ±.12 | 0.3b ±.06 |
| 2 | 0d±0 | 0d±0 | 0d±0 | 0d±0 | 0.3b ±.06 | 0.3b ±.12 |
| 3 | 0d±0 | 0d±0 | 0d±0 | 0d±0 | 0d±0 | 0d±0 |
| 4 | 0d±0 | 0d±0 | 0d±0 | 0d±0 | 0d±0 | 0d±0 |
| 5 | 0d±0 | 0d±0 | 0d±0 | 0d±0 | 0d±0 | 0d±0 |
| 6 | 0d±0 | 0.1cd ±0 | 0.2bc ±.06 | 0.2bc ±.12 | 0.2bc ±.06 | 0.3b ±.12 |

* Treatment 1 (0.1 % EPS), treatment 2 (0.2 % EPS), treatment 3 (0.3 % EPS), treatment 4 (0.4 % EPS), treatment 5 (0.5 % EPS) and treatment 6 (instead of EPS Lb. rhamnosus was used). Means with same letters are not significantly different ($p < 0.05$). Values are the means \pm standard error of triplicate measurements.

or may act synergistically with proteins in retaining water in the gel structure and may affect syneresis levels. Amatayakul *et al.* (2006) reported that yoghurt made using EPS-producing starter cultures had a lower level of syneresis than yoghurt produced with non-EPS producing starter cultures.

Viscosity

A characteristic property, which determines the rate flow of any fluid, is its viscosity, which may be broadly defined as its "internal friction" or resistant to flow. The viscosity depends on the temperature, concentration and molecular weight of solute and suspended matter.

Apparent viscosity

Apparent viscosity is defined as the viscosity of non-Newtonian fluid. This means that the apparent viscosity changes as the shear rate are changed. The existence of a continuous network implies that yoghurt is a gel and a viscoelastic material characterized by a fairly small yield stress.

Figure (1) presented the apparent viscosity of yoghurt samples as affected by EPS concentration (0.1, 0.2, 0.3, 0.4 and 0.5%) during cold storage period. Treatments 4, 5 and 6 showed the highest apparent viscosity compared with other treatments and control at the end of cold storage period. Here, the addition of EPS concentrations affected the apparent viscosity of yoghurt compared with the control. This might be due to the interactions between the EPS and the casein-matrix which play a role on the viscosity of yoghurt. A mixture of polysaccharides and proteins can induce aggregation when associative interactions are operational (Syrbe *et al.*, 1998) and the polysaccharides tend to adsorb onto the protein surfaces. If the amount of polymer is not sufficient to cover the protein surfaces, a polysaccharide may adsorb onto more than one protein surface leading to (bridging) flocculation. In contrast, when the proteins are fully covered the polysaccharide-coated proteins are stabilized, which could be important if the proteins are initially unstable and the polysaccharide can thus act as stabilizer (Michiel *et al.*, 1998). However, when the polysaccharides are very small compared to the protein particles and the volume fraction of particles is sufficiently high, aggregation of particles can lead to a space filling network in a process called gelation (Syrbe *et al.*, 1998).

Sensory evaluation

Table (4) present the organoleptic properties (appearance, body and texture, flavor and total scores) of yoghurt made with different EPS concentrations and with *Lactobacillus rhamnosus* (Treatment, 6) during cold storage. The yoghurt made with EPS concentrations of 0.3, 0.4 or 0.5% and with *Lactobacillus rhamnosus* had higher organoleptic properties than

control and those made with 0.1 and 0.2% of EPS concentrations. These results could be attributed to that adding EPS in concentrations ranged from 0.3 to 0.5% or using *Lactobacillus rhamnosus* (EPS-producing culture) in fermented milk have a greater effect on the physical properties than control and other treatments.

Here, it could be concluded that yoghurt made with 0.4 and 0.5% EPS concentration (Treatments 4 and 5) and product made with *Lactobacillus rhamnosus* can be used to improve the final product of organoleptic and rheological properties.

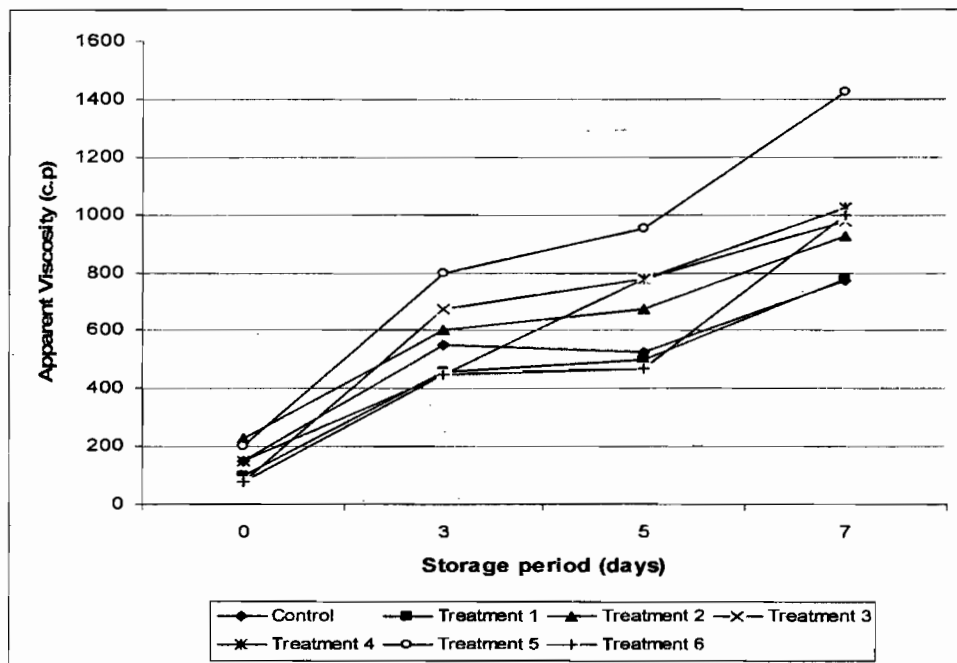


Fig (1): Apparent viscosity of yoghurt during storage period.

Treatment 1 (0.1 % EPS), treatment 2 (0.2 % EPS), treatment 3 (0.3 % EPS), treatment 4 (0.4 % EPS), treatment 5 (0.5 % EPS) and treatment 6 (instead of EPS *Lb. rhamnosus* was used).

Table (4): Sensory evaluation of yoghurt during storage period.

| Yoghurt treatments* | Storage period(days) | Appearance (10) | Body&texture (40) | Flavor (50) | Total scour (100) |
|---------------------|----------------------|-----------------|-------------------|-------------|-------------------|
| Control | 0 | 9 | 30 | 45 | 84 |
| | 3 | 9 | 31 | 48 | 88 |
| | 5 | 9 | 30 | 48 | 87 |
| | 7 | 9 | 30 | 48 | 87 |
| | 10 | 8 | 30 | 48 | 86 |
| | 15 | 8 | 25 | 48 | 81 |
| 1 | 0 | 9 | 32 | 48 | 89 |
| | 3 | 9 | 32 | 48 | 89 |
| | 5 | 9 | 32 | 48 | 89 |
| | 7 | 9 | 30 | 48 | 89 |
| | 10 | 9 | 29 | 48 | 88 |
| | 15 | 8 | 29 | 48 | 85 |
| 2 | 0 | 9 | 30 | 48 | 87 |
| | 3 | 9 | 30 | 48 | 87 |
| | 5 | 9 | 30 | 48 | 87 |
| | 7 | 9 | 30 | 48 | 87 |
| | 10 | 9 | 28 | 48 | 85 |
| | 15 | 8 | 28 | 48 | 84 |
| 3 | 0 | 9 | 33 | 48 | 90 |
| | 3 | 9 | 33 | 48 | 90 |
| | 5 | 9 | 33 | 48 | 90 |
| | 7 | 9 | 30 | 48 | 87 |
| | 10 | 9 | 30 | 48 | 87 |
| | 15 | 8 | 30 | 48 | 86 |
| 4 | 0 | 9 | 34 | 48 | 91 |
| | 3 | 9 | 34 | 48 | 91 |
| | 5 | 9 | 34 | 48 | 91 |
| | 7 | 9 | 34 | 48 | 91 |
| | 10 | 9 | 33 | 48 | 90 |
| | 15 | 8 | 33 | 48 | 89 |
| 5 | 0 | 9 | 34 | 48 | 91 |
| | 3 | 9 | 34 | 48 | 91 |
| | 5 | 9 | 34 | 48 | 91 |
| | 7 | 9 | 34 | 48 | 91 |
| | 10 | 9 | 33 | 48 | 90 |
| | 15 | 8 | 33 | 48 | 89 |
| 6 | 0 | 9 | 34 | 48 | 91 |
| | 3 | 9 | 34 | 48 | 91 |
| | 5 | 9 | 34 | 48 | 91 |
| | 7 | 9 | 34 | 48 | 91 |
| | 10 | 9 | 33 | 48 | 90 |
| | 15 | 8 | 33 | 48 | 89 |

* Treatment 1 (0.1 % EPS), treatment 2 (0.2 % EPS), treatment 3 (0.3 % EPS), treatment 4 (0.4 % EPS), treatment 5 (0.5 % EPS) and treatment 6 (instead of EPS Lb. rhamnosus was used).

Fermented buttermilk

The effect of using *Leuconostoc mesenteroides* strains nos. 10, 12, 16 and *Lactobacillus rhamnosus* as a starter cultures in fermented buttermilk on the chemical (Table, 5 and 6,) and microbiological properties (Table7) of fermented buttermilk compared with control are presented.

4.8.2.1. Chemical properties

Using *Leuconostoc mesenteroides* strains nos. 10, 12, 16 and *Lactobacillus rhamnosus* had no effect on total solids content of all fresh treatments compared to control, whereas they slightly decreased in all treatments during cold storage (Table5). This may be due to fermentation of lactose, and hydrolysis of protein and fat with the formation of volatile substances (Tamime and Deeth, 1980; Abd El Salam *et al.*, 1996).

The changes in pH values of fermented buttermilk during cold storage period are presented in Table (5). All treatments showed decreased in pH value during cold storage period. This might be due to the fermentation of lactose by *Lactococcus* strains and production of lactic acid. No differences effect in pH values between control and fermented buttermilk treatments during storage period.

Acetaldehyde and diacetyl contents

The acetaldehyde and diacetyl contents of fermented buttermilk treatments are presented in Table (6). At fresh time, the acetaldehyde content in fermented buttermilk with *Leuconostoc mesenteroides* no. 12 and the control were higher than those made with *Leuconostoc mesenteroides* nos. 10, 16 and *Lactobacillus rhamnosus*. This might be due to the action of the microorganisms which produce more acetaldehyde than the others. While, during storage period, acetaldehyde content from different treatments and control were decreased. Treatments 2 and 4 showed higher contents of acetaldehyde during storage period. On the other hand, fermented buttermilk treatments 1, and 3 had lower diacetyl content than those made with *Leuconostoc mesenteroides* no. 12, *Lactobacillus rhamnosus* and control at fresh case. Treatments 1 and 3 showed constant content of diacetyl content. This might be attributed to the citrate metabolism into diacetyl may occur more in treatments 2 and 4 than the other treatments and low pH and temperature effect on the citrate metabolism into diacetyl. Moreover, all fermented buttermilk treatments had reached to maximum diacetyl content at 15 days. This is possibly attributed to that the diacetyl and acetoin accumulated because citrate repressed the synthesis of diacetyl reductase (DR) and acetoin reductase (AR) (Drinan *et al.*, 1976). So, there is little or no enzyme to reduce them to acetoin and 2, 3-butylene glycol, respectively.

Once citrate metabolism is finished increased synthesis of DR and AR occurs resulting in decreased level of diacetyl and acetoin (Drinan *et al.*, 1976).

Table (5): Changes in pH and Total solids of fermented buttermilk during storage period.

| Fermented buttermilk treatments * | Storage period (days) | | | | | | | | | | | |
|-----------------------------------|-----------------------|-----------------|------------------|-----------------|-----------------|------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| | 0 | 3 | 5 | 7 | 10 | 15 | 0 | 3 | 5 | 7 | 10 | 15 |
| | pH | | | | | | Total solids % | | | | | |
| Control | 4.78a ±0.01 | 4.57d ±0.01 | 4.39fgh ±0.01 | 4.11mn ±0.01 | 4.24k ±0.02 | 4.36ghl ±0.02 | 12.80fg ±0.04 | 12.83ef ±0.01 | 12.84ef ±0.04 | 12.90c- f±0.01 | 12.95b- e±0.04 | 13.00a- d±0.11 |
| 1 | 4.67bc ±0.01 | 4.49e ±0.02 | 4.41fg ±0.01 | 4.20kl ±0.01 | 4.32ij ±0.02 | 4.32ij ±0.02 | 12.90c- f±0.01 | 12.90c- f±0.03 | 12.90c- f±0.02 | 13.00a- d±0.01 | 13.07a b±0.04 | 13.00a- d±0.01 |
| 2 | 4.64c ±0.01 | 4.45ef ±0.03 | 4.34hl ±0.03 | 4.17lm ±0.02 | 4.23kl ±0.02 | 4.36ghl ±0.01 | 12.70g ±0.04 | 12.85ef ±0.02 | 12.90c- f±0.01 | 12.95b e±0.09 | 12.95b- d±0.04 | 13.00a- d±0.02 |
| 3 | 4.61cd ±0.02 | 4.44ef ±0.02 | 4.31ij ±0.01 | 4.01o ±0.01 | 4.10n ±0.07 | 4.29ij ±0.02 | 12.85ef ±0.09 | 12.90c- f±0.06 | 12.90c- f±0.04 | 13.02a bc±0.02 | 13.00a- d±0.08 | 13.11a ±0.02 |
| 4 | 4.72b ±0.02 | 4.56d ±0.01 | 4.45ef ±0.01 | 4.17lm ±0.01 | 4.26jk ±0.02 | 4.50e ±0.01 | 12.70g ±0.01 | 12.70g ±0.05 | 12.80fg ±0.01 | 12.80fg ±0.03 | 12.90c- f±0.03 | 12.90c- f±0.08 |

*Treatments (1): starter culture + *Leuc. mesenteroides* (10), (2): starter culture + *Leuc. mesenteroides* (12), (3): starter culture + *Leuc. mesenteroides* (16), (4): starter culture + *Lb. rhamnosus*. Means with same letters are not significantly different ($p < 0.05$). Values are the means \pm standard error of triplicate measurements.

Table (6): Effect of EPS-producing starter culture on acetaldehyde and diacetyl contents of Fermented buttermilk.

| Fermented buttermilk treatments* | Storage period (days) | | | | | | | | | | | |
|----------------------------------|------------------------|----------------|-----------------|---------------------|---------------------|----------------|--------------------|-----------------|---------------------|---------------------|---------------------|--------------------|
| | 0 | 3 | 5 | 7 | 10 | 15 | 0 | 3 | 5 | 7 | 10 | 15 |
| | Acetaldehyde mmol/100g | | | | | | Diacetyl mmol/100g | | | | | |
| Control | 24± 1.5 | 18.2± 0.23 | 18± 0 | 14.6fg ± 0.17 | 14.4f g± 0.12 | 14fg± 0.64 | 16.4hi± 0.2 | 16.8hi± 0.17 | 17.2g h± 0.35 | 21ef± 1.15 | 21ef± 1.15 | 30c± 0.58 |
| 1 | 16± 0.29 | 12.2h± 0.12 | 11.2ih± 0.13 | 11ih±0 .07 | 10ij± 0.12 | 9j± 0.17 | 7.3k± 0.17 | 10j± 0.87 | 10j± 1.44 | 10j±0 0.58 | 10j± 0.58 | 21ef± 1.15 |
| 2 | 22.6b± 0.35 | 21c± 0.17 | 14.6fg± 0.4 | 14.4fg ± 0.12 | 14.4f g± 0.17 | 14fg± 0.29 | 15.6hi± 0.23 | 14.6i±0. 17 | 21.8de ± 0.12 | 23.6d ± 0.92 | 23.6 d ± 0.23 | 36.3a ± 1.33 |
| 3 | 15ef± 0.06 | 11ih± 0.58 | 10ij± 1 | 10ij± 1.2 | 10ij±0 0.29 | 9.2j± 0.29 | 7.3k± 0 | 7.3k± 0 | 7.3k± 0.4 | 7.3k± 1 | 10.2j± 0.46 | 19fg± 1.15 |
| 4 | 22.6b± 0.23 | 18.4d± 0.46 | 17.8d± 0.29 | 14.6fg ± 0.35 | 14.6f g± 0.23 | 13.6g± 0.63 | 16.4hi± 0.12 | 21ef± 0.12 | 21.8de ± 0.46 | 21.8d e± 0.69 | 21.8d e± 1.5 | 32.6b ± 0.52 |

* Treatments (1): starter culture + *Leuc. mesenteroides* (10), (2): starter culture + *Leuc. mesenteroides* (12), (3): starter culture + *Leuc. mesenteroides* (16), (4): starter culture + *Lb. rhamnosus*. Means with same letters are not significantly different ($p < 0.05$). Values are the means ± standard error of triplicate measurements.

4.8.2.3. Microbiological properties

The viability of *Leuconostoc mesenteroides* strains nos. 10, 12 and 16 as well as *Lactococci* strain and *Lactibacillus rhamnosus* in fermented buttermilk during 15 day of cold storage are presented in Table (7). Increasing of viable count of all strains was observed during the first three days of storage. This might be attributed to the breaking of the curd and also to the clumps or chains. These results coincide with those stated by Nighswonger *et al.* (1996) and Roushdy *et al.* (1996). Counts of *Leuconostoc mesenteroides*, *Lactococci* strain and *Lactibacillus rhamnosus* gradually decreased during the cold storage Table (7). Levata-Jovanovic and Sandine (1997) found that *Leuconostoc mesenteroides* and *Lc. lactis* ssp. *cremoris* cultures in cultured buttermilk did not grow actively during 7 days storage period, due to low pH and low temperature.

Table (7): The viability of *Leu. mesenteroides*, *Lactococci* strains and *Lb.rhamnosus* in fermented buttermilk during storage period.

| Storage period (days) | Control | | Treatment 1 | | Treatment 2 | | Treatment 3 | | Treatment 4 | |
|-----------------------|---------------------------------------|--|----------------------------------|--|----------------------------------|--|----------------------------------|--|----------------------|--|
| | Log c.f.u/ml | | Log cf.u/ml | | Log c f.u/ml | | Log c f.u/ml | | Log c f.u/ml | |
| | <i>Leu. mesenteroides</i> NRRL B-512f | Total count of <i>lactococci</i> strains | <i>Leuc. mesenteroides</i> No 10 | Total count of <i>lactococci</i> strains | <i>Leuc. mesenteroides</i> No 12 | Total count of <i>lactococci</i> strains | <i>Leuc. mesenteroides</i> No 16 | Total count of <i>lactococci</i> strains | <i>Lb. rhamnosus</i> | Total count of <i>lactococci</i> strains |
| 0 | 9.72 | 9.74 | 9.36 | 9.88 | 9 | 9.96 | 9.29 | 9.45 | 9.62 | 9.66 |
| 3 | 10.1 | 10 | 9.79 | 10.34 | 10.22 | 10.24 | 9.46 | 9.98 | 10.64 | 10.31 |
| 5 | 9.62 | 9.76 | 9.20 | 10.07 | 8.93 | 10.09 | 8.78 | 9.83 | 10.17 | 9.97 |
| 7 | 9.54 | 9.70 | 8.30 | 9.60 | 8.11 | 9.94 | 8.25 | 9.63 | 10.04 | 9.92 |
| 10 | 9.51 | 9.48 | 8.23 | 9.43 | 8.04 | 9.48 | 8.18 | 9.58 | 9.72 | 9.62 |
| 15 | 8.38 | 9.02 | 7.64 | 9.30 | 8 | 8.76 | 7.28 | 8.73 | 9.06 | 9.02 |

Rheological properties

Apparent viscosity

Apparent viscosity of fermented buttermilk samples during storage period are shown in fig. (2). at zero time, insignificant differences were observed in the viscosity of fermented buttermilk made with *Leuconostoc mesenteroides* nos. 10 and 12 (Treatments 1 and 2). On the other hand, significant effects were observed with fermented buttermilk made with *Leuconostoc mesenteroides* no. 16 and *Lactibacillus rhamnosus* (Treatments 3 and 4) compared with control. The most viscous fermented buttermilk produced was with *Leuconostoc mesenteroides* no. 12 and *Lactibacillus rhamnosus*. Levata-Jovanovic and Sandine, (1997) reported that using *ropy Lc. lactis* ssp. *cremoris* strain 352 as the starter of buttermilk resulted in texture improvement. This might be attributed to that exopolysaccharides produced by *Leuconostoc mesenteroides* no. 12 and *Lactibacillus rhamnosus* interact

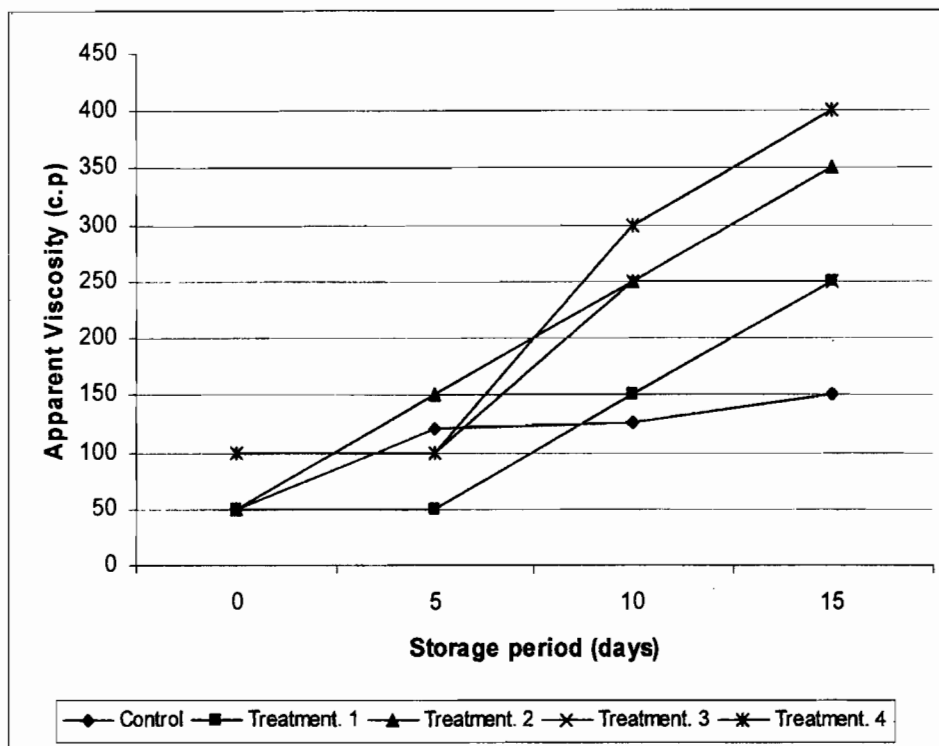


Figure (2): Apparent viscosity of fermented buttermilk treatments during storage period.

Treatments (1): starter culture + Leuc. mesenteroides (10), (2): starter culture + Leuc. mesenteroides (12), (3): starter culture + Leuc. mesenteroides (16), (4): starter culture + Lb. rhamnosus

with the free water in the gel-like structure (De Vuys and Deest, 1999; Hassan *et al.*, 1996 and 2002; Rohm and Kovac, 1994; Skriver *et al.*, 1993; Teggtatz and Morris, 1990; Vlahopoulou and Bell, 1993), or due to the interaction between proteins and polysaccharides, the presence of bacterial cells and filaments of EPS bound to them, the binding of hydration water that reduces the amount of free water molecules and consequently increases the apparent concentration of EPS in the serum phase (Duboc and Mollet, 2001). All these lead to improving the viscosity of fermented buttermilk. The improvement of viscosity was observed during cold storage period. Treatments 2 and 4 (Fermented buttermilk with *Leuconostoc mesenteroides* No 12 and *Lactibacillus rhamnosus*) gave highest viscosity compared with other treatments (1 and 2) and control after 15 day of storage period. Rawson and Marshall (1997) found that a combination of aropy strain of *Lb. bulgaricus* with non-ropy strain of *Str. thermophilus* produced a viscous product, whereas the combination of two ropy strains resulted in a product which lost its viscosity so well.

4.8.2.5. Sensory evaluation

Table (8) presents the sensory evaluation (flavor, body & texture, color and total scores) of fermented buttermilk treatments during storage period. It is obvious that fermented buttermilk with *Leuconostoc mesenteroides* no. 12 and *Lactibacillus rhamnosus* gained the highest scores when being fresh and throughout storage. The lowest total score was reported for fermented buttermilk made with *Leuconostoc mesenteroides* no. 16. No foreign or undesirable flavor was detected in all treatments. However, they had a variable effect on the body and texture of fermented buttermilk. Treatments 2 and 4 had a good body and texture and flavor compared with other treatments and control. This possibly due to high contents of diacetyl and acetaldehyde and high viscosity for treatments 2 and 4 compared with other treatments and control.

Finally, it could be concluded from chemical, microbiological, rheological properties and sensory evaluation that fermented buttermilk made with *Leuconostoc mesenteroides* No 12 and *Lactibacillus rhamnosus* (Treatments 2 and 4) could be used to improve the organoleptic and rheological properties of final product.

Table (8): Sensory evaluation of fermented buttermilk during storage period.

| Fermented buttermilk treatments | Storage period (days) | Flavor (40) | Body&texture (30) | Acid (20) | Color (10) | Total scor (100) |
|--|------------------------------|--------------------|------------------------------|------------------|-------------------|-------------------------|
| Control | 0 | 29 | 20 | 16 | 9 | 74 |
| | 3 | 33 | 24 | 17 | 9 | 83 |
| | 5 | 32 | 27 | 17 | 9 | 85 |
| | 7 | 32 | 27 | 17 | 9 | 85 |
| | 10 | 31 | 26 | 16 | 9 | 82 |
| | 15 | 30 | 26 | 16 | 8 | 80 |
| 1 | 0 | 27 | 19 | 17 | 9 | 72 |
| | 3 | 32 | 25 | 17 | 9 | 83 |
| | 5 | 32 | 27 | 17 | 9 | 85 |
| | 7 | 32 | 27 | 16 | 9 | 84 |
| | 10 | 32 | 26 | 16 | 9 | 83 |
| | 15 | 31 | 26 | 16 | 8 | 80 |
| 2 | 0 | 28 | 20 | 18 | 9 | 75 |
| | 3 | 33 | 25 | 18 | 9 | 85 |
| | 5 | 33 | 26 | 18 | 9 | 86 |
| | 7 | 33 | 26 | 18 | 9 | 86 |
| | 10 | 33 | 26 | 17 | 9 | 85 |
| | 15 | 32 | 25 | 17 | 9 | 84 |
| 3 | 0 | 23 | 17 | 17 | 9 | 66 |
| | 3 | 32 | 24 | 17 | 9 | 82 |
| | 5 | 32 | 25 | 17 | 9 | 83 |
| | 7 | 32 | 25 | 16 | 9 | 82 |
| | 10 | 32 | 25 | 16 | 9 | 82 |
| | 15 | 30 | 24 | 16 | 9 | 82 |
| 4 | 0 | 34 | 22 | 18 | 9 | 83 |
| | 3 | 36 | 26 | 18 | 9 | 89 |
| | 5 | 37 | 28 | 18 | 9 | 92 |
| | 7 | 37 | 28 | 18 | 9 | 92 |
| | 10 | 36 | 28 | 18 | 9 | 91 |
| | 15 | 35 | 27 | 17 | 9 | 88 |

* Treatments (1): starter culture + Leuc. mesenteroides (10), (2): starter culture + Leuc. mesenteroides (12), (3):starter culture + Leuc. mesenteroides (16), (4): starter culture + Lb. rhamnosus.

REFERENCES

- Abd El-Mageed, N.S.E. (1987). Studies on the use of lactose in some dairy products. M.Sc. Thesis. Food Sci. Dep., Fac. Agric., Ain Shams Univ., Cairo, Egypt.
- Abd El- Salam, M.H.A.; M.H. El-Etriby and N.M.Shahein (1996). Influence of some stabilizers on some chemical and physical properties of yoghurt. Egyptian J. Dairy Sci. 24: 25.
- Amatayakul, T.; A.L. Halmos; F. Sherkat and N.P. Shah (2006). Physical characteristics of yoghurt made using exopolysaccharide-producing starter cultures and varying casein to whey protein ratios. Intern. Dairy J. 16: 40-51.
- (APHA) American Public Health Association. (1992). Standard methods for the examination of dairy products. Inc. 16th. Ed. Washington D.C., USA.
- AOAC. (1990) Official Method of analysis. 15 th Ed. Arlington, Virginia, 22201, USA.
- Azzat, B.A.M. (2001). Studies on the utilization of some microorganisms as dietary adjuncts under local conditions. Ph.D. Thesis. Agric. Microbiology Dep., Fac. Agric., Cairo Univ., Giza, Egypt.
- Backer, T. and Z. Puhon (1989). Effect of different process to increase the milk solids non-fat content on rheological properties of yoghurt. Milchwissenschaft. 44: 626-629.
- Barrantes, E.; A.Y. Tamime; G. Davies and M.N.I. Barcly (1994). Production of low-calorie yoghurt using skim milk powder and fat substitute. 2-Composition quality. Milchwissenschaft. 49: 135-139.
- Cerning, J. (1990). Exocellular polysaccharides produced by lactic acid bacteria. FEMS Microbiol. Rev. 87: 113-130.
- De Vuyst, L. and B. Degeest (1999). Heteropolysaccharides from lactic acid bacteria. FEMS Microbiol. Rev. 23: 152-177.
- Drinan, D.F.; S. Tobin and T.M. Cogan (1976). Citric acid metabolism in hetro and homofermentative lactic acid bacteria. Appl. Environ. Microbiol. 31: 481-486.
- Duboc, P. and B. Mollet (2001). Application of exopolysaccharides in the dairy industry. Int. Dairy J. 11: 759-768.
- El- Shafel, K. (2003). Production of probiotic culture buttermilk using mixed cultures containing polysaccharide producing *Leuconostoc mesenteroids*. Egyptian J. Dairy Sci. 31: 43-60.

- El-Soda, M.; M. El.Ziney; S. Awad; G. Osman; N. Omran; G. Gamal; N. Ezzat and H. Shafei (2003). A culture collection of lactic acid bacteria isolated from raw milk and traditional Egyptian dairy products. *Egyptian J. Dairy Sci.* 31: 23-41.
- Farahat, A.M. (1999). Production of some special dairy products. M.Sc. Thesis. Food Sci. Dep., Fac. Agric., Ain Shams Univ., Cairo, Egypt.
- Gomes, K.A. and A.A. Gomes (1984) *Statistical Procedures for Agricultural Research*. John Wiley & Sons ,USA.
- Hammelehle, B.; P. Schkoda and H.G. Kessler (1998). Effect of gel formation conditions on the texture of direct acidified milk gels. *Milchwissenschaft.* 53: 247-251.
- Harwalkar, V.R. and M. Kalab (1986). Relationship between microstructure and susceptibility to syneresis in yoghurt made from reconstituted fat dry milk. *Food Microstruc.* 5: 287-294.
- Hassan, A.N.; M. Corredig and J.F. Frank (2002). Capsule formation by nonropy starter cultures affects the viscoelastic properties of yoghurt during structure formation. *J. Dairy Sci.* 85: 716-720.
- Hassan, A.N.; J.F. Frank; K.A. Schmid and S.I. Shalabi (1996). Rheological properties of yoghurt made with encapsulated nonropy lactic cultures. *J. Dairy Sci.* 79: 2091-2097.
- Kalab, M.; D.B. Emmons and A.G. Sargent (1975). Milk gel structure. IV Microstructure of yoghurts in relation to the presence of thickening agents. *J. Dairy Res.* 42: 453-458.
- Keating, K. and C.H. White (1990). Effect of alternative sweeteners in plain and fruit flavored yoghurt. *J. Dairy Sci.* 37: 54-59.
- Lees ,G.J.and G.R. Jago (1970) The estimation of diacetyl in presence of other carbonyl compounds.*J.Dairy Res.*37:129-132.
- Levata-Jovanovic, M. and W.E. Sandine (1997). A method to use *Leuconostoc mesenteroides* ssp. *cremoris* 9140 to improve milk fermentation. *J. Dairy Sci.* 80: 11-18.
- Marshall, V.M. (1993). Starter cultures for milk fermentation and their characteristics. *J. Soci. Dairy Tech.* 46: 49.
- Marshall, V.M. and A.Y. Tamime (1997). Starter cultures employed in the manufacture of biofermented milks. *Intern. Dairy J.* 50: 351.
- Marshall, V.M., and H.L. Rawson (1999). Effects of exopolysaccharide-producing strains of thermophilic lactic acid bacteria on the texture of stirred yogurt. *Intern.J. Food Sci. &Tech.*, 34, 137-143.

- Michael, B.; B. Kees and D. Lubbert (1998). Mutational analysis of exopolysaccharide biosynthesis by *Lactobacillus sakei* 0-1. *FEMS Microb. Lett.* 169: 241-249.
- Nighswonger, B.D.; M.M. Brashears and S.E. Gilliland (1996). Viability of *Lac. acidophilus* and *Lac. casei* in fermented milk products during refrigerated storage. *J. Dairy Sci.* 79: 212.
- Ravula, R.R. and N.P. Shah (1998). Selective enumeration of *Lactobacillus casei* from yoghurt and fermented milk drinks. *Biotechnol. Techniq.* 12: 819.
- Rawson, H.L. and V.M. Marshall (1997). Effect of ropy strains of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* on rheology of stirred yoghurt. *Intern. J. Food Sci. Techno.* 32: 213.
- Refae, R.I.; G. Amin; H.A. Murad and H.M. Sahar (2006). Exopolysaccharide production by dairy lactic acid bacteria:1. Isolation, characterization of E.P.S.-producing culture and effect of medium supplementation and culture conditions. *J.Agric.Sci. Manssura Univ.* 31:6401-6434.
- Ricciardi, A. and F. Clementi (2000). Exopolysaccharides from lactic acid bacteria: structure, production and technological application (review). *Ital. J. Food Sci.* 12: 23-45.
- Rohm, H. and A. Kovac (1994). Effect of starter cultures on linear viscoelastic and physical properties of yoghurt gels. *J. Texture study*, 25: 311-329.
- Roushdy, I.M.; A.A. Ali and Y.M. Kenany (1996). Production of acidophilus and bifidus buttermilks from buffaloes' milk. *Egypt. J. Food Sci.* 24:233.
- Sahar, H.M. (2000) Production of microbial polysaccharide xanthan gum and its application in some dairy and dairy-like products. Master. Thesis. Agric. Microbiology Dep., Fac. Agric., Cairo Univ., Giza, Egypt
- Schellhaass, S.M., and H.A. Morris (1985). Rheological and scanning electron microscopic examination of skim milk gels obtained lactic acid bacteria. *Food microstruct.* 4: 279-287.
- Shah, N.P. (1997). Bifidobacteria: Characteristics and potential for application in fermented milk products. *Milchwissenschaft*, 52: 16.
- Shah, N.P. (2000). Probiotic bacteria selective enumeration and survival in dairy food. *J. Dairy Sci.* 83: 894.
- Skriver, A.; H. Roemer and K.B. Qvist (1993). Rheological characterization of stirred yoghurt: Viscometry. *J. Texture Study*, 25: 311-329.
- Syrbe, A.; W.J. Bauer and H. Klostermeyer (1998). Polymer science concepts in dairy systems- an overview of milk protein and food colloid interaction. *Intern. Dairy J.* 8: 179-193.

Application of laboratory produced EPS in the manufacture

- Tamime, A.Y. and H.C. Deeth (1980). Yoghurt: Technology and Biochemistry. J.Food Prot. 43: 939-977.
- Tamime, A.Y. and R.K. Robinson (1985). Yoghurt: Science and technology. 1st edition. Pergamon Press, Oxford.
- Tamime, A.Y.; M. Kalab and G. Davis (1984). Microstructure of set-style yoghurt manufactured from cow's milk fortified by various method. Food Microstruc. 3: 83-92.
- Teggatz, J.A. and H.A. Morris (1990). Changes in the rheology and microstructure of ropy yoghurt during shearing. Food Struct. 9: 133-138.
- Van Marle, M.E. and P. Zoon (1995). Permeability and rheological properties of microbiologically and chemically acidified skim-milk gels. Netherlands milk and dairy journal, 49:47-65.
- Vlahopoulou, I. and A. Bell (1993). Effect of various starter cultures on the viscoelastic properties of bovine and caprine yoghurt gels. J. Soci. Dairy Technol. 46: 61-63.
- Walzem, R.L.; C.J. Dillard and J.B. German (2002). Whey components: Millennia of evolution ceate functionalities for mammalian nutrition:What we know and what we may be overlooking. Critical Reviews in Food Sci. and Nutri. 42: 354-375.
- Ziemer, C.J. and G.R. Gibson (1998). An overview of probiotics, prebiotics and synbiotics in the functional food concept: Perspevtives and future strategies. Intern. Dairy J. 8: 473.

استخدام السكريات العديدة المنتجة معمليا في تصنيع اليوجيهورت واللبن الخض المتخمّر

حسين عزاز مراد^١ ، موسى معالي عيد سالم^١ ، رفاعي ابراهيم رفاعي^٢ ،
سحر حسن محمد^١

١ - قسم الالبان-المركز القومي للبحوث

٢ - قسم الميكروبيولوجي-كلية الزراعة-جامعة القاهرة

الملخص العربي

استخدمت السكريات العديدة والسلالات الميكروبية المنتجة لها بصورة منفصلة لتحسين خواص اليوجيهورت واللبن الخض المتخمّر. حيث استخدمت تركيزات مختلفة من هذه السكريات تراوحت ما بين ٠,١ - ٠,٥ % (وزن / حجم) بالنسبة الى اليوجيهورت بعد تلقيح اللبن بمزرعة مختلطة (١:١) من *S.thermophilus*, *L.bulgaricus* كما تم تصنيع لبن الخض المتخمّر على درجة حرارة ٣٠م باضافة ثلاث سلالات من بكتيريا *Luconostoc mesenteroides* بالاشتراك مع سلالات غير منتجة للسكريات العديدة تشمل *Lactococcus lactis ssp. cremores* و *Lactococcus lactis ssp. di acetalactis*

ولقد تحسنت لزوجة اليوجيهورت عند استخدام ٠,٥ % من هذه السكريات وكذلك تحسنت اللزوجة عند اضافة السلالة المنتجة للسكريات العديدة *Luconostoc NRC-12* *Lactobacillus raminosus. mesentroides*

وقد لوحظ عدم حدوث تغير في درجة pH في كل من اليوجيهورت واللبن الخض المتخمّر. وقد تحسن قوام الخثرة مع انخفاض نسبة التثريش عندما زادت نسبة السكريات العديدة الى ٠,٣% فاكتر وقد ازداد العد البكتيري عند نفس النسبة من هذه السكريات العديدة.