Pol. J. Food Nutr. Sci., 2011, Vol. 61, No. 1, pp. 55-60
http://journal.pan.olsztyn.pl

Original Paper
Section: Food Technology

DOI: 10.2478/v10222-011-0005-9

Evaluation of Milk Drinks Fermented by Probiotic Bacteria and Fortified with Zinc Salts

Faten Lotfi Seleet, Wagih Ibrahim El-Kholy, Nabil Samy Abd-Rabou*

Dairy Science Department, National Research Center, Dokki, Giza, Egypt

Key words: fermented milk, zinc salts, fortification, probiotic, sensory evaluation

Fermented milk drinks were made with buffalo's milk produced by three probiotic bacteria *Lactobacillus acidophilus* L-a-5, *Bifidobacterium bifidum* and *Lactococcus lactis ss lactis biovar diacetilactis* MD 099(1:1:1). Four treatments fortified with 10 and 20 mg zinc element/L (as zinc sulphate or zinc acetate), respectively, were studied in comparison with a control sample for 10 days of storage at 5°C. The sensory properties and cell viability of the fermented products were evaluated. The gross chemical composition, pH, flavor components (acetaldehyde and diacetyl) and viscosity were determined. The results showed that zinc salts activated the development of bacterial counts. Counts were increasing gradually during the first day of incubation till the end of the 5th day, then were gradually decreasing. The control and treatment samples contained the recommended levels of survival cells (10^6–10^7 CFU/g) of probiotic bacteria at the end of the 10th day. Statistical analysis of odour intensity, acidity, sweetness, bitterness, saltiness, creaminess, feeling after swallowing for fatty, astringency and metallic taste were sensory evaluated. A correlation was observed between sensory properties characteristics of fortified fermented milk drinks as affected by the addition of different levels of zinc salts.

INTRODUCTION

Fermented milk products are widely consumed for their benefits and refreshing effects. Their popularity is said to be attributed to the effective use of consumer-driven flavors and milder cultures [Jensen & Kroger, 2000]. These products already have a positive health image [Jelen *et al*., 2003; Valli & Traill, 2005], which can be further enhanced by the addition of probiotic bacteria with therapeutic properties [Lourens-Hattingh & Viljoen, 2001].

Probiotics – *i.e.* living microorganisms that when consumed in sufficient amounts provide health benefits beyond basic nutrition – are emerging as important dietary ingredients in functional foods. The majority of probiotics are lactic acid bacteria, especially lactobacilli, and bifidobacteria. Factors related to technological and sensory aspects of the probiotic food products are of utmost importance since only by satisfying the demands of consumers can the food industry succeed in promoting the consumption of functional products in the future [Mattila-Sandholm *et al*., 2002]. Growth of the fermented milk sector represents an opportunity to advance the development of fermented milk fortified with micronutrients into products with interesting nutritional and sensory properties without requiring complicated or costly technology [Sienkiewicz & Riedel, 1990]. Supplementation or fortification with zinc and other micronutrients may be benefic during periods of greatest vulnerability such as pregnancy and early childhood and when diet is low in animal products and based on high-phytate cereals and legumes [Allen, 1998]. Zinc is a metal with great nutritional importance and is particularly necessary in cellular replication and development of the immune response. Therefore, if the growing fetus and infants are at risk of development of zinc deficiency, then an adequate supply of this element is essential for normal growth development [Salgueiro *et al*., 2002]. So, zinc fortification is an important especially because daily intakes appear to be more useful physiologically than intermittent doses [Gidson *et al*., 1998]. Many forms of zinc salts are used in many supplementation trials. The solubility of these compounds is very important and strongly associated to the absorbability. Zinc sulphate is very soluble and zinc acetate is freely soluble [Gidson *et al*., 1998; Allen, 1998]. As reported by Keast [2003], zinc has very little taste (bitter, salty, savory, sour, and sweet), and astringency is the major oral sensation. Also, Lim & Lawless [2006] reported that the oral sensory properties of divalent salts can be described by a combination of four basic tastes with the addition of metallic and astringent. If the product is qualified as astringent, bitter or metallic, it will be rejected. Therefore, the acceptability of zinc-fortified foods has to be considered by panelists first, females specially, which is critical to predict young children acceptability. It seems clear that the prevention of zinc deficiency among young children remains the best policy, not only on moral ground, but also on economic ones. There is a great deal of work yet to be done to find an adequate way to prevent zinc deficiency, but it appears that zinc supplementation or food fortification with an adequate zinc compound may be the key to overcome such a worldwide nu-

* Corresponding author:
E-mail: faten.seleet@gmail.com (F.L. Seleet)

tritional problem [Salgueiro *et al*., 2002]. As zinc having this key role, this study is an attempt to produce fermented milk drinks fortified with zinc sulphate and zinc acetate. The fortification was done by adding little amounts of zinc sulphate or zinc acetate (10 or 20 mg zinc element /L), to offer protection from zinc deficiency for children aged 1–3, 4–8 and 9–13 years who need 3, 5, and 8 mg zinc/day respectively as recommended by the Dietary Reference Intakes [2001]. Each 100 mL of fortified fermented milk drink (10 or 20 mg zinc element/L) provide with 33.35%, 20% and 12% and their duplicate from daily requirement respectively. The duplicate are not to exceed the tolerable daily intakes which were recommended as 7, 12, and 23 mg/day, respectively. In this respect, we investigated the effect of fortification with zinc sulphate and zinc acetate on: physicochemical and sensory properties during refrigerated storage, as well as on the survival and activity of probiotic bacteria.

MATERIALS AND METHODS

Materials

Zinc acetate and zinc sulphate, food grade were obtained from CID, Company for Drugs, Cairo, Egypt.

Fresh buffalo's milk (15.18% TS, 4.10% TP and 6.2% fat) was obtained from the farm of the Faculty of Agriculture, Cairo University, Egypt.

Strains belonging to *Lactobacillus acidophilus* L-a-5 and *Bifidobacterium bifidum* were obtained from the agent of Chr. Hansens Laboratory Denmark A/S. and *Lactococcus lactis ss. lactis biovar diacetilactis* MD 099 were obtained from EZAL Group Rhone-Polanc Z. Ade Buxires BP-10, 88220 Dange Sainaii-Romain-France.

Production of fermented milk drink fortified with zinc salts

Buffalo's milk was used for preparing fermented milk drink fortified with zinc salts. A flow chart of fermented drinks preparation is presented in Figure 1. Milk was heated to 80°C for 15 min, and then rapidly cooled to 40°C. Three probiotic bacteria: *Lactobacillus acidophilus, L. lactis ss. lactis biovar diacetilactis* and *B. bifidum* (1:1:1) were added at the level of 3% (w/v) served as mixed active starter culture into the milk. The milk was divided to five portions as one control and four treatments: (1) the control, fermented milk drink without zinc salts; (2) T1, fermented milk drink fortified with 10 mg Zn element /L, using zinc acetate salt; (3) T2, fermented milk drink fortified with 20 mg Zn element /L, using zinc acetate salt; (4) T3, fermented milk drink fortified with 10 mg Zn element /L, using zinc sulphate salt; and (5) T4, fermented milk drink fortified with 20 mg Zn element /L, using zinc sulphate salt. The samples were incubated at 37°C for 5 h. The resultant coagulates of all treatments were stirred, filled in 250 g glass bottles and then stored in the refrigerator (5°C) for 10 days. Three replicate batches were made from each treatment.

Physicochemical determinations

The total solids, total nitrogen (TN) and non protein nitrogen contents (NPN) of fermented milk were determined according to AOAC [2000]. The pH value of the fermented milk was measured using a digital pH meter (HANNA, Instrument, Italy) with combined glass electrode. Acetaldehyde was determined as mentioned by Less & Jago [1969], while diacetyl content was estimated as described by Less & Jago [1970]. The samples were analysed on day 0, 5, 7 and 10. Viscosity was measured in fresh samples at 7°C using a Brookfield digital viscometer (Model DV-II+VISCOMETER, Spindle-00). The speed was set from 3 to100 rpm. Three readings, 30s apart, were recorded for each sample.

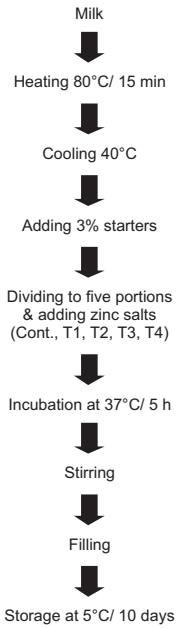

FIGURE 1. Flow chart of the preparation of fermented milk drinks.

Microbiological analysis

Enumeration of fermented milks cultures: samples of fermented milk drinks fortified with zinc were diluted in sterile tryptone diluents (0.1% w/v) and subsequently plated in duplicate onto the following selective media:

(1) viable cell count of *L. lactis ss diacetylactis* were enumerated on M17 agar (Oxoid) after aerobic incubation at 30°C for 48 h [Terzaghi & Sandine, 1975];

(2) *L. acidophilus* was determined on Lactobacillus selective agar plus 0.25 oxgall (LBSO) [Gilliland & Walker, 1990]; the plates were incubated at 37°C for 4 days;

(3) *Bifidobacterium bifidum* was determined according to Blanchett *et al*. [1996] using modified MRS agar (Oxoid) supplemented with 0.05% L-cysteine-HCl (Merck, Germany); the plates were incubated at 37°C for 48 h under anaerobic conditions (BBL Gas pak, Becton Dickinson, Cockeysvile, MA, USA);

(4) coliforms were enumerated according to Harrigan & McCance [1996] using violet Red Bile agar medium; the plates were incubated at 37°C for 24 h

(5) moulds and yeasts were determined according to Standard Methods for Examination of Dairy products [APHA, 1994], using Malt Extract Agar (Oxoid) acidified to pH 3.5; the plates were incubated at 30°C for 5 days.

Sensory evaluation

Eleven trained panelists (4 men and 7 women, between the ages of 28 and 50 years) were selected among the staff of Dairy Science Department, National Research Center, Cairo, Egypt. The entire group were non-smokers. They were asked for sensory evaluation and not to eat or drink for at least 1 h prior to testing. All members were highly trained and had participated in evaluation of dairy products for at least the previous 4 years continuously; they were familiar with terms, standards, and food examples. Scores were on a 5-point scale, where 1 = the absence of the attribute and 5 = exist extremely [Mainfreni *et al.,* 2002]. The sensory terms were adapted as by Gallardo-Escamilla *et al*. [2007] and Rogerio *et al*. [2006].

Statistical analyses

Statistical analyses were performed using the GLM procedure with SAS [1994] software. Duncan's multiple comparison procedure was used to compare the means. A probability to $p \leq 0.05$ was used to establish the statistical significance.

RESULTS AND DISCUSSION

Physicochemical determinations

Total solids content were 15.23, 15.21, 15.25, 15.25, 15.28 for control, T1, T2, T3, and T4 in fresh respectively tended to slight increase as the storage period prolonged. As by Al-Assar *et al*. [2005] and Salem *et al*. [1997], this increase may be attributed to the natural evaporation. The NPN /TN percent increased with the advance of storage period (Table 1), among the probiotic bacteria, *L. acidophilus*, in general, was more proteolytic than *Bifidobacterium* spp, and the differences in proteolytic activity between both groups were significantly different as by Shihata & Shah [2000]. An overall decline in the pH of all fermented milk drink samples occurred during the storage period (Figure 2). Generally, the higher decrease in pH was observed in treatments contained 20 mg zinc element /L (in the form of zinc sulphate or zinc acetate), by the ratio of 15.62% and 13.7%, respectively. However, the higher difference (1.99%) was noticed between control sample and treatment (T4) at the end day of storage period. The increase in acidity and decrease in pH were almost identical for plain yoghurt or fermented milk. Expected results of the activity of LAB were reported by Tamime & Robinson [1999]. The obtained results are in agreement with those reported by Alroubaiya [2004]. The viscosity of fresh samples increased as zinc content was increased in the prepared fermented milk drink (Figure 3). This increase may be attributed to the interaction between zinc salts and protein.

Acetaldehyde contents of both control and treatments were gradually increased until the 7th day (more pronounced in treatments with 20 mg zinc element/L) then suddenly decreased (Figure 4). In case of diacetyl (Figure 5), a slight increase occurred until the 7th day then decreased except the

TABLE 1. Change in NPN content (% of N total) in fermented milk drinks during the storage period at 5°C.

Treatments	Storage period (days)				
	Fresh	3	5	7	10
Control	7.86	9.01	10.12	15.00	17.13
Treatment 1	7.26	8.41	9.53	13.34	16.33
Treatment 2	7.55	8.94	10.50	14.38	17.56
Treatment 3	7.71	8.64	9.42	15.81	17.66
Treatment 4	7.63	8.53	10.22	15.81	17.19

T1 = 10 mg Zn/L (as zinc acetate); T2 = 20 mg Zn/L (as zinc acetate); T3 = 10 mg Zn/L (as zinc sulphate); and T4 = 20 mg Zn/L (as zinc sulphate).

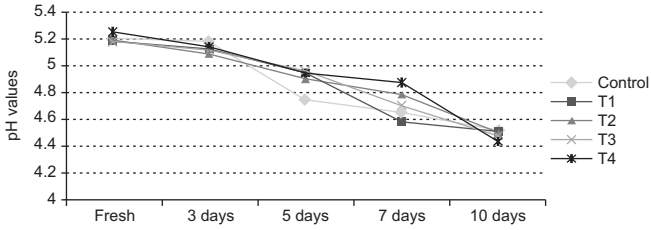

FIGURE 2. Changes of pH value of fortified fermented milk drinks during the storage at 5°C.

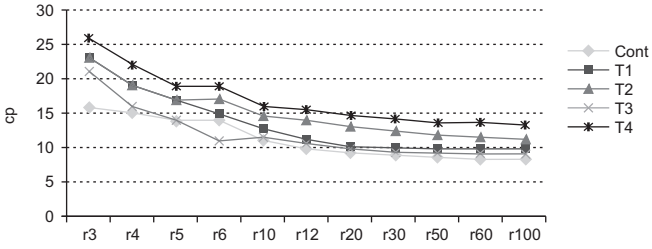

FIGURE 3. Effect of zinc fortification on the viscosity of fresh fermented milk drinks.

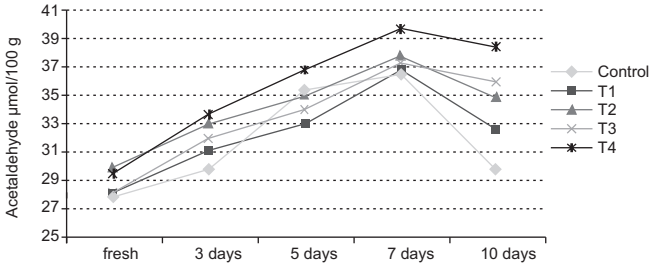

FIGURE 4. Changes of acetaldehyde content (μmol/100 g) in fermented milks drinks during storage at 5°C.

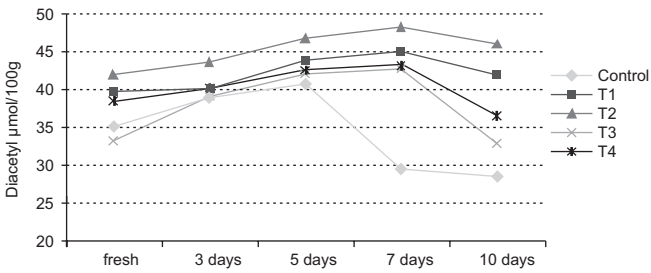

FIGURE 5. Changes of diacetyl content (μmol/100g) in fermented milks drinks during storage at 5°C.

control sample which showed a sharp decrease after 5th day of storage. However, it seems to be that the addition of zinc salts enhances formation of both acetaldehyde and diacetyl. These data are in agreement with those obtained by Aumara [2000] and Aumara & Hassan [2007].

Microbiological analysis

The effect of fortification by zinc salts on cell viability of probiotic bacteria is presented in Figure 6. Higher cell viability was observed in treatments fortified with 10 and 20 mg Zn element/L (the form of zinc sulphate) from the first day till the end of storage. Counts gradually increased from the first day of incubation till the end of the 5th day then decreased again, which may be due to the elevated pH values. In turn, the control sample reached the maximum level of cell viability in the 3rd day and gradually decreased at the end of storage. The reduction in the number of probiotic strains may be due to the sensitivity of these bacteria to the acid produced during the storage period. Higher cell viability of *B. bifidum* and *L. acidophilus* and *L. diacetylactis* could be due to the effect of zinc salts whish activated their growth. In this respect; these results are in agreement with Magdoub *et al.* [1993] who reported that zinc accelerated the growth rate of bacteria strains were tested (*L. lactis* spp. *lactis*, *S. thermophilus*, *L. delbruckii* spp. *bulgaricus*). Generally, numbers of all probiotic bacteria remained more than 10^6 CFU/mL in all treatments until the end of storage period. A minimum of 10^6–10^7 viable microorganisms per gram or milliter should be present in food product in order to meet the requirements of a probiotic food, as by the Japanese Fermented Milk and Lactic Acid Bacteria Beverages Association [Ishibashi & Shimanura, 1993]. All samples were free of coliforms, moulds and yeasts when fresh and throughout storage period (10 days) at refrigerator temperature of 5°C as result of hygienic condition during the preparation with, or by the fact that diacetyl is generally considered to be an antimicrobial agent because of the presence of two reactive carbonyl moieties in the molecule as reported by Lopez de Felipe *et al*. [1998].

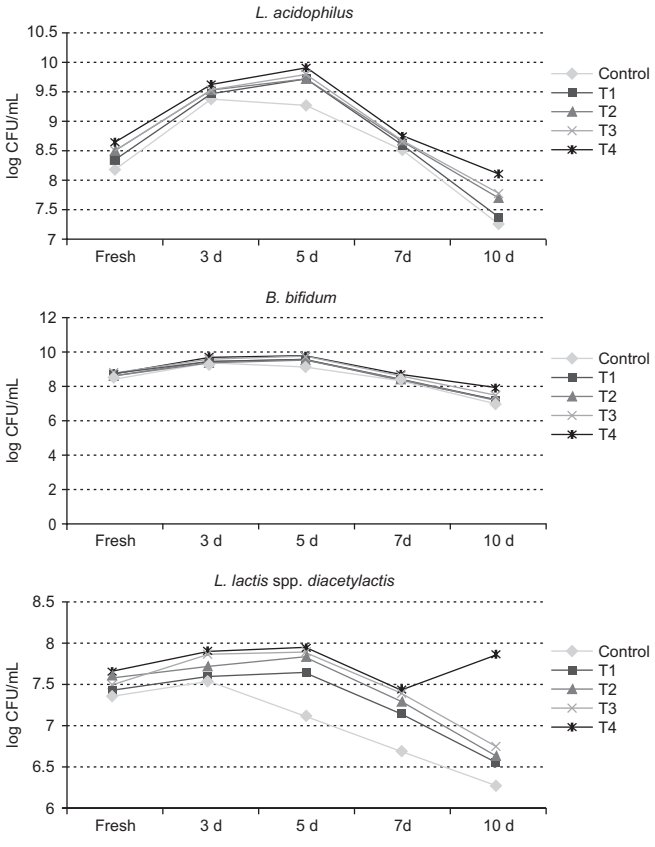

FIGURE 6. Effect of zinc fortification on: (A) *L. acidophilus*, (B) *B. bifidum* and (C) *L. lactis* ssp. *diacetylactis* in fermented milks during storage at 5°C.

Sensory evaluation

The mean scores of the sensory properties perceived in each sample and the statistical significance of the effect of the fortification with different levels of zinc (10 or 20 mg zinc/L) were shown in Table 2. Both levels of zinc salts (zinc acetate and zinc sulfate) caused a slight increase ($p>0.05$) in odor intensity, feeling of acidity, saltiness and metallic properties of fermented milk at different storage periods. Astringency property increased with addition of different levels of zinc salts, which were more pronounced ($p>0.05$) at 20 mg zinc/L in the form of zinc sulfate, whereas slight astringency was observed after swallowing in control sample. In this respect, Sano *et al*. [2005] reported that the elicitation of astringency induced by whey protein under acidic conditions would be caused by aggregation and precipitation of protein molecules in the mouth. For bitterness, there was no significant effect ($p>0.05$) of different levels of zinc salts, except for 20 mg zinc element/L (in the form of zinc sulfate) when changes were more pronounced after 10 days. These results were achieved as by Keast [2003], who suggested that the zinc salts had very little bitter taste. Conversely, sweetness and creaminess were gradually decreasing ($p>0.05$) with increasing levels of zinc element addition at different storage days. A negative correlation was observed between astringency and creaminess (Table 3). This suppression may be due to the zinc ion. Also, zinc sulfate is a potent inhibitor of the sweetness of most sweeteners, but does not affect salty taste quality [Keast *et al*., 2004; Keast, 2003]. It seems that probiotic cultures produced

TABLE 3. Correlation coefficient between characteristics of fortified fermented milk drinks as affected by addition of different levels of zinc salts.

Characteristics	Correlation coefficient
Acidity $_x$ odor	+0.37**
Acidity $_x$ saltiness	+0.20*
Creaminess $_x$ sweetness	+0.23**
Saltiness $_x$ feeling of acidity	+0.20*
Saltiness $_x$ bitterness	+0.27**
Saltiness $_x$ astringency	+0.17 *
Saltiness $_x$ metallic	+0.22**
Metallic $_x$ bitterness	+0.25**
Astringency $_x$ creaminess	-0.25**
Astringency $_x$ fatty	-0.21**
Astringency $_x$ bitterness	+0.17*

* – significant; ** – highly significant; NS – not significant.

TABLE 2. Sensory properties of fortified fermented milk drink as affected by addition of different levels of zinc salts.

Properties	Storage (days)	Treatments				
		Control	T1	T2	T3	T4
Odor intensity	Fresh	$3.82^{Aa} \pm 0.23$	$4.00^{Aa} \pm 0.23$	$4.09^{Aa} \pm 0.16$	$4.18^{Aa} \pm 0.12$	$4.27^{Aa} \pm 0.24$
	5	$4.00^{Aa} \pm 0.13$	$4.09^{Aa} \pm 0.21$	$4.18^{Aa} \pm 0.18$	$4.27^{Aa} \pm 0.24$	$4.36^{Aa} \pm 0.16$
	10	$3.91^{Aa} \pm 0.21$	$4.18^{Aa} \pm 0.24$	$4.27^{Aa} \pm 0.19$	$4.27^{Aa} \pm 0.19$	$4.45^{Aa} \pm 0.15$
Acidity	Fresh	$2.64^{Aa} \pm 0.24$	$3.00^{Aa} \pm 0.19$	$3.18^{Aa} \pm 0.23$	$2.73^{Aa} \pm 0.19$	$2.91^{Aa} \pm 0.31$
	5	$2.73^{Aa} \pm 0.24$	$3.27^{Aa} \pm 0.19$	$3.27^{Aa} \pm 0.14$	$3.00^{Aa} \pm 0.19$	$3.09^{Aa} \pm 0.37$
	10	$3.00^{Aa} \pm 0.27$	$3.36^{Aa} \pm 0.26$	$3.27^{Aa} \pm 0.24$	$3.18^{Aa} \pm 0.12$	$3.27^{Aa} \pm 0.24$
Sweetness	Fresh	$1.73^{Aa} \pm 0.27$	$1.18^{Ab} \pm 0.12$	$1.09^{Ab} \pm 0.09$	$1.09^{Ab} \pm 0.09$	$1.00^{Ab} \pm 0.00$
	5	$1.36^{Aa} \pm 0.20$	$1.00^{Ab} \pm 0.00$	$1.00^{Ab} \pm 0.00$	$1.09^{Ab} \pm 0.09$	$1.00^{Ab} \pm 0.00$
	10	$1.27^{Aa} \pm 0.19$	$1.00^{Ab} \pm 0.36$	$1.00^{Ab} \pm 0.00$	$1.00^{Ab} \pm 0.00$	$1.00^{Ab} \pm 0.00$
Bitterness	Fresh	$1.00^{Aa} \pm 0.00$	$1.00^{Aa} \pm 0.00$	$1.00^{Aa} \pm 0.00$	$1.00^{Aa} \pm 0.00$	$1.09^{Aa} \pm 0.09$
	5	$1.00^{Aa} \pm 0.00$	$1.00^{Aa} \pm 0.00$	$1.00^{Aa} \pm 0.00$	$1.09^{Aa} \pm 0.09$	$1.18^{Aa} \pm 0.12$
	10	$1.00^{Ab} \pm 0.00$	$1.00^{Ab} \pm 0.18$	$1.00^{Ab} \pm 0.00$	$1.09^{Ab} \pm 0.09$	$1.45^{Aa} \pm 0.21$
Saltiness	Fresh	$1.00^{Aa} \pm 0.00$	$1.18^{Aa} \pm 0.00$	$1.09^{Aa} \pm 0.09$	$1.18^{Aa} \pm 0.12$	$1.18^{Aa} \pm 0.12$
	5	$1.00^{Aa} \pm 0.00$	$1.00^{Aa} \pm 0.00$	$1.09^{Aa} \pm 0.09$	$1.18^{Aa} \pm 0.12$	$1.18^{Aa} \pm 0.12$
	10	$1.00^{Aa} \pm 0.00$	$1.00^{Aa} \pm 0.12$	$1.18^{Aa} \pm 0.12$	$1.27^{Aa} \pm 0.14$	$1.27^{Aa} \pm 0.14$
Creaminess	Fresh	$3.36^{Aa} \pm 0.15$	$3.27^{Aa} \pm 0.14$	$2.82^{Aa} \pm 0.26$	$2.82^{Aa} \pm 0.23$	$2.91^{Aa} \pm 0.25$
	5	$3.18^{ABa} \pm 0.16$	$3.09^{Ab} \pm 0.16$	$2.55^{Ac} \pm 0.16$	$2.64^{Abc} \pm 0.20$	$2.73^{Abc} \pm 0.19$
	10	$2.73^{Ba} \pm 0.19$	$2.82^{Aa} \pm 0.24$	$2.45^{Aa} \pm 0.16$	$2.55^{Aa} \pm 0.21$	$2.45^{Aa} \pm 0.25$
Fatty	Fresh	$3.09^{Aa} \pm 0.16$	$2.91^{Aa} \pm 0.25$	$2.73^{Aa} \pm 0.33$	$2.73^{Aa} \pm 0.27$	$2.64^{Aa} \pm 0.24$
	5	$3.00^{Aa} \pm 0.19$	$2.91^{Aa} \pm 0.21$	$2.45^{Aa} \pm 0.21$	$2.55^{Aa} \pm 0.21$	$2.45^{Aa} \pm 0.25$
	10	$2.36^{Ba} \pm 0.24$	$2.64^{Aa} \pm 0.31$	$2.36^{Aa} \pm 0.31$	$2.36^{Aa} \pm 0.24$	$2.27^{Aa} \pm 0.27$
Astringency	Fresh	$1.27^{Ab} \pm 0.14$	$1.91^{Ab} \pm 0.25$	$1.91^{Aa} \pm 0.39$	$1.82^{Ab} \pm 0.35$	$2.18^{Ab} \pm 0.35$
	5	$1.36^{Aab} \pm 0.20$	$2.00^{Aa\,b} \pm 0.38$	$2.00^{Aab} \pm 0.27$	$2.00^{Aab} \pm 0.33$	$2.36^{Aa} \pm 0.34$
	10	$1.36^{A\,b} \pm 0.15$	$2.09^{Aab} \pm 0.42$	$2.18^{Aab} \pm 0.30$	$2.73^{Aa\,b} \pm 0.47$	$2.55^{Aa} \pm 0.39$
Metallic	Fresh	$1.00^{Aa} \pm 0.00$	$1.00^{Aa} \pm 0.00$	$1.09^{Aa} \pm 0.09$	$1.09^{Aa} \pm 0.09$	$1.09^{Aa} \pm 0.09$
	5	$1.00^{Aa} \pm 0.00$	$1.09^{Aa} \pm 0.09$	$1.18^{Aa} \pm 0.12$	$1.27^{Aa} \pm 0.14$	$1.27^{Aa} \pm 0.14$
	10	$1.00^{Aa} \pm 0.00$	$1.27^{Aa} \pm 0.20$	$1.27^{Aa} \pm 0.14$	$1.27^{Aa} \pm 0.14$	$1.45^{Aa} \pm 0.28$

Means with the same letters between columns or rows are not significantly ($p \leq 0.05$). T1 = 10 mg Zn/L (as zinc acetate); T2 = 20 mg Zn/L (as zinc acetate); T3 = 10 mg Zn/L (as zinc sulphate); and T4 = 20 mg Zn/L (as zinc sulphate).

more intense odor with zinc sulfate than with zinc acetate and control (Table 2). The attributes astringency and bitterness showed positive correlation in between (Table 3), while a negative correlation was observed between the amount of fat perceived in the mouth after swallowing. In turn, a significant correlation was observed between astringency, bitterness, saltiness, metallic and fatty taste. The presence of astringency, metal or bitter taste is undesirable in dairy products. But, it could be considered that adding fruits may improve flavors produced by zinc salts, especially by zinc sulphate.

CONCLUSIONS

The obtained results suggested that fortification of fermented milk drinks with zinc acetate or zinc sulphate affected advantageously the sensory profile of the resultant products and promoted the growth of probiotic bacteria. All samples (both control and treatments) contained the recommended levels (10^6–10^7 CFU/g) of survival cells of probiotic bacteria at the end of storage. Therefore, the use of zinc element in the form of zinc acetate or sulphate at the levels of 10 or 20 mg Zn/L could be recommended as stimulating the growth and activity of probiotic bacteria already resident in the colon. Thus application of zinc acetate or zinc sulphate in fermented milk drink production will result in obtaining dairy products with new functional properties and healthy benefits.

REFERENCES

1. A.O.A.C. Official Methods of Analysis. Association Official Analytical Chemists, 2000, 17th Ed. Washington, DC, USA.
2. Al-Assar M.A., El-Abd M.M., El-Sabie W.B., Attia M., Characteristics of low cholesterol Rayeb milk during storage. Egypt. J. Appl. Sci., 2005, 20, 739–750.
3. Allen LH., Zinc and micronutrient supplements for children. Am. J. Clin. Nutr., 1998, 68, 495–498.
4. Alroubaiya K.A.S., Studies on some factors affecting the rheological, structural and organoleptical properties of stirred yo-

ghourt. 2004. M.Sc. Thesis, Dairy Sciences and Technology, Fac. Agric., Cairo University, pp. 86–94.
5. APHA, American Public Health Association. Standard methods of the examination of Dairy products. 1994. 16 th Ed, Washington, USA.
6. Aumara I.E., Studies on the use of Bifidobacteria in manufacture of some dairy products. 2000, M Sci. Thesis. Fac. of Agric. Ain Shams University, pp. 135–150.
7. Aumara I.E., Hassan Z.M.R., More healthy fermented milk manufactured with Doum palm fruit powder. 2007, Conf. Dairy Sci. & Tech., Proc. 10th Egypt, pp. 283–300.
8. Blanchette I., Roy D., Belanger G., Gauthier F.S., Production of cottage cheese using dressing fermented by bifidobacteria. J. Dairy Sci., 1996, 79:8–11.
9. Dietary Reference Intakes (DRIs), Recommended Intakes for Individual Elements. 2001. Food and Nutrition Board, Institute of Medicine, National Academies.
10. Gallardo-Escamilla F.J., Kelly A.L., Delahunty C.M., Mouthfeel and flavour of fermented whey with added hydrocolloids. Int. Dairy. J., 2007, 17, 308–315.
11. Gidson R.S., Yeudall F., Drost N., Mititimuni B., Cullinan T., Dietary interventions to prevent zinc deficiency. Am. J. Clin. Nutr., 1998, 68, 484.
12. Glliland S.E., Walker K., Factors to consider when selecting a culture of L. acidophilus as a dietary adjunct to produce a hypochlesteralnic effect in humans. J. Dairy Sci., 1990, 73, 905–911.
13. Harrigan W.E., McCance M.E., Laboratory Methods in Microbiology. 1996, Academic Press, London& New York, pp. 292–294.
14. Ishibashi N., Shimanura S., Bifidobacteria: research and development in Japan. Food Technol., 1993, 47, 126.
15. Jelen P., Gallmann P., Coolbear T., Current and future applications of fermentation technology in the dairy industry. 2003, IDF Seminar on Aroma and Texture of Fermented Milk, p. 10.
16. Jensen R.G., Kroger M., The importance of milk and milk products in the diet. 2000, *in:* Handbook of Dairy Foods and Nutrition (Eds. G.D. Miller, J.K. Jarvis, L.D. McBean), 2nd ed., Boca Raton, Florida, pp. 51–52.
17. Keast R.S.J., The effect of zinc on human taste perception. J. Food Sci., 2003, 68, 1871–1877.
18. Keast R.S.J., Canty T.M., Breslin P.A.S., Oral zinc sulfate solutions inhibit sweet taste perception. Chem. Senses, 2004, 29, 513–521.
19. Less G.J., Jago G.R., Methods for the estimation of acetaldehyde in cultured dairy products. Aust. J. Dairy Technol., 1969, 12, 181–185.
20. Less G.J., Jago G.R., The estimation of diacetyl in the presence of other carbonyl compounds. J. Dairy Res., 1970, 37, 129.
21. Lim J., Lawless H.T., Detection thresholds and taste qualities of iron salts. Food Qual. Pref., 2005, 17, 513–521.
22. Lopez de Felipe F., Kleerebezem M., de Vos W. M., Hugenholtz J., Co factor engineering: a novel approach to metabolic engineering in *Lactococcus lactis* by controlled expression of NADH oxidase. J. Bacteriol., 1998. 180, 3804–3808.
23. Lourens-Hattingh A., Viljoen B.C., Yoghurt as probiotic carrier food. Int. Dairy J., 2001, 11, 1–17.
24. Magdoub M.N.I., Fayed M.E., Zidain A.A., Abou-arab A.A.K., Milk preparedness for bacterial growing in relation to environmental contaminants. 4th Conf. Agric. Dev. Res, Ain-Shams Univ., Cairo, Feb.13–18. Annals Agric. Sci., 1993, 1, 97–110.
25. Mainfreni M., Marino M., Pittia P., Rondinini G., Textural and sensorial characterization of Montasio cheese produced using proteolytic starters. Milchwissenchaft, 2002, 57, 23–26.
26. Mattila-Sandholm T., Myllarinen P., Crittenden R., Mogensen G., Fonde´nR Saarela M., Technological challenges for future probiotic foods. Int. Dairy J., 2002, 12, 173–182.
27. Rogerio P., Matia-Merino L., Jones V., Singh H., Influence of fat on the perceived texture of set acid milk gels: a sensory perspective. Food Hydrocoll., 2006, 20,305–313.
28. Salem O.M., Hamed A.J., Kebary Y.K.M.K., EL Sisi A.S., Influence of attenuated lactococci on the quality of Kareish cheese made by direct acidification. Egypt. J. Dairy Sci., 1997, 25, 253–268.
29. Salgueiro M.J., Zubillaga M.B., Lysionek A.E., Caro A.R., Weill R., Boccio J.R., The role of zinc in the growth and development of children. Nutrition, 2002, 18, 510–519.
30. Sano H., Egashira T., Kinekawa Y., Kitabatake N., Astringency of bovine milk whey protein. J. Dairy Sci., 2005, 88, 2312––2317.
31. SAS. Statistical Analyses System. SAS User's. Statistics. SAS Institute Inc., 1994.
32. Sienkiewicz T., Riedel C.-L., Whey and Whey Utilization. 1990, Verlag Th. Mann, Gelsenkirchen-Buer, Germany, pp. 44–88.
33. Shihata A., Shah N.P., Proteolytic profiles of yogurt and probiotic bacteria. Int. Dairy. J., 2000, 10, 401–408.
34. Tamime A.Y., Robinson R.K., Yoghurt: Science and Technology. 1999, UK, Woodhead Publishing Limited, pp. 384–385.
35. Terzaghi B.E., Sandine W.E., Improved medium for lactic streptococci and their bacteriophages. App. Microbiol., 1975, 29, 807–813.
36. Valli C., Traill W. B., Culture and food: A model of yoghurt consumption in the EU. Food Qual. Pref., 2005, 16, 291–304.

Received December 2009. Revision received April and accepted June 2010.