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EFFECT OF FORTIFICATION WITH ZINC, IRON AND ASCORBIC ACID ON THE CHEMICAL, MICROBIOLOGICAL AND ORGANOLEPTIC PROPERTIES OF BUFFALO'S BIO-YOGHURT

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ABSTRACT

Buffalo's bio-yoghurt was manufactured with or without fortification with zinc, iron and/or ascorbic acid. There was non-significant differences between treatments of buffalo's bio-yoghurt in pH, titratable acidity, total solids, fat, protein, acetaldehyde, total volatile fatty acids contents, the viable numbers of *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Lactobacillus acidophilus*. While, significant differences between treatments were noticed in water-soluble nitrogen/total nitrogen, ascorbic acid contents, the viable number of *Bifidobacterium lactis* and total organoleptic score. During storage at 4°C for 10 days, non-significant differences were noticed in total solids, protein, acetaldehyde contents and the viable number of *Lactobacillus acidophilus* in all samples. Also, the pH value; fat and ascorbic acid contents; the viable numbers of *Streptccoccus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Bifidobacterium lactis* and total organoleptic score significantly decreased during storage period. While, significant increased in titratable acidity, water soluble nitrogen/total nitrogen and total volatile fatty acids contents were noticed. Lactic acid bacteria consumed ascorbic acid during fermentation (5.5 hours) with an average retention 4-8 % in fresh bio-yoghurt. The numbers of yoghurt and bio starter bacteria were > 10^7 cfu g^{-1} in fresh and stored bio-yoghurt. Fortification of the bio-yoghurt with zinc had the highest organoleptic score followed by control and iron-treatments, ascorbic acid treatment then zinc + iron + ascorbic acid treatment in order. The results indicated the possibility of fortification of bio-yoghurt with zinc and iron without any inhibition on the counts of bio starter bacteria and any defects on the organeloptic properties of the resultant product.

It is suggested that the ingestion of 125 g of zinc or iron-enriched bio-yoghurt would provide 17 to 83 % or 14 to 25 %, respectively of recommended dietary allowances of both essential trace elements for infants, children and other population groups in Egypt in addition to health benefits of probiotic bacteria.

Keywords: Bio-yoghurt, probiotic, zinc, iron, ascorbic acid.

INTRODUCTION

Milk and dairy products are considered as ideal and good source for calcium and phosphorus in human balanced diets. In addition, milk and dairy products are rich in proteins, lipids, carbohydrates and some vitamins, but its poor in some essential trace elements such as iron and zinc (Jarrett, 1979 and Jayasekarel *et al.*, 1992) and some vitamins such as ascorbic acid. Therefore, consumers need to obtain their requirements of trace elements and ascorbic acid from other products rather than milk and dairy products.

Iron is one of the most studied elements because its deficiency affects about one-third of the world's population (Boccio *et al.*, 1997). Iron

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deficiency anemia is still the most prevalent nutritional problem in Egypt. The recommended dietary allowance (RDA) of iron is 10 to 18 mg for adults, 10 to 15 mg for children and infants. Menstruating and pregnant women have considerably higher requirements (National Academy of Science, 1980). Cow's milk is containing 0.45 mg iron kg^{-1} milk (Webb *et al.*, 1987). The contribution of iron from milk to the total diet iron of adults is only about 3% (Jarrett, 1979). The most common iron fortification compound is ferrous sulfate which can be completely dissolved and thus provide very high bio-available iron (Boccio *et al.*, 1997).

Zinc is essential to the normal growth and development of all forms of life. It participates in protein, nucleic acid, carbohydrate and lipid metabolism through enzymes. It's also involved in tissue synthesis, gene expression and embryogenesis (Fayed and Abou-Zikri, 1997). Zinc content in whole, skim milk; full fat, low fat and low fat/fruit yoghurt were 4, 4, 7, 6 and 5 ppm, respectively (Tamime and Robinson, 1999). The RDA's for zinc is 15 mg for adults, 10 mg for children and 3 to 5 mg for infants (National Academy of Science, 1980). The contribution of zinc from milk to the total diet zinc of adults is only about 13 and 25% in Canada and United States (Jarrett, 1979). Fermentation of milk by yoghurt starter significantly increased zinc bioavailability to 12.5% compared with 6.3% for raw milk (Degheidi, 1998). The problem, which may be appear (at high concentration levels), with fortification of some dairy products with iron and zinc is increasing fat oxidation during storage or ripening (Degheidi and Abd Rabou, 1998 and Abd Rabou *et al.* 1999).

Ascorbic acid is required in the formation and maintenance of intercellular material, such as the collagen of fibrous tissue and the matrices of bone, cartilage and dentin. Ascorbic acid also play an important roles in the synthesis of neurotransmitters, steroid hormones, carnitine, conversion of cholesterol to bile acids, tyrosine degradation and metal ion metabolism. This vitamin also may enhance iron bioavailability. The role of ascorbic acid as a biological reducing agent may be linked to its prevention of degenerative diseases, such as cancer and cardiovascular diseases. Scurvy is resulting from severe ascorbic acid deficiency (Webb *et al.*, 1987 and National Institutes of Health, 1991). The RDA's of the National Academy of Science (1980) for this vitamin for adults ranges from 50 to 60 mg. Ascorbic acid contents in whole, skim milk; full fat, low fat and low fat/fruit yoghurt was reported to be 1 mg 100 g^{-1} (Tamime and Robinson, 1999). Dairy products are estimated to contribute about 5% of the total vitamin C in the American diet (Webb *et al.*, 1987). Also, ascorbic acid was used as an oxygen scavenger for improving viability of porbiotic bacteria in yoghurts made with commercial starter cultures and to prevent fat oxidation (Dave and Shah, 1997).

Over the past decade, considerable interest has developed in the use of probiotic organisms [*Lactobacillus* (*L.*) *acidophilus* and bifidobacteria] in food, pharmaceutical and feed products. The consumption of probiotic products has increased dramatically in most European, Asia-Pacific and American countries, and >90 products containing *L. acidophilus*, or bifidobacteria or both are available in the market worldwide (IDF, 1984 and

1988). Some of the proposed health benefits are thought to be conferred by live bacteria contained in the products. Suggested minimum numbers of porbiotic bacteria at consumption are 10^5-10^6 cfu g^{-1}. The therapeutic properties of fermented milks and bio-yoghurt discussed by Robinson (1991) and Tamime and Robinson (1999).

The objective of this study was to elucidate the effect of fortification of buffalo's bio-yoghurt with iron, zinc and/or ascorbic acid on the properties of the resultant products.

MATERIALS AND METHODS

Starter cultures and additives

Yoghurt starter culture (Yo-Flex, YC-350, DVS) consists of *Streptococcus* (*S.*) *thermophilus*, *L. delbrueckii* subsp. *bulgaricus*; probiotic bacteria, *L. acidophilus* La-5 (DVS) and *Bifidobacterium* (*B.*) *lactis* Bb-12 (DVS) were obtained from Chr. Hansen's Lab A/S Copenhagen, Denmark. Zinc acetate and ferrous sulfate (food grade) were obtained from Chemical Industries Development Company for Drug. L-ascorbic acid was obtained from EL-Nasr Pharmaceutical Chemicals Company, Egypt.

Bio-yoghyurt manufacture

Fresh buffalo's milk was (from the herd of Fac. of Veterinary, Suez Canal Univ., Ismailia, Egypt) standardized to 5.5% fat, heated at 90°C/5 min, then cooled to 37°C; inoculated with yoghurt starter, *L. acidophilus* La-5 and *B. lactis* Bb-12 at 0.01, 0.025 and 0.025%, respectively. The milk was divided into 5 portions: the first portion was regarded as control (T1), the second portion (T2) was fortified with zinc acetate at a level of 20 mg kg^{-1}, the third portion (T3) was fortified with ferrous sulfate at a level of 20 mg kg^{-1}, the fourth portion (T4) was fortified with L-ascorbic acid at a level of 100 mg kg^{-1} and the fifth portion (T5) was fortified with zinc acetate (at a level of 20 mg kg^{-1}) + ferrous sulfate (at a level of 20 mg kg^{-1}) + L-ascorbic acid (at a level of 100 mg kg^{-1}). Each portion was distributed into 120 mL in plastic cups, the cups closed and incubated at 37°C until a firm curd was formed (5.5 h). The resultant bio-yoghurt was kept in a refrigerator (3-4°C) for 10 days. Three replicates of each treatment were conducted.

Chemical properties

Bio-yoghurt samples were analyzed chemically in fresh and after 3, 7 and 10 days of refrigerated storage.

Titratable acidity, total solids, total nitrogen (TN), water-soluble nitrogen (WSN) and fat contents were determined according to Ling (1963). Total volatile fatty acids (TVFA) were determined by the method of Kosikowski (1978). Thiobarbituric acid value (TBA) was estimated according to Pearson (1976). Acetaldehyde was estimated according to Robinson *et al.* (1977). Ascorbic acid was estimated according to James (1995). pH value was measured using a pH meter (PTI-15, Aqua Chemical Co., England).

Microbiological properties

The counts of viable yoghurt and probiotic starter bacteria were determined according to Chr. Hansen (1995) in fresh samples and after 3, 7 and 10 days of refrigerated storage. Lee's agar medium (composition g L^{-1} distilled water: tryptone 10, yeast extract 10, lactose 5, sucrose 5, K_2HPO_4

0.5, agar 12; pH 7.0; sterilized at 121°C/15 min) was used for the enumeration of *S. thermophilus* after incubation at 43°C for 3 days under aerobic condition. MRS agar medium (Biolife, Italy) acidified to pH 5.4, with approximately 1.32 mL of glacial acetic acid per liter of MRS agar medium, was used for the enumeration of *L. delbrueckii* subsp. *bulgaricus* after incubation at 44°C for 3 days under anaerobic condition (BBL anaerobic jar containing Gas Generating kit, Oxoid, England). MRS maltose agar medium; composition is the same for MRS agar medium, except that the glucose has been removed and replaced by maltose; was used for the enumeration of *L. acidophilus* after incubation at 37°C for 3 days under anaerobic condition. MRS agar medium (supplemented with 5 mL of NNLP solution/100 mL medium) was used for the enumeration of *B. lactis* after incubation at 37°C for 3 days under anaerobic condition. NNLP solution consisted of lithium chloride (Merck, Germany), 6.00 g; nalidixic acid (Sigma, USA), 0.20 g and paromomycin sulfate (Sigma), 0.03 g; neomycin sulfate (Sigma), 0.25 g suspended in 100 mL distilled water, the pH was adjusted to 7.5 with 0.1 N NaOH and filter sterilized using a 0.45 µm cellulose nitrate filter (Sartorius, AG. 37070 Goettingen, Germany).

Antibiotic standard plate count agar medium was used for the enumeration of yeast and molds according to the American Public Health Association (1993).

Organoleptic properties

Organoleptic properties of bio-yoghurt samples were evaluated according to Tamime and Robinson (1999) in fresh and after 3, 7 and 10 days of refrigerated storage. Bio-yoghurt was examined for flavour (0-10 points), body and texture (0-5 points) and appearance and colour (0-5 points) by a panel of 20 persons.

Statistical analysis

All data were analyzed by ANOVA using statistical program V. 6.12 according to SAS (1989). Least significant differences (LSD) test was performed to determine differences in means at significant levels of $p< 0.05$, $p< 0.01$ and $p< 0.001$. The data presented in the Tables are the mean (± standard deviation) of 3 replicate experiments. The values presented in the Figures are the mean of 3 replicate experiments and the error bars represented standard deviation.

RESULTS AND DISCUSSION

Chemical properties

The mean values for titratable acidity, total solids, fat, protein and acetaldehyde contents of each treatment were presented in Table 1. The differences between treatments of bio-yoghurt on titratable acidity, pH, total solids, fat, protein and acetaldehyde contents were not significant (Tables 1, 3 and Fig. 1). Titratable acidity % and pH values significantly increased ($p< 0.01$) and decreased ($p< 0.001$) respectively during refrigerated storage of the bio-yoghurt. This may be due to fermentation of lactose, which produces lactic and acetic acid during fermentation and storage period. The differences in total solids, protein and acetaldehyde contents during storage of bio-

yoghurt were not significant. Significant decreases in fat content were observed during storage period ($p < 0.001$). Tamime and Robinson (1999) mentioned an appreciable loss of lipids, namely a decrease of 3.4% in the fat of yoghurt stored for 21 days at 4°C. Our results are in agreement with those of Degheidi (1998), Abd Rabou *et al.* (1999) and El-Loly and El-Hofi (1999). They reported that no differences were observed in the gross chemical composition of yoghurt and acidophilus milk samples fortified with various sources and levels of zinc or iron either fresh or during storage while the differences were more clear in acidity, pH and acetaldehyde.

Table (1): Chemical properties of bio-yoghurt fortified with zinc, iron and/or ascorbic acid

Treatments	Storage period (day)	Properties*				
		Acidity %	Total solids %	Fat %	Protein %	Acetaldehyde (ppm)
T1 (Control)	Fresh	1.11 ± 0.07	15.23 ± 0.17	5.5 ± 0.1	4.73 ± 0.07	3.23 ± 1.10
	3	1.12 ± 0.09	15.19 ± 0.29	5.4 ± 0.1	4.66 ± 0.11	4.20 ± 0.46
	7	1.13 ± 0.08	15.54 ± 0.67	5.3 ± 0.1	4.71 ± 0.11	4.20 ± 0.46
	10	1.16 ± 0.10	15.23 ± 0.87	5.3 ± 0.1	4.69 ± 0.10	3.20 ± 1.00
T2 (Zinc acetate)	Fresh	1.00 ± 0.02	15.12 ± 0.28	5.5 ± 0.2	4.62 ± 0.15	6.70 ± 4.48
	3	1.03 ± 0.07	15.19 ± 0.38	5.4 ± 0.1	4.73 ± 0.04	3.27 ± 0.40
	7	1.04 ± 0.08	15.19 ± 0.99	5.3 ± 0.1	4.66 ± 0.10	3.27 ± 0.40
	10	1.09 ± 0.04	15.38 ± 0.62	5.3 ± 0.1	4.73 ± 0.06	3.30 ± 1.40
T3 (Ferrous sulfate)	Fresh	1.00 ± 0.03	15.29 ± 0.38	5.5 ± 0.0	4.72 ± 0.06	3.00 ± 0.60
	3	1.07 ± 0.50	15.62 ± 0.51	5.4 ± 0.1	4.64 ± 0.14	3.00 ± 0.79
	7	1.12 ± 0.12	15.80 ± 0.41	5.3 ± 0.2	4.70 ± 0.07	3.00 ± 0.79
	10	1.16 ± 0.09	15.96 ± 0.39	5.3 ± 0.2	4.72 ± 0.06	3.57 ± 0.95
T4 (Ascorbic acid)	Fresh	1.07 ± 0.09	15.41 ± 0.48	5.5 ± 0.1	4.87 ± 0.11	2.47 ± 0.55
	3	1.06 ± 0.15	15.20 ± 0.34	5.4 ± 0.1	4.74 ± 0.04	3.40 ± 0.53
	7	1.20 ± 0.14	15.29 ± 0.91	5.3 ± 0.1	4.74 ± 0.16	3.40 ± 0.53
	10	1.23 ± 0.12	15.42 ± 0.58	5.4 ± 0.1	4.72 ± 0.06	3.10 ± 1.50
T5 (Zinc acetate+ Ferrous sulfate+ Ascorbic acid)	Fresh	1.00 ± 0.12	15.54 ± 0.59	5.4 ± 0.1	4.64 ± 0.13	2.17 ± 0.25
	3	1.01 ± 0.19	15.56 ± 0.26	5.3 ± 0.1	4.68 ± 0.04	3.03 ± 0.76
	7	1.14 ± 0.17	15.42 ± 0.71	5.4 ± 0.1	4.74 ± 0.16	3.03 ± 0.76
	10	1.16 ± 0.13	15.49 ± 0.62	5.3 ± 0.1	4.72 ± 0.06	5.07 ± 0.65

*** Thiobarbituric acid value was not detected in fresh bio-yoghurt samples and during storage period.**

The results of thiobarbituric acid test indicated that no oxidative rancidity had been detected in fresh bio-yoghurt and during refrigerated storage samples. This result is in agreement with Abd Rabou *et al.* (1999) who reported that enrichment of milk with zinc acetate at 10 and 20 mg L^{-1} milk had no effect on the fat oxidation of the resultant yoghurt.

Significant ($p < 0.01$) increase in WSN/TN contents was observed in ascorbic acid treated bio-yoghurt (T4 and T5) comparing with other treatments (T1, T2 and T3). These results indicated that addition of ascorbic acid might be enhanced proteolytic activity of starter cultures. During storage period, the WSN/TN contents were significantly ($p < 0.001$) increased in bio-yoghurt and the increases were more pronounced at 7 and 10 days (Table 3 and Fig. 2). This increase was related to significant ($p < 0.001$) decrease in the count of viable *S. thermophilus* after 3 days of storage (Table 3 and Fig. 5). The increase of WSN/TN contents may be due to proteolytic activity (endopeptidase) of *L. delbrueckii* subsp. *bulgaricus* which hydrolyzed casein to polypeptides then, the later was hydrolyzed to amino acids with exopeptidases produced by *S. thermophilus* (Tamime and Robinson, 1999).

The differences in TVFA contents between treatments of bio-yoghurt were non-significant, while significant ($p< 0.001$) increase was observed during refrigerated storage (Table 3 and Fig 3). These increases may be due to small degree of lipolysis exhibited by *L. delbrueckii* subsp. *bulgaricus*, *L. acidophilus* and *S. thermophilus*. *Lactobacillus* produces more TVFA than *S. thermophilus*. The increases of TVFA contents also may be due to oxidative deamination and decarboxylation of amino acids, which convert the amino acids into its corresponding volatile fatty acids (Tamime and Robinson, 1999).

Ascorbic acid contents significantly ($p< 0.001$) decreased from 100 mg kg^{-1}, in buffalo's milk to 4-8 mg kg^{-1} respectively in fresh ascorbic acid containing treatments, T4 and T5, (Fig. 4). Loss of ascorbic acid occurred during fermentation step (5.5 hours), with an average retention 4-8 %. Significant ($p< 0.001$) decrease in ascorbic acid content was more pronounced in treatment T5 (zinc, iron and ascorbic acid) than that in treatment T4 (ascorbic acid only) and during storage period. Enrichment of bio-yoghurt with ascorbic acid to a level of 500 mg kg^{-1} milk was not a solution to the problem of bacterial consumption of the vitamin, noting that its taste at this level was sensible (data not shown). These decreases may be due to bacterial consumption and/or oxidation, which increased with the presence of iron, of the vitamin during fermentation step and storage period (Tamime and Robinson, 1999). The reduced form of vitamin C is slowly converted to the reversibly oxidized but still biologically active form, dehydroascorbic acid, which in turn is further slowly oxidized irreversibly to the biologically inactive diketogluconic acid. This acid itself is unstable; the oxidation proceeds to oxalic acid and threonic acid. Iron catalyses the oxidation of ascorbic acid to dehydroascorbic acid and not to the irreversible oxidation of dehydroascorbic acid itself (Webb *et al.*, 1987). Dave and Shah (1997) reported that a loss of ascorbic acid in bio-yoghurt was occurred during manufacture and storage, with an average retention of only 15-20 % after about 35 days storage at 4°C. Comparing the results of ascorbic acid contents in our experiment with Dave and Shah (1997), the differences may be due to bacterial strains.

Microbiological properties

There were non-significant differences between treatments of bio-yoghurt in the viable numbers of *S. thermophilus*, *L. delbrueckii* subsp. *bulgaricus* and *L. acidophilus* (Table 3). While, pronounced significantly ($p< 0.001$) decrease were observed in the viable number of *B. lactis* in treatment T5 (zinc, iron and ascorbic acid). The viable numbers of *S. thermophilus* in all treatments of bio-yoghurt were significantly ($p< 0.001$) decreased by about 1.0 - 1.5 log cycle during storage (Fig. 5). This decrease, which was more pronounced in all treatments after 3 days of storage, may be due to the increase of acidity %. The viable numbers of *L. delbrueckii* subsp. *bulgaricus* in all treatments of bio-yoghurt were significantly ($p< 0.05$) decreased during storage period (Table 3 and Fig. 6). During storage, there were non-significant differences in the viable numbers of *L. acidophilus* in bio-yoghurt (Table 3 and Fig. 7) while, the viable numbers *B. lactis* of bio-yoghurt were significantly ($p< 0.05$) decreased (Table 3 and Fig. 8).

The numbers of yoghurt and bio starter bacteria were > 10^7 cfu g^{-1} in all treatments of bio-yoghurt when fresh and during storage period (Figs. 5-8). The results from different studies on the health benefits of viable and non-viable bacteria gave indication that viable bacteria had more health benefits than that non-viable (Ouwehand and Salminen, 1998). Dave and Shah (1997) reported that the viable counts of *S. thermophilus* were lower, whereas those of *L. delbrueckii* subsp. *bulgaricus* were higher, with increasing concentration of ascorbic acid in bio-yoghurt during storage at 4°C for 35 days. Also, they reported that the counts of *L. acidophilus* during storage period decreased less rapidly with increasing concentration of ascorbic acid, whereas the count of bifidobacteria remained unchanged.

Yeast and molds were not detected (nil in 0.1 g^{-1}) in all treatments of fresh bio-yoghurt and during storage period due to good hygienic condition during manufacturing and storage period.

Organoleptic properties

There were significant ($p < 0.001$) differences in the flavour scores between treatments of bio-yoghurt (Tables 2 and 3).

Table (2): Organoleptic properties of bio-yoghurt fortified with zinc, iron and/or ascorbic acid

Treatments	Storage period (day)	Flavour (10)	Body & texture (5)	Appearance & colour (5)	Total (20)
T1 (Control)	Fresh	9.2 ± 0.1	4.8 ± 0.1	4.8 ± 0.1	18.8 ± 0.2
	3	9.0 ± 0.5	4.7 ± 0.2	4.8 ± 0.2	18.4 ± 0.9
	7	7.7 ± 0.2	4.3 ± 0.1	4.7 ± 0.3	18.7 ± 0.3
	10	7.1 ± 0.4	4.4 ± 0.2	4.3 ± 0.1	15.9 ± 0.5
T2 (Zinc acetate)	Fresh	9.3 ± 0.2	4.8 ± 0.1	4.9 ± 0.1	18.9 ± 0.2
	3	8.9 ± 0.8	4.8 ± 0.0	4.8 ± 0.1	18.4 ± 0.7
	7	8.0 ± 0.4	4.4 ± 0.1	4.7 ± 0.3	17.1 ± 0.7
	10	7.6 ± 0.6	4.6 ± 0.1	4.3 ± 0.1	16.3 ± 0.6
T3 (Ferrous sulfate)	Fresh	9.1 ± 0.1	4.7 ± 0.1	4.9 ± 0.0	18.7 ± 0.2
	3	8.4 ± 0.5	4.8 ± 0.0	4.8 ± 0.1	18.0 ± 0.8
	7	7.5 ± 0.3	4.1 ± 0.3	4.8 ± 0.1	16.2 ± 0.3
	10	7.3 ± 1.0	4.3 ± 0.1	4.3 ± 0.1	15.9 ± 1.0
T4 (Ascorbic acid)	Fresh	8.7 ± 0.3	4.7 ± 0.1	4.9 ± 0.2	18.3 ± 0.4
	3	7.9 ± 0.1	4.7 ± 0.1	4.9 ± 0.1	17.4 ± 0.2
	7	6.9 ± 0.2	3.9 ± 0.3	4.6 ± 0.2	15.3 ± 0.3
	10	6.3 ± 0.4	4.2 ± 0.1	4.2 ± 0.1	14.7 ± 0.5
T5 (Zinc acetate + Ferrous sulfate+ Ascorbic acid)	Fresh	8.4 ± 0.1	4.5 ± 0.1	4.8 ± 0.1	17.7 ± 0.1
	3	7.2 ± 0.4	4.5 ± 0.3	4.7 ± 0.2	16.3 ± 0.7
	7	6.0 ± 0.2	3.8 ± 0.4	4.4 ± 0.1	14.2 ± 0.4
	10	5.9 ± 0.9	4.2 ± 0.2	4.2 ± 0.2	14.3 ± 1.3

Treatment T2 (zinc) had the highest score for flavour followed by T1 (control), T3 (iron), T4 (ascorbic acid) and T5 (zinc, iron and ascorbic) in order. Some panelists noticed the characteristic taste of ascorbic acid in treatments T4 and T5, noting that this taste was more pronounced in the latter might be due to metal addition. Flavour scores were significantly ($p < 0.001$) decreased as storage progressed may be due to the increase of acidity % during storage period (Table 3). Body and texture scores of bio-yoghurt were significantly ($p < 0.001$) affected with fortification with zinc, iron and/or ascorbic acid. Treatment T2 (zinc) had the highest body and texture score followed by T1 (control), T3 (iron), T4 (ascorbic acid) and T5 (zinc, iron

order. Some panelists noticed granular coagulum in the texture of treatments T4 (ascorbic acid) and T5 (zinc, iron and ascorbic acid) in bio-yoghurt. Body and texture scores were significantly ($p < 0.001$) decreased as storage progressed (Table 3). Appearance and colour scores of bio-yoghurt were not affected by fortification with zinc, iron and/or ascorbic acid (Tables 2 and 3). Whereas, significantly ($p < 0.001$) decreased in appearance and colour scores were noticed when storage progressed. Total organoleptic scores of bio-yoghurt were significantly ($p < 0.001$) decreased in treatments T4 (ascorbic acid) and T5 (zinc, iron and ascorbic acid) as compared with other treatments (T1, T2 and T3). This may be due to the characteristic taste of ascorbic acid in treatments T4 and T5, which was noticed by some panelists. Significant ($p < 0.001$) decreases in the total organoleptic scores of bio-yoghurt were noticed when storage period progressed. The rate of the decrease, during and at the end of storage period, were more pronounced in treatment T5 (zinc, iron and ascorbic acid) and treatment T4 (ascorbic acid) in order. Treatment T2 had the highest total organoleptic score followed by equal scores for treatments T1 (control) and T3 (iron) at the end of storage period. Our results are in agreement with Degheidi (1998), Abd Rabou, *et al.* (1999) and El-Loly and El-Hofi (1999). They reported that yoghurt and acidophilus milk enriched with zinc acetate, zinc sulfate and iron had highest organeloptic score comparing with that without enrichment. The results indicated that the possibility of fortifying bio-yoghurt with zinc or iron without any remarkable effect on the chemical properties, viable counts of yoghurt and bio starter bacteria and organeloptic properties in the resultant product. While, fortification with ascorbic acid was negatively affected the organeloptic properties. It is suggested that the ingestion of 125 g of zinc or iron-enriched bio-yoghurt would provide 17 to 83 % or 14 to 25 %, respectively of recommended dietary allowances of both essential trace elements for infants, children and other population groups in Egypt in addition to health benefits of probiotic bacteria.

Table (3): Statistical analysis of the composition of bio-yoghurt fortified with zinc, iron and/or ascorbic acid

Properties	Mean Squares	Effect of treatments[1] Multiple Comparison					Mean Squares	Effect of storage period (days) Multiple Comparison			
		T1	T2	T3	T4	T5		Fresh	3	7	10
Chemical:											
pH	0.0055[ns]	4.39	4.40	4.35	4.35	4.38	0.0950***	4.47[a]	4.41[ab]	4.34[bc]	4.28[c]
Titratable acidity %	0.0210[ns]	1.13[a]	1.04[b]	1.09[ab]	1.15[a]	1.08[ab]	0.0490**	1.04[c]	1.06[bc]	1.13[ab]	1.16[a]
Total solids %	0.390.3[ns]	15.30	15.22	15.67	15.33	15.50	0.1013[ns]	15.319	15.353	15.45	15.50
Fat %	0.0008[ns]	5.38	5.37	5.38	5.38	5.37	0.1015***	5.49[a]	5.37[b]	5.33[b]	5.31[b]
Protein %	0.0011[ns]	4.70	4.69	4.70	4.72	4.70	0.0047[ns]	4.68	4.91	4.71	4.72
WSN/TN %	0.2286[ns]	12.25[bc]	12.24[bc]	12.36[bc]	12.42[ab]	12.57[a]	17.238***	11.48[c]	11.64[c]	12.54[b]	13.82[a]
Acetaldehyde (ppm)	2.3048[ns]	3.708	4.133	3.142	3.092	3.325	0.2444[ns]	3.513	3.380	3.380	3.647
TVFA (ml. 0.1 N NaOH 100 g^{-1})	0.5514[ns]	6.771[b]	7.199[a]	7.015[ab]	7.248[a]	7.292[a]	4.3592***	6.437[d]	6.954[c]	7.329[b]	7.699[a]
Ascorbic acid (mg kg^{-1})	23.3994***	0.308[c]	0.342[c]	0.192[d]	3.500[b]	1.217[b]	17.928***	2.633[a]	1.173[b]	0.400[c]	0.240[d]
Microbiological:											
S. thermophilus (x 10^6 cfu mL^{-1})	45.41 x 10^8[ns]	223.93[a]	211.98[a]	153.30[ab]	189.70[ab]	71.02[b]	12.18 x 10^{11}***	597.40[a]	33.71[b]	21.33[b]	27.49[b]
L. del. ssp. bulgaricus (x 10^6 cfu mL^{-1})	49.76 x 10^8[ns]	206.29	149.79	171.50	178.63	170.63	44.32 x 10^9*	205.00[ab]	235.73[a]	142.43[bc]	118.30[c]
L. acidophilus (x 10^6 cfu mL^{-1})	20.62 x 10^9[ns]	166.92[b]	238.45[ab]	276.45[a]	226.15[ab]	256.29[ab]	28.60 x 10^9[ns]	188.33[b]	285.33[a]	250.69[ab]	207.07[ab]
B. lactis (x 10^6 cfu mL^{-1})	18.23 x 10^{11}***	950.83[a]	992.50[a]	366.67[c]	742.92[b]	90.43[d]	45.08 x 10^9*	668.10[a]	628.51[ab]	551.20[b]	666.86[a]
Organoleptic:											
Flavour (10)	76.5922***	8.41[ab]	8.50[a]	8.12[b]	7.56[c]	6.98[d]	231.8742***	8.94[a]	8.19[b]	7.13[c]	6.65[d]
Body & texture (5)	3.2066***	4.59[ab]	4.63[a]	4.56[ab]	4.47[b]	4.29[c]	16.5476***	4.70[a]	4.66[a]	4.15[c]	4.29[b]
Appearance & colour (5)	0.9771[ns]	4.67	4.68	4.69	4.69	4.52	13.8827***	4.84[a]	4.76[a]	4.56[b]	4.26[c]
Total (20)	129.5170***	17.67[a]	17.81[a]	17.37[a]	16.72[b]	15.79[c]	504.1893***	18.48[a]	17.61[b]	15.84[c]	15.19[d]

[1] Treatments of bio-yoghurt: T1, control; T2, zinc; T3, iron; T4, ascorbic acid and T5, zinc + iron + ascorbic acid.

ns: non-significant. * Significant at p<0.05. ** Significant at p< 0.01. *** Significant at p< 0.001.

For each effect the different letters in the means the multiple comparison are different from each. Letters a is the highest means followed by b, c .....etc.

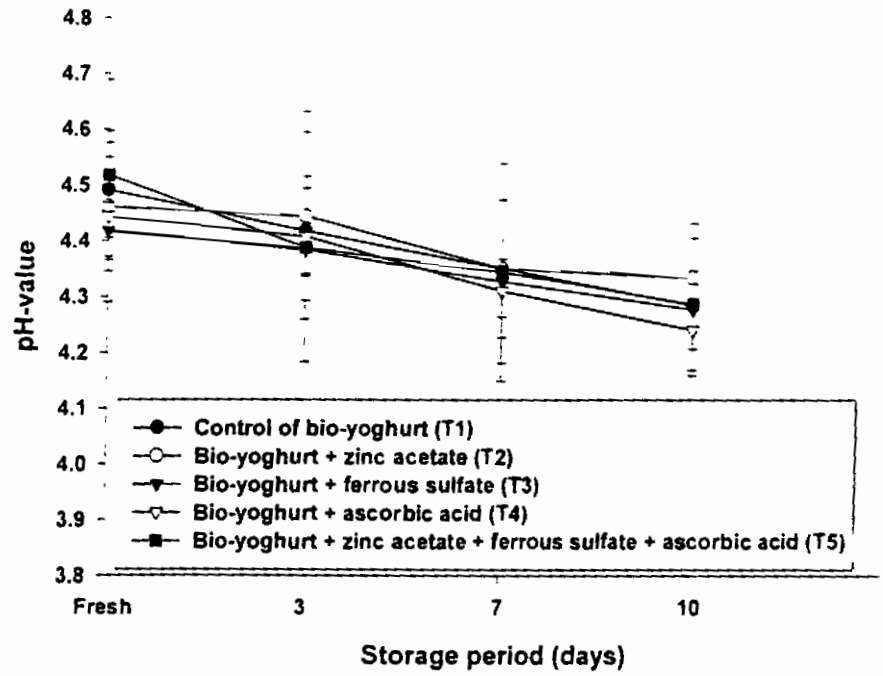

Fig. (1): Changes in pH-value during storage of bio-yoghurt

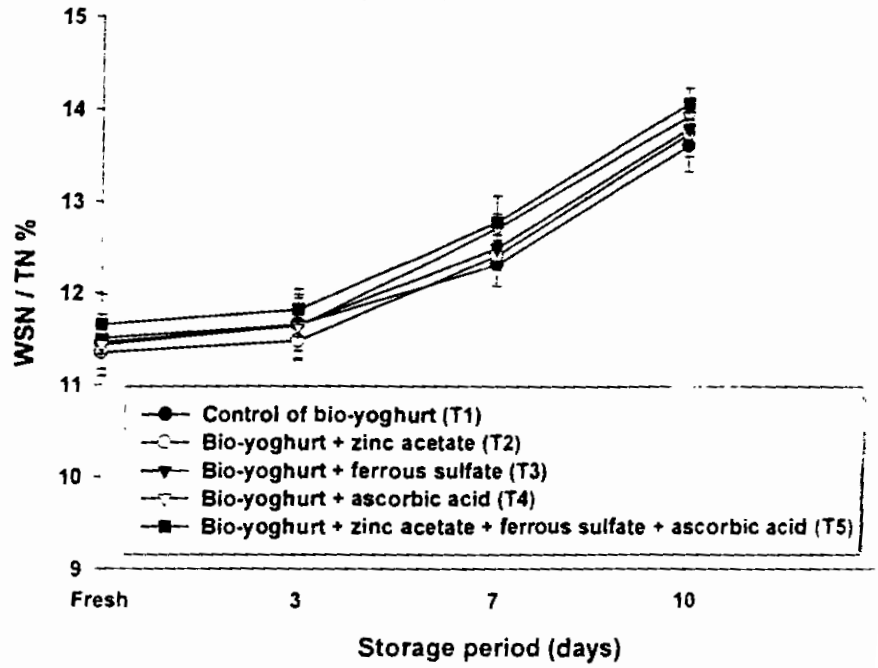

Fig. (2): Changes in WSN/TN % during storage of bio-yoghurt

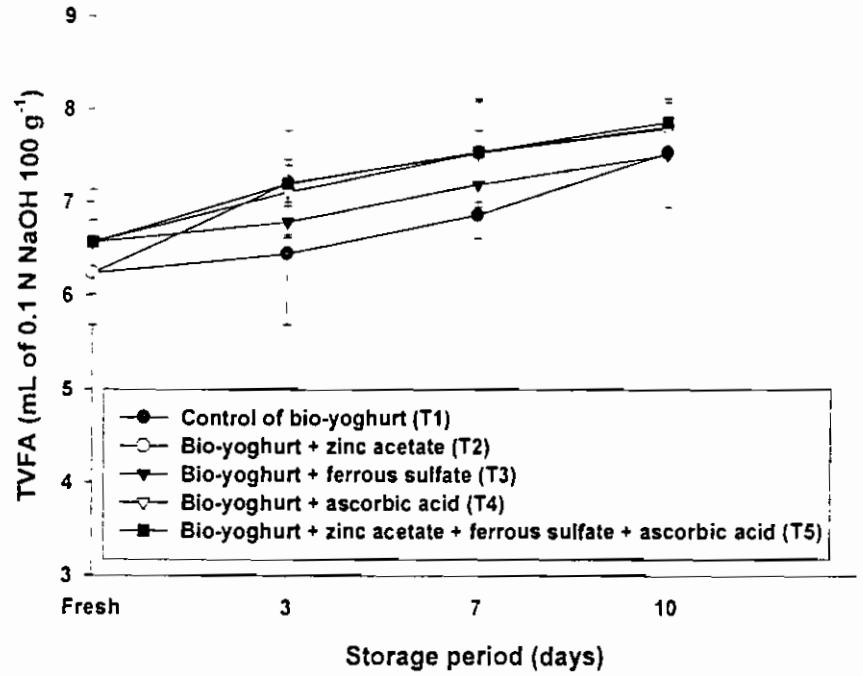

Fig. (3): Changes in TVFA during storage of bio-yoghurt

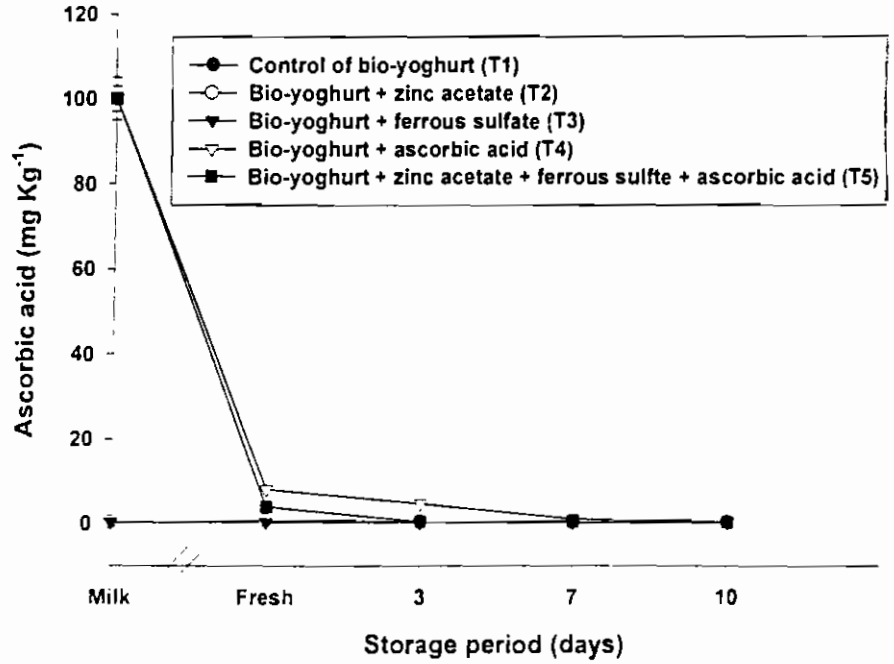

Fig. (4): Changes in ascorbic acid content of bio-yoghurt during fermentation and storage period

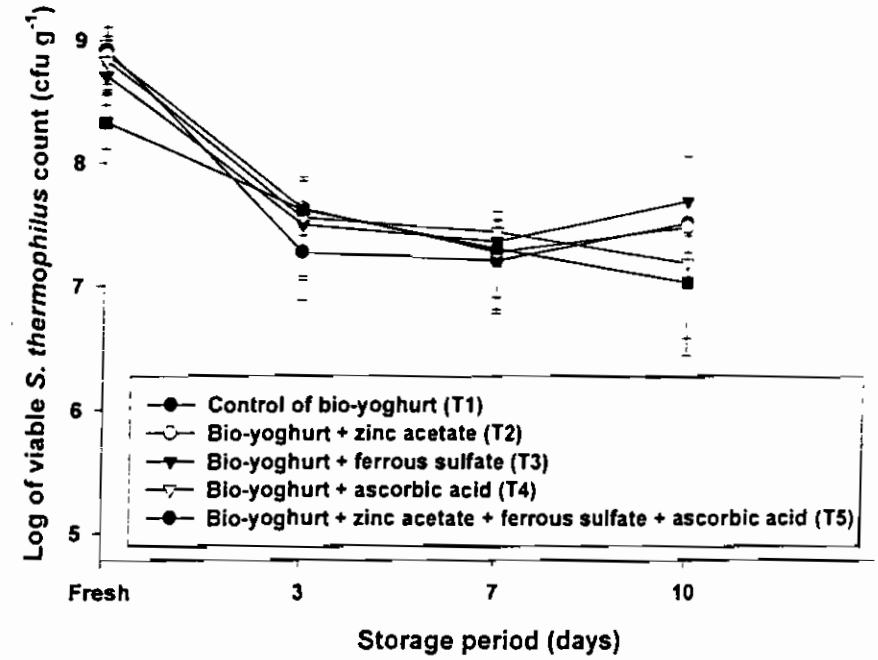

Fig. (5): Changes in the viable count of *S. thermophilus* during storage of bio-yoghurt

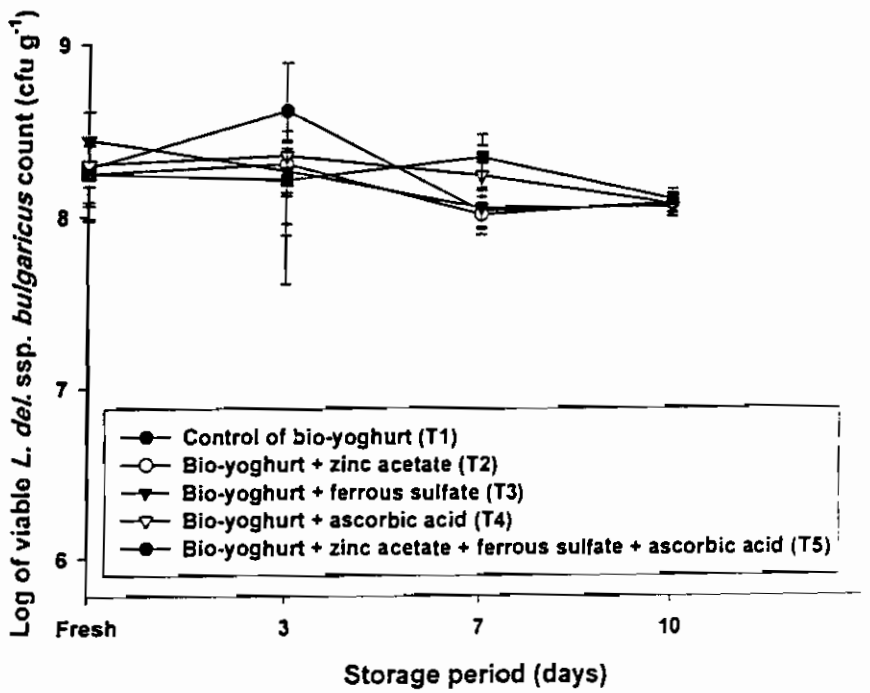

Fig. (6): Changes in the viable count of *L. delbrueckii* subsp. *bulgaricus* during storage of bio-yoghurt

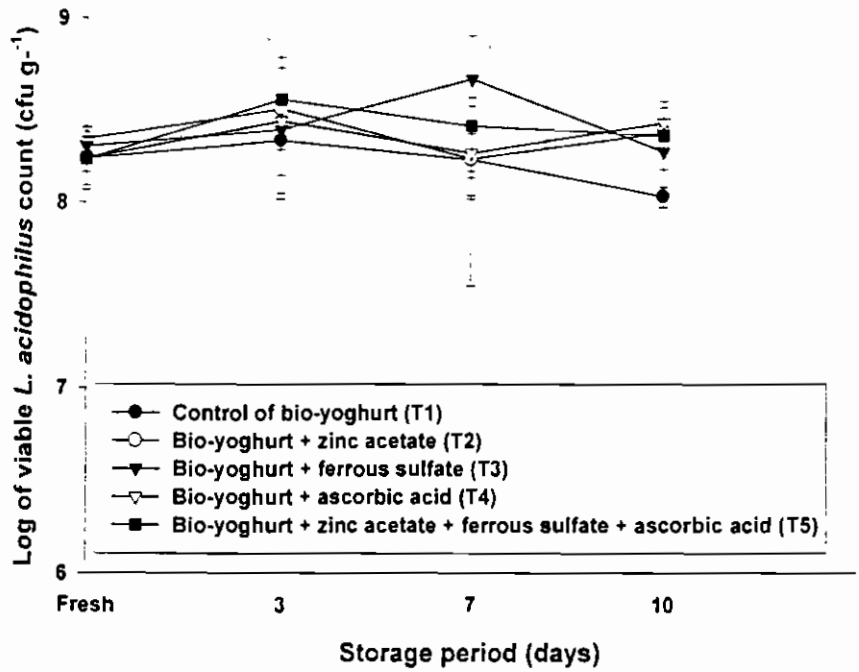

Fig. (7): Changes in the viable count of *L. acidophilus* during storage of bio-yoghurt

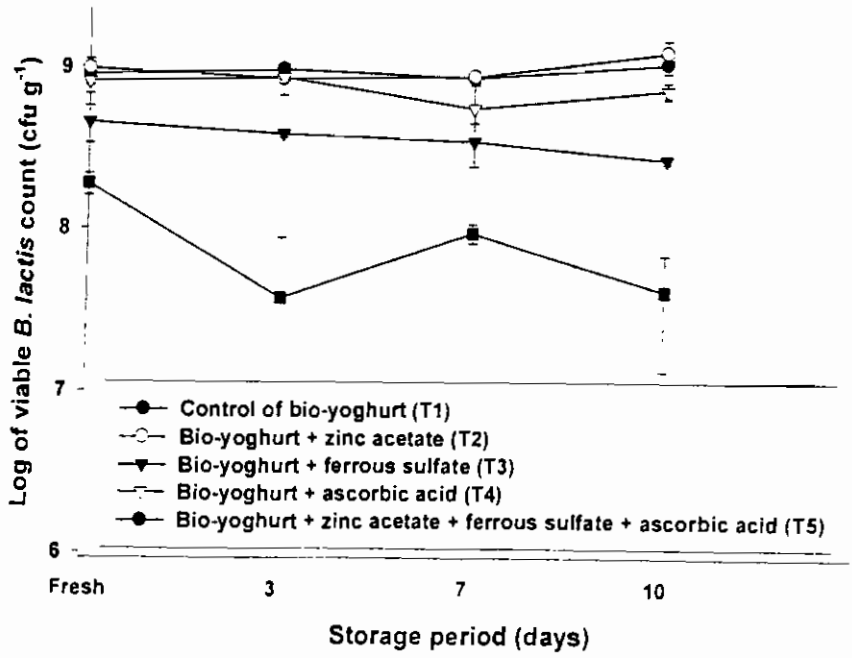

Fig. (8): Changes in the viable count of *B. lactis* during storage of bio-yoghurt

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REFERENCES

Abd Rabou, N. S.; M. A. Hofi and M. H. Abbas (1999). Effect of milk enrichment with different sources of zinc on the properties of yoghurt. J. Agric. Sci. Mansoura Univ., 24: 837-845.

American Public Health Association (1993). Standard Methods for the Examination of Dairy Products, 16th ed. APHA. Washington, DC.

Boccio, J. R.; M. B. Zubillaga; R. A. Caro; C. A. Gotelli; M. J. Gotelli and R. Weill (1997). A new procedure to fortify fluid milk and dairy products with high-bioavailable ferrous sulfate. Nutrition Reviews, 55: 240-246.

Chr. Hansen's (1995). Nu-trish cultures. Methods of analysis for the identification of *Lactobacillus acidophilus* and bifidobacteria. Seminar, Chr. Hansen Copenhagen Pectin, January 18th Cairo, Egypt, pp. 29-30.

Dave, R. I. and Shah, N. P. (1997). Effectiveness of ascorbic acid as an oxygen scavenger in improving viability of probiotic bacteria in yoghurts made with commercial starter cultures. International Dairy Journal, 7:435-443.

Degheidi, M. A. (1998). Influence of enriching yoghurt with zinc sulphate on some chemical and physicochemical properties during storage. J. Agric. Sci. Mansoura Univ., 23: 6099-6113.

Degheidi, M. A. and Abd N. S. Rabou (1998). Effect of zinc salts on the ripening process of Ras cheese. Proc. 7th Egyptian Conf. Dairy Sci. and Techn., 7-9 November, Cairo, Egypt, pp. 403-416.

El-Loly, M. M. and M. A. El-Hofi (1999). Effect of fortification with zinc and iron on the properties of buffaloes acidophilus milk. J. Agric. Sci. Mansoura Univ., 24: 5757-5767.

Fayed, M. I. and M. E. Abou-Zikri (1997). Zinc supplementation in Diarrhea. Egypt. Society for Pediatric Gastroenterology and Nutrition, 1: 1-5.

IDF, International Dairy Federation (1984). Fermented Milks. IDF Bull. No. 179. Int. Dairy Fed., Brussels, Belgium.

IDF, International Dairy Federation (1988). Starters with selected intestinal bacteria. In: Fermented Milks-Science and Technology. Ch. 2, Sec. 5. IDF Bull. No.227. Int. Dairy Fed., Brussels, Belgium.

James, C. S. (1995). Analytical Chemistry of Foods, 1st ed., Chapman and Hall, London, UK.

Jarrett, W. D. (1979). A review of the important trace elements in dairy products. The Australian Journal of Dairy Technology 3: 28-34.

Jayasekarel, S.; U. Samarajeewa and A. N. Jayakody (1992). Trace metals in food and animals origin in Srilanka. Asian-Food J., 7: 105-107.

Kosikowski, F. V. (1978). Cheese and Fermented Milk Foods. 2nd ed. Cornell Univ. Ithaca, New York.

Ling, E. R. (1963). A Text - Book of Dairy Chemistry. Vol. 2 Practical, 3rd ed., Champan and Hall Ltd., London, UK.

National Academy of Science (1980). Recommended Dietary Allowances, 9th ed., Food and Nutrition Board, National Research Council, Washington, D. C., USA.

National Institutes of Health (1991). Ascorbic acid: biologic functions and relation to cancer. Proceedings of a conference held at the National Institutes of Health, Bethesda, Md., September 10-12, 1990. American Journal of Clinical Nutrition, 54: 1113-1327.

Ouwehand, A. C. and S. J. Salminen (1998). The health effect of cultured milk products with viable and non-viable bacteria. International Dairy J., 8: 749-758.

Pearson, D. (1976). The Chemical Analysis of Foods, 7th ed., Churchill Livingstone, New York.

Robinson, R. K. (1991). Therapeutic Properties of Fermented Milks. Elsevier Science Ltd. England.

Robinson, R. K.; A. Y. Tamime and L. W. Cubb (1977). Acetaldehyde as an indicator of flavour intensity in yogurt. The Milk Industry, 4: 4-6.

SAS (1989). SAS/Stat ™ V. 6.12 Guide for personal computer. Institute Inc., Cary, NC.

Tamime, A. Y. and R. K. Robinson (1999). Yoghurt Science and Technology, 2nd ed., CRC Press LLC, Washington, DC.

Webb, B. H.; A. H. Johnson and J. A. Alford (1987). Fundamentals of Dairy Chemistry, 2nd ed., CBS, Delhi, India.

تأثير التدعيم بالزنك والحديد وفيتامين ج على الخواص الكيماوية والميكروبيولوجية والحسية للزبادي الحيوي

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تم تصنيع زبادي حيوي بدون إضافة (كنترول) أو بإضافة زنك أو حديد أو فيتامين ج أو زنك + حديد + فيتامين ج للبن المعد لصناعة الزبادي. أوضحت النتائج عدم وجود فروق معنوية بين المعاملات للزبادي الحيوي في النسبة المئوية للحموضة والـ pH والمحتوى من المواد الصلبة الكلية والدهن والبروتين والأسيتالدهيد والأحماض الدهنية الطيارة الكلية وأعداد البكتريا الحية لـ *Streptococcus thermophilus, Lactobacillus delbrueckii* subsp. *bulgaricus, Lactobacillus acidophilus*. بينما وجدت فروق معنوية بين المعاملات في المحتوى من النتروجين الذائب في الماء للنتروجين الكلي وفيتامين ج وأعداد البكتريا الحية لـ *Bifidobacterium lactis* والخواص الحسية الكلية. لم تحدث تغيرات معنوية في المحتوى من المواد الصلبة الكلية والبروتين والأسيتالدهيد وأعداد البكتريا الحية لـ *L. acidophilus* أثناء تخزين الزبادي الحيوي على ٤ °م لمدة ١٠ أيام. ولقد حدث انخفاض معنوي في قيم الـ pH والمحتوى من الدهن وفيتامين ج وأعداد البكتريا الحية لـ *S. thermophilus, L. delbrueckii* subsp. *bulgaricus, B. lactis* والخواص الحسية الكلية أثناء التخزين. بينما حدثت زيادة معنوية في النسبة المئوية للحموضة والمحتوى من النتروجين الذائب في الماء للنتروجين الكلي والأحماض الدهنية الطيارة الكلية أثناء فترة التخزين. استهلكت بكتريا حامض اللاكتيك فيتامين ج أثناء فترة التخمر (٥،٥ ساعة) ولم يتبقى منه سوى ٤-٨% فقط بالزبادي الحيوي الطازج. كانت أعداد بكتريا الزبادي والبكتريا الحيوية أكثر من ١٠^٧ خلية/جرام أثناء التخزين. حصل الزبادي الحيوي المدعم بالزنك على أعلى قيم في التقييم الحسي وتلاه المدعم بالحديد ثم الكنترول معا ثم المدعم بفيتامين ج وأخيرا المدعم بالزنك + الحديد + فيتامين ج.