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Properties of Yoghurt Made from Fortified Buffalo's Milk with Iron and Zinc Salts

[1]*Samy A. El-Behairy and* [2]*Ezzat A. Mohamed*

[1]National Organization of Drug Control and Research, Egypt
[2]Department of Dairy, Faculty of Agriculture, Al-Azhar University, Egypt

Abstract: Milk is rich in lipids, protein, carbohydrate and some minerals such as calcium, magnesium and phosphorus, but it is poor in some other elements particularly, zinc and iron. The study was carried out to determine whether utilization of iron and zinc salts may affect the quality and storage stability of fortified yoghurt. Buffalo milk was fortified with ferrous chloride, ferrous sulphate, zinc sulphate and zinc acetate salts at the rate of 20, 40 and 60 mg/kg. The milk was used for making yoghurt. The results of this trials showed that yoghurt fortified with ferrous salts or zinc salts showed no effect on total solids, protein content, ash and fat content, while a decrease in lactose content was observed. Zinc salts caused an increase in the acidity of yoghurt more than iron. Samples fortified with 60 mg/kg zinc acetate were not acceptable in organoleptic assessment. Gradually decrease in total and lactic acid bacterial count was observed after three days of storage in fortified yogurt with iron salts or zinc salts.

Key words: Milk · Fortification · Iron and zinc salt · Yoghurt · Properties

INTRODUCTION

Milk and other dairy products are close to ideal food that contains all nutrients required for newborns, adults and elders. Milk is considered as good source of proteins, fat, carbohydrates as well as vitamins, calcium and phosphorus. However, it is generally poor source for trace elements. Iron deficiency is considered to be the commonest worldwide nutritional deficiency and affects approximately 20% of the world population. Women and young children are especially at risk. It is stated that adverse effects include lower growth rate and impaired cognitive scores in children and poor pregnancy outcome and lower working capacity in adults [1]. As iron, zinc is one of the most important elements for body metabolism, where it has structural and regulatory roles in many enzymes like retinal dehydrogenize alkaline phosphatase, nucleic acid polymerase and many others. As zinc having this role for metabolism in the body, its deficiency will markedly affect the growth of human body. To meet the needs of the practically all healthy persons, the recommended daily allowances (RDA) for adult men are set at 15 mg/day. The recommended daily intakes of dietary iron for normal infants are 1 mg/kg and for children, male or female adolescents, need 10, 12 and 15 mg per day, respectively. Women during reproductive years, 15 mg per day [2]. Yoghurt is among the most common dairy products eaten in Egypt. An active and intensive research has concerned fortification of yoghurt [3-7]. All of these studies concerned mostly exploration fortification effect of these elements on quality and storage stability of fortified products.

In view of the aforementioned, the objectives of this study were to investigate the effect of fortification yoghurt with some iron and zinc salts at different rates on yoghurt properties in order to receive the best rate of fortification which should be used.

MATERIALS AND METHODS

Materials

Source of Milk: Fresh whole buffalo's milk was obtained from the herd of Faculty of Agriculture, Al-Azhar University, Mostorod, Cairo, Egypt.

Yoghurt Starter: Mixed starter culture, consisted of *Lactobacillus delbrueckii* subsp. *Bulgaricus* and *Lactococcus salivarius* susp. *Thermophilus* were purchased by CHR-Hansen's la A/S Copenhagen, Denmark.

Iron and Zinc Salts

The Following Food Grade Salts Were Used: Ferrous chloride from (Merck chemicals company, Germany); Ferrous sulphate from (Merck Chemicals Company, Germany); Zinc sulphate (El-Nasr Pharmaceutical Chemicals Company, Egypt) and Zinc acetate (El-Nasr Pharmaceutical Chemicals Company, Egypt).

Corresponding Author: Samy A. El-Behairy, National Organization of Drug Control and Research, Egypt

Iron and Zinc Salts Preparations: In order to achieve the accurate and complete distribution of fortified salts, preparation of 10000 mg/kg of iron and zinc salts were prepared by dissolving these salts in distilled water. Then the real concentration of iron and zinc salts was determined using atomizer these preparations were kept in refrigerator, where renewed monthly.

Methods

Yoghurt Manufacture [8]: The full fat fresh buffalo's milk of (5.5-6%) fat was heated to 85°C for 20 min. then iron and zinc salt concentrations were added to milk while heating. Milk was cooled to 42°C inoculated with 3% yoghurt starter and incubated for about three hours. Yoghurt samples were chemically, microbiologically and organoleptically examined when fresh and after 3, 6, 10 and 14 days of refrigerating at 5°C.

Chemical Analysis:

- Moisture content was determined at 105°C [9] by air oven.
- Titratable acidity and pH value were determined according to the methods reported by Ling [10].
- Total and soluble nitrogen contents were determined according to A.O.A.C. [9].
- Fat content of milk was determined by modified Gerber method as described by Ling [10].
- Ash content was measured according to A.O.A.C. [9].
- Iron (Fe) and zinc (Zn) was determined using atomic absorption spectrophotometer (Perkin Elmer Instrument Model 2380).
- Thiobarbituric acid (TAB) was estimated according to Keeny [11].
- Lactose content was colorimetrically determined as described by Barnett and Abd El-Tawab [12].

Microbiological Analysis:

- Total viable bacteria count was determined using tryptone glucose-yeast extract agar medium according to Richardson [13].
- Lactic acid bacteria count was determined using (M.R.S) medium according to Deman *et al.* [14].
- Mould and yeast counts were determined using PDA medium according to Difco Manual [15].
- Coliform bacterial count was determined using violet red bile glucose agar medium (V.R.G.G.A) according to Hartman and Lagrange [16].

Organoleptic Assessment

Yoghurt: Yoghurt was organoleptically evaluated according to the score suggested by Nelson and Trout [17] with total score of 100 points as follows:

- Flavour (50 points).
- Body & texture (40 points).
- Appearance (10 points).

Experimental Procedure: Samples were examined chemically and microbiologically after 3, 6, 10 and 15 days.

Statistical Analysis: Data obtained was statistically analysis according to Bernsein and Weatherall [18].

RESULTS AND DISCUSSION

Data presented in Tables 1-4 show the effect of iron and zinc salts fortification on total solids, total protein, ash content and fat content of yoghurt during storage respectively. The obtained data revealed that fortified yoghurt with either iron or zinc salts did not change significantly ($P > 0.05$) in the preceding parameters during storage period. These results are in agreements with those reported by Difco Manual [15], Abd Rabou *et al.* [19], Fayed *et al.* [20] and El-Sayed *et al.* [21] who reported the acceptability of fortified yoghurt with iron and zinc salts. In case of lactose content (Table 5), the results showed a slight decrease in lactose content along storage period for all fortified studied yoghurt samples with 20, 40 and 60 μg/kg of salts under study. This finding might be attributed to the acceleration effect of added salts concentrations on bacterial growth. The attained results were in agreement with those reported by Degheidi [22] who found that the increasing of zinc level added caused an increase in the total acidity was associated with a decrease in pH and lactose content throughout the storage period.

In respect of titratable acidity (Table 6), the obtained data showed that the fortification of yoghurt with zinc caused an increase in acidity more than samples manipulated with iron. In general an increase in acidity in all fortified samples with zinc or iron salts was noticed significantly ($P< 0.05$) as storage period proceeded. On the other hand a slight decrease in pH values (Table 7) was noticed ($P > 0.05$). These results are in agreement with those reported by Abd Rabou *et al.* [19], Kolodkin *et al.* [23], Badran *et al.* [24], Abd Rabou [25], Salama and Hassan [26], Kebary [27] and Kebary and Hussein [28].

Table 1: Effect of both iron and zinc salts fortification on the Total Solids (%) of yoghurt during storage

		Treatments (mg/kg)											
		Ferrous chloride			Ferrous sulphate			Zinc sulphate			Zinc acetate		
		20	40	60	20	40	60	20	40	60	20	40	60
Storage period (days)	Control	Total solids (%)											
Fresh	16.20	16.22	16.24	16.26	16.23	16.24	16.25	16.22	16.20	16.21	16.23	16.24	16.25
3	16.24	16.26	16.30	16.33	16.25	16.27	16.29	16.25	16.27	16.29	16.25	16.30	16.33
6	16.27	16.30	16.33	16.34	16.27	16.30	16.33	16.33	16.35	16.37	16.28	16.33	16.37
10	16.30	16.34	16.38	16.40	16.32	16.34	16.38	16.35	16.40	16.42	16.33	16.35	16.42
15	16.33	16.36	16.41	16.43	16.36	16.40	16.44	16.40	16.42	16.45	16.35	16.42	16.50

LSD: (A = Storage period = 0.009, B = Salts = 0.009, AB= 0.021, C = Concentrate = 0.007. AC = 0.016, ABC = N.S and BC = 0.016), N.S. = No significant

Table 2: Effect of both iron and zinc salts fortification on the Total Protein (%) of yoghurt during storage

		Treatments (mg/kg)											
		Ferrous chloride			Ferrous sulphate			Zinc sulphate			Zinc acetate		
		20	40	60	20	40	60	20	40	60	20	40	60
Storage period (days)	Control	Total protein (%)											
Fresh	5.22	5.23	5.23	5.24	5.23	5.24	5.24	5.22	5.23	5.23	5.23	5.24	5.24
3	5.22	5.24	5.23	5.25	5.23	5.24	5.25	5.23	5.24	5.24	5.24	5.24	5.24
6	5.23	5.24	5.25	5.26	5.24	5.25	5.26	5.24	5.25	5.25	5.25	5.25	5.25
10	5.25	5.26	5.26	5.27	5.25	5.26	5.27	5.26	5.28	5.27	5.25	526	5.26
15	5.26	5.27	5.28	5.28	5.26	5.27	5.28	5.26	5.28	5.29	2.27	5.28	5.30

LSD: (A = Storage period = 0.017, B = Salts = N.S., AB= 0.037, C = Concentrate = N.S. AC = N.S., BC = N.S and ABC = N.S)

Table 3: Effect of both iron and zinc salts fortification on the Ash content (%) of yoghurt during storage

		Treatments (mg/kg)											
		Ferrous chloride			Ferrous sulphate			Zinc sulphate			Zinc acetate		
		20	40	60	20	40	60	20	40	60	20	40	60
Storage period (days)	Control	Ash content (%)											
Fresh	0.97	0.970	0.974	0.977	0.973	0.975	0.977	0.972	0.974	0.976	0.971	0.974	0.976
3	0.970	0.972	0.973	0.978	0.974	0.975	0.978	0.973	0.976	0.976	0.973	0.976	0.976
6	0.972	0.974	0.975	0.979	0.975	0.977	0.979	0.974	0.977	0.978	0.975	0.976	0.978
10	0.975	0.975	0.976	0.981	0.976	0.976	0.977	0.976	0.978	0.976	0.976	0.976	0.977
15	0.976	0.974	0.977	0.980	0.978	0.978	0.979	0.978	0.977	0.980	0.978	0.988	0.974

LSD: (A = Storage period = 0.0002, B = Salts = 0.0002, AB= 0.0005, C = Concentrate = 0.0002., AC = 0.0004, BC = 0.0004 and ABC = 0.001)

Table 4: Effect of both iron and zinc salts fortification of yoghurt on the Fat content (%) during storage.

		Treatments (mg/kg)											
		Ferrous chloride			Ferrous sulphate			Zinc sulphate			Zinc acetate		
		20	40	60	20	40	60	20	40	60	20	40	60
Storage period (days)	Control	Fat content (%)											
Fresh	6.2	6.1	6.2	6.1	6.2	6.2	6.0	6.2	6.1	6.2	6.1	6.2	6.0
3	6.2	6.1	6.1	5.8	6.2	6.1	6.0	6.0	6.1	6.1	6.0	6.1	6.0
6	6.0	6.0	6.0	5.6	6.0	5.9	5.9	6.1	5.9	5.8	6.0	5.9	5.9
10	6.0	5.8	5.8	5.8	5.8	5.8	5.8	6.0	5.8	5.8	5.9	5.8	5.6
15	5.8	5.6	5.8	5.8	5.8	5.8	5.8	5.8	5.7	5.6	5.9	5.6	5.6

LSD: (A = Storage period = 0.17, B = Salts = N.S., AB= N.S., C = Concentrate = N.S., AC = N.S., BC = N.S and ABC = N.S.)

Table 5: Effect of both iron and zinc salts fortification on the lactose content (%) of yoghurt during storage

		Treatments (mg/kg)											
		Ferrous chloride			Ferrous sulphate			Zinc sulphate			Zinc acetate		
		20	40	60	20	40	60	20	40	60	20	40	60
Storage period (days)	Control	Lactose content (%)											
Fresh	4.30	4.28	4.29	4.28	4.30	4.29	4.29	4.30	4.31	4.30	4.29	4.29	4.28
3	3.76	3.80	3.90	3.80	3.83	3.77	3.69	3.90	3.85	3.80	3.80	3.96	3.50
6	3.40	3.15	3.70	3.50	3.38	3.30	3.15	3.60	3.50	3.40	3.42	3.60	3.20
10	3.15	3.10	3.40	3.20	3.18	3.10	3.05	3.23	3.15	3.15	3.30	3.25	3.00
15	3.10	3.10	3.00	3.00	3.05	3.00	3.00	3.13	3.10	3.00	3.00	3.00	2.90

LSD: (A = Storage period = 0.009, B = Salts = 0.009, AB= 0.019, C = Concentrate = 0.007, AC = 0.015, BC = 0.015 and ABC = 0.034)

Table 6: Effect of both iron and zinc salts fortification on the Acidity (%) of yoghurt during storage.

		Treatments (mg/kg)											
		Ferrous chloride			Ferrous sulphate			Zinc sulphate			Zinc acetate		
		20	40	60	20	40	60	20	40	60	20	40	60
Storage period (days)	Control	Acidity (%)											
Fresh	0.78	0.80	0.81	0.83	0.83	0.84	0.85	0.84	0.85	0.86	0.86	0.78	0.87
3	0.87	0.91	0.94	0.96	0.95	0.98	1.02	0.97	1.00	1.04	1.08	1.15	1.17
6	1.05	1.10	1.14	1.17	1.15	1.18	1.24	1.17	1.20	1.27	1.32	1.34	1.43
10	1.18	1.20	1.25	1.29	1.33	1.36	1.39	1.28	1.33	1.38	1.43	1.48	1.55
15	1.48	1.50	1.55	1.66	1.63	1.66	1.71	1.65	1.70	1.76	1.79	1.84	1.95

LSD: (A = Storage period = 0.037, B = Salts = 0.037, AB= 0.082.C = Concentrate = 0.028, BC = N.S and ABC = N.S.)

Table 7: Effect of both iron and zinc salts fortification on pH values of yoghurt during storage

		Treatments (mg/kg)											
		Ferrous chloride			Ferrous sulphate			Zinc sulphate			Zinc acetate		
		20	40	60	20	40	60	20	40	60	20	40	60
Storage period (days)	Control	pH											
Fresh	4.61	4.60	4.59	4.58	4.58	4.57	4.56	4.57	4.56	4.55	5.55	5.54	5.53
3	4.57	4.55	4.53	4.52	4.50	4.47	4.46	4.45	4.42	4.40	4.35	4.30	4.25
6	4.42	4.41	4.40	4.38	4.37	4.35	4.35	4.33	4.32	4.30	4.27	4.20	4.15
10	4.35	4.33	4.31	4.92	4.27	4.25	4.23	4.20	4.18	4.16	4.13	4.06	4.00
15	3.95	3.93	3.91	3.90	3.86	3.83	3.81	3.79	3.75	3.73	3.68	3.64	3.60

LSD: (A = Storage period = 0.007, B = Salts = 0.007, AB= 0.04,C = Concentrate = 0.01, AC = 0.001, BC = 0.011 and ABC = 0.026)

Table 8a: Organoleptic assessment of yoghurt fortified with iron and zinc salts

			Treatments (mg/kg)											
			Ferrous chloride			Ferrous Sulphate			Zinc sulphate			Zinc acetate		
Storage period (days)	Organoleptic Properties	Control	20	40	60	20	40	60	20	40	60	20	40	60
Fresh	Flavor (50)	48	48	40	37	44	36	31	38	31	R	38	25	R
	Body & texture (40)	34	32	34	33	35	34	27	33	30	R	34	27	R
	Appearance (10)	9	9	7	7	9	8	7	9	9	R	9	8	R
	Total (100)	91	89	81	77	88	78	65	80	70	R	81	60	R
3	Flavor (50	48	47	38	36	40	33	25	35	29	R	38	25	R
	Body & texture (40)	32	31	32	34	34	31	27	32	30	R	31	27	R
	Appearance (10)	9	8	7	6	9	8	7	8	9	R	9	8	R
	Total (100)	89	86	77	67	83	72	59	75	68	R	78	60	R

Table 8b: Organoleptic assessment of yoghurt fortified with iron and salts

			Treatments (mg/kg)											
			Ferrous chloride			Ferrous sulphate			Zinc sulphate			Zinc acetate		
Storage period (days)	Organoleptic Properties	Control	20	40	60	20	40	60	20	40	60	20	40	60
6	Flavor (50)	42	46	34	33	35	28	25	32	29	R	34	23	R
	Body & texture (40)	32	30	27	27	30	22	20	27	27	R	27	27	R
	Appearance (10)	9	8	6	6	9	7	8	7	7	R	7	5	R
	Total (100)	83	84	67	66	74	57	53	66	63	R	68	55	R
10	Flavor (50)	40	44	34	30	33	26	22	30	26	R	32	20	R
	Body & texture (40)	31	31	24	27	30	20	21	25	25	R	26	25	R
	Appearance (10)	8	7	6	6	7	7	7	7	6	R	7	5	R
	Total (100)	79	82	64	63	70	53	50	62	57	R	65	50	R
15	Flavor (50	38	42	34	29	31	22	23	28	21	R	30	20	R
	Body & texture (40)	30	29	23	25	28	21	20	25	23	R	24	25	R
	Appearance (10)	8	7	6	5	6	7	7	6	6	R	6	5	R
	Total (100)	76	78	63	59	65	50	50	59	50	R	60	50	R

R = Rejected

Table 9: Effect of both iron and zinc salts fortification on the total bacterial count (CFu/mL x 10^7) of yoghurt during storage

		Treatments (mg/kg)											
		Ferrous chloride			Ferrous sulphate			Zinc sulphate			Zinc acetate		
		20	40	60	20	40	60	20	40	60	20	40	60
Storage period (days)	Control	(CFu/mL x 10^7)											
Fresh	14.00	17.10	19.20	19.00	15.50	17.00	20.00	16.00	17.00	18.00	19.40	20.60	22.10
3	17.50	19.20	20.00	22.00	18.10	21.60	24.60	21.00	22.40	24.00	21.40	24.20	27.10
6	12.20	13.10	10.50	13.50	9.60	11.60	12.00	10.80	11.60	12.60	10.60	13.30	16.80
10	9.50	10.00	8.40	9.50	7.80	7.00	6.00	9.00	9.40	5.50	8.80	7.50	6.20
15	7.30	7.50	6.80	6.60	7.00	7.05	6.00	7.30	6.00	5.90	6.10	7.400	5.00

(Cfu/mL) = Colony Forming Unit.

Table 10: Effect of both iron and zinc salts fortification on lactic acid bacterial count (CFu/mL x 10^7) of yoghurt during storage

		Treatments (mg/kg)											
		Ferrous chloride			Ferrous sulphate			Zinc sulphate			Zinc acetate		
		20	40	60	20	40	60	20	40	60	20	40	60
Storage period (days)	Control	(CFu/mL x 10^7)											
Fresh	7.30	10.10	10.50	13.60	8.60	10.20	14.40	10.80	13.20	15.20	11.00	13.20	17.20
3	9.50	12.00	12.60	16.20	11.80	13.10	16.10	16.60	20.10	26.20	18.00	19.00	22.00
6	10.20	9.20	8.20	9.20	8.80	8.10	7.50	8.20	11.60	10.80	15.10	8.60	7.00
10	6.30	8.50	7.60	6.20	6.70	7.60	5.00	7.50	7.00	4.50	8.00	6.10	5.00
15	5.00	7.00	6.00	5.00	5.00	5.00	4.00	5.40	5.00	3.50	3.60	3.00	3.60

(Cfu/mL) = Colony Forming Unit

Data in Tables 8 a and b show the effect of fortification on organoleptic assessment. Fortified samples with 60 mg/kg zinc acetate and zinc sulphate were not acceptable. In general organoleptic score showed that the fortification with iron of zinc salts could be acceptable, when fortified with ferrous chloride at the rate of 20 and 40 mg/kg and also upon 20 mg/kg of ferrous sulphate, zinc acetate and zinc sulphate. Close results were obtained by Nelson and Trout [17]. Data in Tables 9 and 10 show the microbiological properties of fortified yoghurt with salts understudy. An increase in total and lactic acid bacterial count was noticed during the first three days of storage, then gradually decreased. On the other hand the fortification by zinc concentrations used caused an increase in the count of total and lactic acid bacteria more than that observed with iron in the first

period of storage. The obtained data are agreement with those reported by Kebary [27], Kebary and Hussein [28] and Badawi and El-Sonbaty [29].

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