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Impact of Zinc and Iron Salts Fortification of Buffalo's Milk on the Dairy Product

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Abstract: Buffalo's milk was fortified with different sources of iron and zinc salts to manufacture of Domiati cheese. It was fortified with ferrous chloride, ferrous sulphate, zinc sulphate and zinc acetate at levels of 40, 60 and 120 mg Fe or Zn/kg milk. All fresh and stored samples (30 and 90 days) of Domiati cheese were analyzed for pH, acidity moisture, TN, SN, fat, lactose, ash and TBA number. No obvious significantly effects were observed in the chemical composition analysis and organoleptic evaluation on Domiati cheese fortified with all mineral salts. Organoleptic evaluation indicated that low level (40 mg/kg milk) for both of iron and zinc salts had no significant effect on cheese quality and most suitable sources for fortification. Whereas fortification with high levels of these salts had diminished significantly score of flavor, body and texture and total score.

Key words: Buffalo's milk · Domiati cheese · Zinc · Iron · Fortifications

INTRODUCTION

Milk is a key contribution to improving nutrition and food security. Particularly in developing countries, this may offer the most promise in reducing malnutrition [1]. Fortified milk contains added mineral and vitamins that are not naturally in this food or may be found insufficient amounts. Fortification is used to help prevent widespread occurrence of many health problems that can occur as a result of certain nutritional deficiencies. Minerals cannot be produced by the body; they should be taken in the daily food. Fortified milk with trace minerals namely zinc and iron are needed in small amounts [2]. Most adults require roughly similar amount of each of both trace nutrients every day being 11 mg/day [3]. Body needs zinc to metabolize carbs, fats, protein to synthesize DNA and RNA to boost the immune system, support growth, development during pregnancy and childhood. As for iron, it helps blood cells to carry oxygen to all parts of the body. Inadequate intake can lead to iron deficiency anemia, which results in weakness, fatigue and lightheadedness [4]. Cheese is actually a really ancient food and of course is made from this fortified milk [5].

The objective of this study is to investigate the effect of mineral salts fortification on the quality and storage of this fortified cheese.

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.

Rennet: The powdered Rennet was of CHR-Hansen's Laboratory, Copenhagen, Denmark was used.

Iron and Zinc Salts: Food grade salts were used: Ferrous chloride and ferrous sulphate were obtained from Merck Chemicals Company, Germany, Zinc sulphate and zinc acetate from El-Nasr Pharmaceutical Chemicals Company, Egypt.

Iron and Zinc Salts Preparation: 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.

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Table 1: Effect of heating on the used mineral in the fortification

Type of salt 40 mg/kg permeate	Observation
Ferrous chloride	Clear
Ferrous sulphate	Clear
Zinc sulphate	Clear
Zinc acetate	Clear
Ferrous chloride + Zinc sulphate	Turbid
Ferrous chloride + zinc acetate	Clear
Ferrous sulphate + Zinc sulphate	Turbid
Ferrous sulphate + zinc acetate	Turbid

Then the real concentrations of iron and zinc salts were determined using atomizer and kept in refrigerator, where renewed monthly.

Solubility of Iron and Zinc Salts Against Heating: The solubility of any fortified element is considered as a limiting factor for its absorption in body. Heating is essential treatment for dairy industry. Therefore, this experiment was devoted to investigate the effect of heating on solubility of iron and zinc salts either in separate addition or in combinations. In order to accomplish this object, a fresh permeate was heated to 85°C and filtered through filter paper, then fortified with salts, Ferrous chloride, Ferrous sulphate, Zinc sulphate and Zinc acetate were added separately and in combinations between iron and zinc salts at the rate of 40 mg/kg. The fortified permeate was transferred in to test tubes and heated to 85°C for 5 min and cooled to 30°C. All test tubes were examined for any turbidity by comparing them against control (clear permeate without fortification). Table 1 show that iron and zinc salts were added to milk and dairy products as separate salt, however only ferrous chloride and zinc acetate were added together without expecting their precipitation.

Methods

Domiati Cheese Manufacture: Fresh buffalo's milk was standardized to (5.5% fat), then heated to 75°C for 5 minutes. The iron and zinc salts were added while heating. The heated milk was cooled to 40°C Domiati cheese was manufactured according to traditional method described by Fahmi and Sharara [6]. Cheese samples (one cups for each analysis) were chemically, microbiologically and organoleptically examined immediately manufacture and after 30 and 90 days of storage.

Chemical Analysis:

- Moisture content was determined according to the method recommended by A.O.A.C. [7]
- Titratable acidity and pH value were determined according to the methods reported by Ling [8].
- Total and soluble nitrogen contents were determined according to A.O.A.C. [7].
- Fat content of milk was determined by modified Gerber method as described by Ling [8]
- Ash content was measured according to A.O.A.C. [7]
- Thiobarbituric acid (TBA) was estimated according to Keeny [9].
- Lactose content was calorimetrically determined as described by Barnett *et al.* [10].

Organoleptic Evaluation:

- The organoleptic properties of cheese were assessed according to scoring sheet proposed by El-Koussy [11]
- Iron (Fe) and zinc (Zn) was determined using atomic absorption spectrophotometer (Perkin Elmer Instrument Model 2380) [6].

Statistical Analysis: Data were statistically analyzed using "t" test according to the method described by Bernstein and Weatherall [12].

RESULTS AND DISCUSSION

Moisture Content: Data presented in Table 2 indicated that there was no significant differences ($p > 0.05$) could be noticed among cheese treatments, which means that neither the type of iron salt nor its concentration affected significantly the moisture content of Domiati cheese. Moisture content of all cheese treatments stored at room temperature revealed a little decrease along pickling period. This decrease could be attributed to the contraction of curd as a result of developed acidity during pickling period, which helps to expel the whey out the curd. Similar results were reported by Hussein [13], Farag *et al.* [14], Ezzat [15], Kebary *et al.* [16], Badawi and Kebary [17] and Badawi and Kebary [18]. The moisture content of cheese fortified with zinc salts had affected with the type of zinc salt, its concentration and pickling period than fortified with iron salts.

Ash Content: Data in Table 3 indicated that the fortification of cheese milk with iron or zinc salts at the rate of 40, 60 and 120 mg /kg had no noticeable effect on ash content of all cheese samples either fresh or after storage to 90 days. So the ash content of cheese treatments fortified with iron and or zinc salts were around those of control samples with a slight fluctuation. The ash content of all fortified cheese including control samples

Table 2: Effect of iron and zinc fortification on the moisture of Domiati cheese during storage at room temperature.

	Treatment (mg ion/kg milk)												
		Ferrous chloride			Ferrous sulphate			Zinc sulphate			Zinc acetate		
Storage period (days)	Control	40	60	120	40	60	120	40	60	120	40	60	120
						Moisture (%)							
Fresh	59.69	59.68	59.68	59.66	59.69	59.69	59.68	59.68	59.68	59.68	59.68	59.68	59.67
30	57.40	57.35	57.33	57.29	57.30	57.27	57.24	57.20	57.17	57.14	57.10	57.06	57.00
90	52.22	52.18	52.13	52.10	52.03	53.96	53.90	53.88	53.85	53.83	53.79	53.75	53.68

LSD :(A= Storage period = 0.007, B=Salts = 0.009, AB = 0.016, C = Concentrate = 0.007, AC = 0.012, BC = 0.015 and ABC = 0.027).

Table 3: Effect of iron and zinc fortification on the ash content of Domiati cheese during storage at room temperature (25-30oC).

	Treatment (mg ion/kg milk)												
		Ferrous chloride			Ferrous sulphate			Zinc sulphate			Zinc acetate		
Storage period (days)	Control	40	60	120	40	60	120	40	60	120	40	60	120
						Ash content (%)							
Fresh	5.20	5.12	5.20	5.20	5.22	5.21	5.20	5.22	5.22	5.21	5.22	5.20	5.22
30	5.45	5.48	5.50	5.53	5.48	5.50	5.52	5.48	5.50	5.50	5.49	5.52	5.52
90	5.55	5.58	5.60	5.60	5.53	5.60	5.62	5.66	5.62	5.62	5.58	5.62	5.00

LSD: (A= Storage period = 0.0024, B=Salts = 0.003 , AB = 0.005 , C = Concentrate = 0.0023, AC = 0.004, BC = 0.005 and ABC = 0.009).

Table 4: Effect of iron and zinc fortification on the acidity of Domiati cheese during storage at room temperature (25-30oC).

	Treatment (mg ion/kg milk)												
		Ferrous chloride			Ferrous sulphate			Zinc sulphate			Zinc acetate		
Storage period (days)	Control	40	60	120	40	60	120	40	60	120	40	60	120
						Acidity (%)							
Fresh	0.30	0.36	0.38	0.36	0.35	0.36	0.39	0.37	0.38	0.39	0.37	0.39	0.41
30	0.96	0.98	0.01	1.05	1.10	1.12	1.15	1.18	1.20	1.23	1.27	1.33	1.42
90	1.36	1.39	1.44	1.50	1.51	1.53	1.57	12.61	1.64	1.66	1.73	1.79	1.85

LSD: (A= Storage period = 0.004, B=Salts = 0.006, AB = 0.009, C = Concentrate = 0.004, AC = 0.0076, BC = 0.0094 and ABC = 0.017).

Table 5: Effect of iron and zinc fortification on the pH value of Domiati cheese during storage at room temperature (25-30oC).

	Treatment (mg ion/kg milk)												
		Ferrous chloride			Ferrous sulphate			Zinc sulphate			Zinc acetate		
Storage period (days)	Control	40	60	120	40	60	120	40	60	120	40	60	120
						pH value							
Fresh	6.20	6.13	6.11	6.10	6.08	6.10	6.11	6.07	6.10	6.11	6.09	6.10	6.03
30	6.00	5.43	5.41	5.38	5.36	5.30	5.26	5.23	5.20	5.17	4.98	4.93	4.86
90	3.70	4.18	4.15	4.13	4.11	4.08	4.06	4.05	4.00	3.96	3.94	3.90	3.83

LSD: (A= Storage period = 0.0036, B=Salts = 0.0.0048, AB = 0.008, C = Concentrate = 0.0037, AC = 0.006, BC = 0.008 and ABC = 0.014).

Table 6: Effect of iron and zinc fortification on the thiobarbituric acid number of Domiati cheese during storage at room temperature (25- 30oC).

	Treatment (mg ion/kg milk)												
		Ferrous chloride			Ferrous sulphate			Zinc sulphate			Zinc acetate		
Storage period (days)	Control	40	60	120	40	60	120	40	60	120	40	60	120
						Thiobarbituric acid							
Fresh	0.009	0.019	0.023	0.022	0.015	0.029	0.034	0.012	0.011	0.012	0.010	0.010	0.011
30	0.009	0.012	0.013	0.014	0.013	0.016	0.025	0.013	0.014	0.018	0.012	0.015	0.014
90	0.010	0.023	0.027	0.031	0.026	0.036	0.044	0.027	0.038	0.044	0.023	0.026	0.029

LSD: (A= Storage period = 0.00021, B=Salts = 0.0003, AB = 0.0005, C = Concentrate = 0.00021, AC = 0.00036, BC = 0.0005 and ABC = 0.0008).

Table 7: The iron content in fresh whey and stored Domaiti cheese.

Storage period (days)		Treatment (mg ion/kg milk)											
			Ferrous chloride						Ferrous sulphate				
		Control	40	Recovery	60	Recovery	120	Recovery	40	Recovery	60	Recovery	120	Recovery
Fresh	Cheese	1.50	135	84.40	210	87.50	464	96.66	130	81.25	191	79.58	419	87.29
	Whey	0.20	8	**	10	**	5	**	10	**	16	**	20	**
30	Cheese	1.20	130	81.30	198	82.50	457	95.21	120	75.00	180	75.00	410	85.42
90	Cheese	0.83	125	78.13	190	79.20	448	93.33	115	71.90	174	72.50	399	83.13

Table 8: The zinc content in fresh whey and stored Domaiti cheese.

Storage period (days)		Treatment (mg ion/kg milk)											
			Ferrous chloride						Ferrous sulphate				
		Control	40	Recovery	60	Recovery	120	Recovery	40	Recovery	60	Recovery	120	Recovery
Fresh	Cheese	2.85	142	88.75	205	85.42	430	89.58	143	89.40	199	82.92	411	85.63
	Whey	0.50	6	**	11	**	16	**	6	**	13	**	23	**
30	Cheese	12	135	84.38	200	83.33	424	88.33	133	83.13	186	77.50	400	83.33
90	Cheese	8.5	132	82.50	195	81.25	417	86.88	122	76.25	182	75.83	395	82.29

gained a gradual increase, which proceeded with storage period this increase might be attributed to the loss of moisture content. A similar trend was noticed in Tallaga cheese [19].

Titratable Acidity: The results in Table 4 indicated that the fortification of cheese milk with iron salts at the rate of 40mg /kg increased acidity % of cheese slightly ($p>0.05$). The increase was more noticeable in case of fortification with ferrous sulphate. Also, it could be seen that increasing fortification rate from 40 to 120 mg /kg led to slight increase in acidity. The same observation was corresponded with Abd Rabou [19]. However, the fortification of cheese milk with zinc salts increased significantly the acidity of cheese ($p<0.05$). This increase may be due to the stimulation of lactic acid bacterial growth and subsequently the developing of acidity or may leading to stimulate enzymes which sharing in lactic acid formation. The acidity of all cheese treatments were increased significantly ($p<0.05$) throughout pickling period, whereas the fortification revealed more increase than that in the control samples. Also, the increase in acidity during first month of storage period was more than the corresponding induced increase of two latter months. These results are in accordance with those reported by Farag *et al.* [14], Kebary *et al.* [16], Badawi and Kebary [18] and Badwi and Hussein [20].

pH Values: Data presented in Table 5 showed that fortification of cheese milk with iron and zinc salts affected pH values as of acidity; however this effect was not pronounced as acidity because of the buffering capacity of milk components. The pH values of all treated cheese decreased significantly ($p>0.05$) as in advanced pickling period. Similar results were obtained by Farag *et al.* [14], Ezzat [15], Kebary *et al.* [16], Badawi and Kebary [17].

Thiobarbituric Acid Number (TBA Number): The addition of some trace elements especial iron and copper to cheese milk might induce oxidation of milk fat and some other components at long-range of storage. Therefore, the determination of thiobarbituric acid number should give an indication about that oxidation TBA number indicated that fortification of cheese-milk with iron or zinc salts led to an increases in TBA number with more observed increase in case of Iron salts, especially iron sulphate. Also, TBA number of cheese samples fortified with iron salts revealed mild decrease after 30 days of storage then reincreased to more at the end of storage as compared with fresh cheese (Table 6). This might be attributed to the oxidized effect of iron salts, especially iron sulphate, whereas, this effect could be diminished or neutralized due to some compounds produced through the first month of ripening, after which, this effect might be disappeared due to degradation of these compounds. On the contrary, the addition of zinc salts caused a regular slight increase which was very close to the control, it means that zinc salts has a slight effect for inducing oxidized off-flavor. As known, the TBA number is an indication for oxidized off flavor; hence there are an expected correlation between the TBA number and cheese

flavor. An important observation was that the TBA number determinations of all cheeses samples were still within the range reported in other cheese [21]. The attained results are in agreement with those reported by Manson and Cannon [22], who reported that the formation of iron-casein complexes induces the oxidation of iron from the ferrous to the ferric state. Also, fortification of cheddar cheese-milk with 40 mg/kg had no effect on TBA numbers [21].

Distribution of Iron and Zinc Salts Between Whey and Cheese: In Egypt the whey is mostly considered as wastage, so any compounds could draw in it should not be usable as a nutrient. Dairy products has an advantage, whose the combination of iron and zinc minerals is mostly happened with milk proteins, especially casein, whereas zinc is associated with colloidal calcium phosphate of casein micelle while the iron is bound to amino acids present in casein micelle. The results in Tables 7 and 8 indicated that the distribution of fortified iron and zinc salts between Domiati cheese curd and its released whey. The results showed that generally most of added iron and zinc salts were recovered in cheese curd by about 80% taking in to account the amount of the curd and whey. Also the results revealed that the content of zinc in the whey were slightly less than iron ($p>0.05$). This may be attributed to association of zinc with colloidal calcium phosphate of casein micelle while iron is associated with amino acids especially those containing (OH) group. The results also revealed that the amounts (mg/kg) of both iron and zinc in cheese curd were more than that added initially to cheese-milk. This may be attributed to recovering mostly all casein into cheese curd. The amounts of both iron and zinc were reduced regularly along storage period (Tables 7 and 8). Also this may be attributed to release of these minerals in whey due to shrinking of cheese curd, but their concentration was still high as compared with control samples. These results are in agreement with those reported by Abd-Rabou [23] and Khader [24].

Fat Content: The results in Table 9 indicated that the fat content of all cheese treatments were slightly increased as pickling period proceeded ($p> 0.05$). This increase in fat content might be due to the decrease in moisture content. These results are in agreement with those reported by Ezzat [15], Abd Rabou [19] and Kamaly [25]. The fat content of cheese fortified with iron or zinc salts at all used rates were not significantly different from each other ($p>0.05$), which means that both of the type of salt or its concentration did not affect significantly the fat content of cheese.

Total Nitrogen Content: There were significant differences among cheese treatments at the same time of storage($p>0.05$), which indicated that fortification of cheese with iron at the rate of 40, 60 and 120 mg/kg did not affect significantly the total nitrogen content of fresh cheese (Table 10). Also, fortification of cheese with zinc at the used rates have no significant effect on total nitrogen content of fresh cheese ($p>0.05$). On the other had, fortification of Domiati cheese milk with iron or zinc at the used rates (40, 60 and 120 mg/kg) had no significant effect on total nitrogen content throughout storage period. However, the total nitrogen content of all cheese (control and those fortified with either iron or zinc) decreased slightly throughout pickling period ($p>0.05$). This decrease may be due to the degradation of proteins into water soluble nitrogen, hence its release in pickling solution. These results are in agreement with those reported by Hussein [13], Farag *et al.* [14], Kebary *et al.* [16], Badawi and Kebary [18], Abd Rabou [19] and Kamaly [25].

Soluble Nitrogen Content: As shown in Table 11 a general gradual increase in soluble nitrogen which proceeded with storage. Also, the fortification with iron or zinc salts at the rate of 40, 60 and 120 mg/kg caused a slight increase in soluble nitrogen content in case of fresh cheese samples as compared with control($p>0.05$). Whilst, after 9 days of storage the added fortification salts caused

Table 9: Effect of iron and zinc fortification on the fat content of Domiati cheese during storage at room temperature (25-30oC).

		Treatment (mg ion/kg milk)											
		Ferrous chloride			Ferrous sulphate			Zinc sulphate			Zinc acetate		
Storage period (days)	Control	40	60	120	40	60	120	40	60	120	40	60	120
		Fat content (%)											
Fresh	22.77	22.58	22.77	22.77	22.68	22.77	22.77	22.58	22.58	22.76	22.68	22.68	22.68
30	23.62	22.68	23.15	23.33	22.96	23.15	23.52	22.76	22.86	22.43	23.20	23.29	23.36
90	24.57	24.37	24.28	24.28	24.28	24.28	24.28	24.09	24.18	24.18	24.37	24.37	24.37

LSD: (A= Storage period = 0.07, B=Salts = 0. 09, AB = 0.16, C = Concentrate = 0.07, AC = 0.12, BC = 0.161 and ABC = 0.277).

Table 10: Effect of iron and zinc fortification on the total nitrogen of Domiati cheese during storage at room temperature (25-30oC).

		Treatment (mg ion/kg.milk)											
		Ferrous chloride			Ferrous sulphate			Zinc sulphate			Zinc acetate		
Storage period (days)	Control	40	60	120	40	60	120	40	60	120	40	60	120
						Fat content (%)							
Fresh	2.34	2.37	2.35	2.37	2.34	2.34	2.36	2.40	2.41	2.38	2.32	2.34	2.37
30	2.30	2.31	2.32	2.32	2.32	2.32	2.33	2.35	2.34	2.32	2.31	2.29	2.30
90	2.12	2.22	2.26	2.26	2.21	2.23	2.25	2.32	2.33	2.32	2.20	2.22	2.27

LSD: (A= Storage period = 0.0003, B=Salts = 0. 0003, AB = 0.0006, C = Concentrate = 0.0003, AC = 0.0004, BC = 0.0006 and ABC = 0.0009).

Table 11: Effect of iron and zinc fortification on the soluble nitrogen of Domiati cheese during storage at room temperature (25-30oC).

		Treatment (mg ion/kg milk)											
		Ferrous chloride			Ferrous sulphate			Zinc sulphate			Zinc acetate		
Storage period (days)	Control	40	60	120	40	60	120	40	60	120	40	60	120
						Fat content (%)							
Fresh	0.215	0.220	0.222	0.230	0.225	0.230	0.220	0.220	0.230	0.225	0.230	0.232	0.230
30	0.260	0.325	0.332	0.342	0.330	0.340	0.350	0.326	0.332	0.342	0.340	0.346	0.355
90	0.395	0.420	0.440	0.450	0.430	0.450	0.480	0.432	0.438	0.440	0.440	0.465	0.485

LSD: (A= Storage period = 0.0002, B=Salts = 0. 0003 , AB = 0.0005 , C = Concentrate = 0.0002, AC = 0.0041, BC = 0.0005 and ABC = 0.0009).

Table 12: Organoleptic assessment of Domiati cheese fortified with iron or zinc during storage at room temperature.

			Treatments (mg ion/kg milk)											
			Ferrous chloride			Ferrous sulphate			Zinc sulphate			Zinc acetate		
Storage period (days)	Organoleptic Properties	Control	40	60	120	40	60	120	40	60	120	40	60	120
Fresh	Flavour (50)	38	39	35	36	39	36	36	36	36	36	39	30	25
	Body & Text. (40)	40	37	37	33	36	32	30	36	34	32	36	36	33
	Appearance (10)	9	9	7	8	9	8	8	9	7	7	9	9	9
	Total (100)	87	85	79	77	84	76	74	81	77	75	84	75	67
30	Flavour (50)	38	44	33	30	45	30	34	34	31	28	43	31	23
	Body & Text. (40)	40	34	33	32	38	27	31	36	31	28	36	30	31
	Appearance (10)	9	9	7	8	7	9	8	9	8	8	7	9	6
	Total (100)	87	87	73	70	90	66	74	79	70	64	86	70	60
90	Flavour (50)	43	46	25	32	45	26	20	35	29	23	44	27	28
	Body & Text. (40)	38	37	26	28	38	24	28	33	24	21	38	26	20
	Appearance (10)	9	9	7	6	7	8	8	8	7	6	7	7	7
	Total (100)	90	92	85	66	90	58	56	76	60	50	89	60	55

a significant increase in soluble nitrogen content for most rates of addition, which continued tell the end of storage. A slight difference ($p>0.05$)in soluble nitrogen content could be observed in between samples due to the increased fortification rates of iron and zinc salts. This means that increasing rate of fortification from 40 to 120 mg/kg either for iron or zinc salts did not affect significantly the rate of protein degradation. The obtained results are in agreement with those reported by Farag *et al.* [14], Kebary *et al.* [16], Badawi and Kebary [17] and Badawi and Kebary [18].

Organoleptic Properties: The score of organoleptic properties (flavor, body texture, appearance and total score) are presented in Table 12 showed that all organoleptic properties of control and all cheese samples fortified at the rate of 40 mg/kg were enhanced with proceeding of storage, so, they gained higher score at the end of storage period. However, Domiati cheese fortified with 40 mg/kg of all mineral salts were promising efficient and acceptable. But the samples fortified with high levels of all mineral salts gained the lowest score for flavor because they imparted rusty or oxide or metallic taste.

It could be concluded that the addition of mineral salts namely iron and zinc upon the recommended daily allowance led to acceptable oxidative stability and textural profile which is agreement with those obtained by Stathopuls *et al.* [26].

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