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Fortification of Yoghurt with Iron

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Abstract: Standardized buffaloe´s milk (4% fat) was fortified with 20 mg iron /Kg milk using five different sources of iron salts.Chemical, microbiological and sensory characteristics of the yoghurts as well as lipid oxidation were monitored over 10 days of storage at 4±2°C. The results showed no clear differences in the total solids content of all fresh fortified yoghurt by different iron salts. No significant differences on fat content, pH value, syneresis, acetaldehyde and diacetaldehyde content and total lactic acid bacteria counts among iron fortified fresh yoghurt. Fortification of yoghurt with Ammonium ferric sulphate and ammonium ferrous sulphate significantly increases oxidation (as measured by the TBA test) in comparison to unfortified yoghurt. Sensory characteristics scores did not detect any significant differences in the flavours, appearance or overall quality between fortified and unfortified yoghurt. All yoghurt samples were acceptable, suggesting that yoghurt is a suitable vehicle for iron fortification.

Key words: Iron fortification · Milk · Yoghurt

INTRODUCTION

Yoghurt has gained widespread consumer acceptance. It is an excellent source of calcium and protein but as is typical of all dairy products, contains very little iron. Therefore, dairy products are logical vehicles for iron fortification because they have high nutritive values, reach target population and are widely consumed. The quality of iron-fortification dairy products depends on the iron sources used, levels of iron and properties of dairy products utilized for iron fortification. Fortification with iron is technically more difficult than with other nutrients because iron reacts chemically with several food ingredients. Therefore, the ideal iron compound for food fortification should be one that supplies highly bioavailability iron and in the mean time does not affect the nutritional value or sensory properties of the food and should be stable during food processing and of low cost. Many studies have been carried out on iron fortification of yoghurt [1-6]. It is well known that tow major off-flavours may be associated with fortified dairy products: oxidized flavour resulting from catalysis of lipid oxidation by iron and metallic flavour contributed by iron salts [7]. No oxidative rancidity had been detected in fresh bio-yoghurt and during storage samples [8, 4] while, Mehanna *et al*. [2] concluded that fresh yoghurt fortified with iron from different sources seem to be slightly affected by iron concentration. In this respect, it was reported that the sensory quality of iron-fortified dairy foods has been shown to be affected by the type of iron used, the amount of iron added and the properties of dairy products being fortified [9-11]. Therefore, the purpose of this study was to identify potential iron fortification sources that would cause minimal sensory deterioration in yoghurt. Five iron salts (20 mg /Kg milk) have been selected out of 10 salts after a preliminary studies on yoghurt at different salts concentration.

MATERIALS AND METHODS

Materials: Bufflaloe's milk (9% SNF and 6% fat) was obtained from a private farm in Ismailia Governorate. Ammonium ferric sulphate, ammonium ferrous sulphate were obtained from Loba Chemie PVT. LTD and ferrous lactate was obtained from Brolabo, Egypt, The Fe-Casein complex (Fe-CN) was prepared according to Zhang and Mahoney [12] by adding 50 ml 0.2 M $FeCl_3$ to 250 ml skim milk, Fe-casein complex was precipitated by adjusting the pH to 4.6 with HCl or NH_4OH. The supernatants were separated by centrifugation at 3000 rpm. Fe-casein complex was freezed and stored in plastic bottle. The Fe-WP complex was made by adding 330 ml 0.5 M $FeCl_3$ into 4000 ml Karish cheese whey and adjusting pH to 3.5 with NaOH to precipitate Fe-WP. The precipitate was washed

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twice with lactic acid solution. The Fe-WP complex was freezed and stored in plastic bottles until use Zhang and Mahoney [10]. Direct Vat Starter (DVS) yoghurt culture was obtained from CHR- Hansen`s laboratories, Denmark, under commercial name type (FD–DVS–YC–X11) containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* in the amount of 50 unit /250 cm^3 of the processed milk, which corresponded to 2% of activated working starter.

Yoghurt Making Procedure: Fresh buffalo's milk was standardized to 4% fat, heated to 95°C/5 min, the milk was divided into 6 portions: The first portion was not fortified with iron and regarded as control. The rest 5 portions were fortified with (ammonium ferric sulphate, ammonium ferrous sulphate, ferrous lactate, Fe-casein complex and Fe-whey protein complex) respectively at a level of 20 mg iron/ Kg milk. The milk was cooled to 42°C, inoculated with yoghurt culture and filled into 120 m1 plastic cups, covered and incubated at 42°C until a firm curd was formed. The resultant yoghurt was kept in a refrigerator (4±2°C) for 10 days. Three replicates of each treatment were conducted. Yoghurt samples were analyzed chemically, microbiologically and organolepticly when fresh and after 3, 7 and 10 days of cold storage.

Methods of Analysis: Total solids content and Fat content (Gerber's method) were determined according to AOAC [13]. The pH values were measured using Jenway pH meter (Jenway limited, England).Whey separation was measured by centrifugation method, The volume of supernat was determined as synersis index and expressed in percent according to Abou El- nour *et al.* [14]. Acetaldehyde and diacetyl content of yoghurt samples were estimated as described by Lee and Jago [15] with some modification, one big Petri dish with cover and another small one without cover instead of the Conway micro diffusion cell. Big dish is used instead of the outer compartment while the small one instead of the inner wall of a Conway micro diffusion cell. The small was destabilized in the centre of the big one. Thiobarbaturic acid was estimated as given by Pearson [16]. Elliker agar medium Elliker *et al*. [17] was used for the enumeration of total lactic acid bacteria after incubation at 37°C for 3 days under aerobic condition. Antibiotic standard plate count agar medium Marshall [18] was used for the enumeration of yeast and molds after incubation at 25°C for 4 days under aerobic condition.

Sensory Evaluation: Organoleptic properties of yoghurt samples were evaluated according to Tamime and Robinson [19]. Yoghurt was examined for flavour (10 points), body and texture (5 points) and appearance and colour (5 points).

Statistical Analysis: All measurements were done in triplicate and analysis of variance with two factorial (treatments and storage period) were conducted by the procedure of General Linear MODEL (GLM) according to Snedcor and Cochran [20] using Costate under windows software version 6.311 and least significant difference (LSD) test were employed to determine significant difference at $p<0.05$.

RESULTS AND DISCUSSION

Chemical Analysis: Data presented in Tables 1-3 show the effect of iron salts fortification on total solids, fat content and pH values of yoghurt during storage, respectively. The differences in total solids content of all yoghurt treatments are not clear (Table 1). The storage period significantly ($p < 0.05$) affected the total solid content of all yoghurt treatments. Mehanna and Gonc [21] mentioned similar trend and attributed these increase to evaporation of some yoghurt water during cold storage.

The fat content of iron fortified yoghurt in all treatments was quite closed. The variations in fat contents during storage period may be due to the change in moisture of different yoghurt treatments (Table 2). There was a significant ($p < 0.05$) effect of storage period on the fat content and no significant ($p < 0.05$) difference among the iron salt treatments this is in agreement with Mehanna *et al*. [2] who reported that the fat content seems to be not affected by fortifying yoghurt with iron.

The changes in the pH values for all yoghurt treatments were not significantly ($p < 0.05$) different. These results are in agreement with those given by Hekmat and McMahon [1]. They reported that iron fortification had no effect on the incubation time required for the yoghurt mixes to reach pH 4.3 and the starter culture growth was independent of iron fortification. A slight decrease in pH values (Table 3) was noticed as storage period proceeded. These results are in agreement with those reported by Abd Rabou et al. [8]. The obtained data in Tables (1-3) revealed that fortified yoghurt with iron salts did not significantly ($p < 0.05$) change in the preceding parameters during storage period.

Table 1: Effect of using different iron salts on Total solids (%) of iron fortified yoghurt during storage period at 4± 2°C

	Storage period (day)				
Treatments*	Fresh	3	7	10	Mean**
T 1	14.4	14.69	14.89	15.17	14.78^{A}
T 2	13.98	14.32	14.57	14.83	14.42^{D}
T 3	14.05	14.39	14.59	14.91	14.49^{CD}
T 4	14.15	14.50	14.69	14.99	14.58^{BC}
T 5	14.32	14.66	14.87	15.19	14.76^{A}
T 6	14.30	14.65	14.66	15.18	14.70^{Ab}
Mean**	14.20^{d}	14.53^{c}	14.71^{b}	15.04^{a}	

*T1: Control, T2: Ammonium ferric sulphate, T3: Ammonium ferrous sulphate, T4: Ferrous lactate, T5: Fe-CN and T6: Fe - WP

** a, b, c & d and A, B, C & D: means with the same letter among the treatments storage period respectively are not significantly different ($p<0.05$)

Table 2: Effect of using different iron salts on Fat (%) of iron fortified yoghurt during storage period at 4± 2°C (average of 3replicates)

	Storage period (day)				
Treatments*	Fresh	3	7	10	Mean*
T 1	4.40	4.50	4.57	4.67	4.53^{A}
T 2	4.32	4.40	4.47	4.57	4.44^{C}
T 3	4.33	4.43	4.50	4.57	4.46^{BC}
T 4	4.37	4.47	4.50	4.60	4.48^{ABC}
T 5	4.37	4.47	4.57	4.63	4.51^{AB}
T 6	4.37	4.47	4.53	4.63	4.5^{AB}
Mean*	4.36^{d}	4.46^{c}	4.52^{b}	4.61^{a}	

* a, b, c & d and A, B, & C: means with the same letter among the treatments and storage period respectively are not significantly different ($p<0.05$)

Table 3: Effect of using different iron salts on pH values of iron fortified yoghurt during storage period at 4± 2°C

	Storage period (day)				
Treatments*	Fresh	3	7	10	Mean*
T 1	4.43	4.23	4.10	4.05	4.20^{A}
T 2	4.47	4.27	4.17	4.03	4.23^{A}
T 3	4.4	4.20	4.17	4.07	4.21^{A}
T 4	4.37	4.20	4.10	4.07	4.18^{A}
T 5	4.40	4.27	4.13	4.07	4.22^{A}
T 6	4.33	4.17	4.13	4.00	4.16^{A}
Mean*	4.4^{a}	4.22^{b}	4.13^{c}	4.05^{c}	

* a, b, c & d and A, B, & C: means with the same letter among the treatments and storage period respectively are not significantly different ($p<0.05$)

Table 4: Effect of using different iron salts on Syneresis of iron fortified yoghurt during storage period at 4± 2°C

	Storage period (day)				
Treatments*	Fresh	3	7	10	Mean*
T 1	10.17	9.67	9.67	9.57	9.77^{B}
T 2	10.83	10.33	9.67	9.93	10.19^{A}
T 3	10.33	9.83	9.83	10.00	10.00^{AB}
T 4	10.67	10.33	9.83	9.50	10.08^{A}
T 5	11.00	10.33	9.33	9.17	9.96^{AB}
T 6	11.00	9.83	9.60	9.60	10.00^{AB}
Mean*	10.66^{a}	10.06^{b}	9.66^{c}	9.63^{c}	

* a, b, c & d and A, B, & C: means with the same letter among the treatments and storage period respectively are not significantly different ($p<0.05$)

Table (4) shows the whey separated from yoghurt fortified by different iron salts. The result showed that syneresis decreased in all samples during storage, as a result of improve the protein hydration of some of the free water Dave and Shan [22]. There was no significant ($p < 0.05$) difference in syneresis among most of yoghurt treatments fortified with different iron salts. On the other hand, Achanta *et al*. [23] reported that fortified yoghurt with iron improved the water holding capacity. The storage time significantly ($p < 0.05$) affected the syneresis up to 3 days, after that the effect was not significant.

Gallardo-Escamilla *et al*. [24] reported that the volatile compounds such as acetaldehyde and diacetyl are a key compound for typical yoghurt aroma. Tables (5) and (6) show the changes in acetaldehyde and diacetyl contents of iron fortified yoghurt during cold storage. There were no significant ($p < 0.05$) differences in acetaldehyde among most iron treatments. Also, there were no significant ($p < 0.05$) difference between all treatments after 7 and 10 days of cold storage. The result showed that the values of acetaldehyde decreased during storage probably due to its conversion into another organic compound which reduced to ethanol or diacetyl El-Loly and Hofi [25]. On the other hand, Osman and Ismail [4] reported that the differences in acetaldehyde contents during storage of bio yoghurt were not significant. Diacetyl content took an opposite trend to that of acetaldehyde, the diacetyl content increased sharply ($p < 0.05$) up to the end of storage period Table (6). The action of iron on diacetyl content was observed ($p < 0.05$) when yoghurt milk was fortified with ferrous lactate and Fe- wp.

The TBA test has been extensively applied to both milk and milk fat systems, in which the absorbance of TBA reaction products correlates positively with the organoleptic evaluation Hegenauer *et al*. [26]. Fortification of yoghurt with iron significantly ($p < 0.05$) increases oxidation particularly yoghurt fortified with ammonium ferric sulphate and ammonium ferrous sulphate in comparison to unfortified yoghurt (Table 7) during 10 days of storage. However, the differences in TBA among iron treatments were not significant. The slight increase in oxidation may be due to the high acidity of yoghurt which prevent or greatly reduce oxidation potency and formation of iron hydroxides. Treatment 5 (Fe-CN) and treatment 6 (Fe-WP) showed the least TBA reading along the storage period. Iron bound to milk protein that probably reduce its ability to participate in iron catalyzed hydroxyl radical formation and peroxidation Hekmat and McMahon [1] and Azzam [6].

Abd Rabou *et al*. [8] and Osman and Ismail [4] indicated that no oxidative rancidity had been detected in fresh bio-yoghurt and during storage samples while, Mehanna *et al*. [2] reported that fresh yoghurt fortified with iron from different sources seem to be slightly affected by iron concentration.

Total Lactic Acid Bacteria (LAB): The total lactic bacterial count in yoghurt fortified with iron are shown in Table (8). Fortification of yoghurt with different iron salts had no effect on the total lactic acid bacteria in all treatments when fresh and during cold storage. These results are in agreement with those of Hekmet and McMahon [1] and El-Nagar and Shenana [27], they reported that iron fortification had no effect on the incubation time required for the yoghurt mixes. No significant ($p < 0.05$) effect was found when the fortified yoghurt was partitioned by sources of iron fortification. The same pattern occurred in yoghurts fortified with the protein-complexes forms of iron (Fe-CN or FeWP).

Osman and Ismail [4] reported that there were non-significant differences between treatments of bio-yoghurt in the viable numbers of *S. thermophillus*, *L. delbrueckii* ssp. *bulgaricus* and *L. acidophilus*. They concluded that the numbers of yoghurt and bio starter bacteria were $> 10^7$ cfg^{-1} in all treatments of bio-yoghurt when fresh and during storage period.

Yeast and molds data not show were absent in 0.1g^{-1} in all treatments either when fresh or during storage period, with exception of few samples which had a count but less than 10 cfu in 10^{-1} dilution which may be due to the post contamination in these samples after manufacturing from polyethylene containers or during storage period. This confirmed the finding of El-Nagar and Shenana [27].

Sensory Evaluation: The effect of different iron salts on the sensory evaluation of yoghurt during storage period at 4± 2°C for 10 days are shown in Table (9). The score of flavour for all yoghurt samples was stable up to 3 days then gradually decreased along the storage period. However the highest flavour score was for control (T1) and yoghurt fortified with Fe-WP (T6) when fresh and during the storage time. The lowest score was for yoghurt fortified with ammonium ferrous sulphate (T3) which showed a slight metallic flavour.

Also, the iron treatments did not affect significantly ($p<0.05$) the body and texture of yoghurt. While the effect of storage period was significant. In all iron treatments the body and the texture improved within the first 3 days of

Table 5: Effect of using different iron salts on acetaldehyde (ppm) of iron fortified yoghurt during storage period at 4± 2°C

	Storage period (day)				
Treatments*	Fresh	3	7	10	Mean*
T 1	15.13	8.87	8.50	7.77	10.07^{C}
T 2	16.60	11.53	10.23	9.50	11.97^{AB}
T 3	15.57	12.10	12.53	8.27	12.12^{AB}
T 4	18.17	13.70	10.93	10.27	13.27^{A}
T 5	16.40	11.57	9.83	8.03	11.46^{BC}
T 6	18.90	13.20	9.80	9.60	12.88^{AB}
Mean*	16.79^{a}	11.83^{b}	10.31^{c}	8.91^{c}	

* a, b, c & d and A, B, & C: means with the same letter among the treatments and storage period respectively are not significantly different ($p<0.05$)

Table 6: Effect of using different iron salts on diacetyl (ppm) of iron fortified yoghurt during storage period at 4± 2°C

	Storage period (day)				
Treatments*	Fresh	3	7	10	Mean*
T 1	2.53	3.53	8.17	8.63	5.72^{C}
T 2	5.37	5.13	7.47	9.77	6.93^{C}
T 3	3.77	6.37	6.57	8.27	6.24^{C}
T 4	6.10	7.93	13.00	17.17	11.05^{A}
T 5	2.40	5.50	6.57	9.10	5.88^{C}
T 6	2.80	6.60	10.13	15.77	8.83^{B}
Mean*	3.83^{d}	5.84^{c}	8.65^{c}	11.44^{a}	

* a, b, c & d and A, B, & C: means with the same letter among the treatments and storage period respectively are not significantly different ($p<0.05$)

Table 7: Effect of using different iron salts on TBA (absorbance at 538 nm) of iron fortified yoghurt during storage period at 4± 2°C

	Storage period (day)				
Treatments*	Fresh	3	7	10	Mean*
T 1	0.045	0.047	0.053	0.050	0.049^{C}
T 2	0.054	0.056	0.060	0.059	0.057^{A}
T 3	0.049	0.054	0.060	0.058	0.055^{AB}
T 4	0.046	0.051	0.060	0.060	0.053^{ABC}
T 5	0.045	0.052	0.049	0.059	0.051^{BC}
T 6	0.047	0.051	0.050	0.057	0.051^{BC}
Mean*	0.048^{C}	0.052^{b}	0.054^{ab}	0.057^{a}	

* a, b, c & d and A, B, & C: means with the same letter among the treatments and storage period respectively are not significantly different ($p<0.05$)

Table 8: Effect of using different iron salts on total lactic acid bacteria ($x10^{8}$ $cfug^{-1}$) of iron fortified yoghurt during storage period at 4± 2°C

	Storage period (day)				
Treatments*	Fresh	3	7	10	Mean*
T 1	20.72	17.07	22.45	23.18	20.58^{A}
T 2	23.63	22.00	18.00	18.58	20.55^{A}
T 3	18.3	18.05	14.47	17.13	16.99^{A}
T 4	18.27	15.72	18.58	17.23	17.45^{A}
T 5	19.77	16.40	18.50	17.38	18.01^{A}
T 6	21.63	15.99	17.38	19.93	18.73^{A}
Mean*	20.37^{a}	17.54^{a}	18.23^{a}	18.9^{a}	

* a, b, c & d and A, B, & C: means with the same letter among the treatments and storage period respectively are not significantly different ($p<0.05$)

Table 9: Sensory evaluation of iron fortified yoghurt during storage period at 4± 2°C (average of 3replicates)

	Storage period (day)				
Treatments*	Fresh	3	7	10	Mean*
Flavour (10 points)					
T 1	9.00	9.00	8.70	7.80	8.63^{A}
T 2	8.70	8.30	7.50	7.30	7.95^{C}
T 3	8.00	8.00	7.30	6.80	7.53^{D}
T 4	8.50	8.30	7.80	7.30	8.00^{C}
T 5	8.80	9.00	8.00	7.30	8.28^{B}
T 6	9.00	9.00	8.20	7.70	8.48^{AB}
Mean*	8.67^{a}	8.61^{a}	7.92^{b}	7.39^{c}	
Body & Texture (5 points)					
T 1	4.00	4.50	4.30	4.20	4.25^{A}
T 2	4.00	4.50	4.30	4.00	4.20^{A}
T 3	4.20	4.50	4.30	4.00	4.25^{A}
T 4	4.20	4.50	4.30	4.20	4.30^{A}
T 5	4.00	4.50	4.30	4.00	4.20^{A}
T 6	4.20	4.50	4.30	4.00	4.25^{A}
Mean**	4.08^{c}	4.50^{a}	4.33^{b}	4.06^{c}	
Appearance and colour (5 points)					
T 1	4.50	4.30	4.00	4.00	4.20^{A}
T 2	4.30	4.30	4.00	4.00	4.15^{A}
T 3	4.30	4.30	4.00	4.00	4.15^{A}
T 4	4.30	4.30	4.00	4.00	4.15^{A}
T 5	4.20	4.20	4.00	4.00	4.10^{A}
T 6	4.20	4.20	4.00	4.00	4.10^{A}
Mean*	4.31^{a}	4.28^{a}	4.00^{b}	4.00^{b}	
Total acceptance (20 points)					
T 1	17.50	17.80	17.00	16.00	17.08^{A}
T 2	17.00	17.20	15.80	15.30	16.33^{C}
T 3	16.50	16.80	15.70	14.80	15.93^{D}
T 4	17.00	17.20	16.20	15.50	16.45^{BC}
T 5	17.00	17.70	16.30	15.30	16.58^{BC}
T 6	17.30	17.70	16.50	15.70	16.83^{AB}
Mean*	17.06^{b}	17.39^{a}	16.25^{c}	15.44^{d}	

* a, b, c & d and A, B, & C: means with the same letter among the treatments and storage period respectively are not significantly different ($p<0.05$)

storage, as a result of improve the protein hydration. Similar observation was reported by Augustin [28]. Also, the appearance of yoghurt fortified with iron was not affected by the sources of iron or storage period up to 3 days which appeared natural yoghurt colour. These was in agreement with those given by Abd Rabou [29]. Augustin [28] and Hekmat and McMahon [1] reported that the consumer panels did not observe significant difference in the appearance of yoghurt fortified with iron.

The total score of yoghurt fortified with iron from different sources showed that the addition of Fe-WP (T6) resulted yoghurt quite similar to that of control samples, this observation is in agreement with Hekmat and McMahon [1], they reported that yoghurt fortified with Fe-WP complex caused no or minor effect on yoghurt quality. Also, the yoghurt fortified with Fe-CN (T5) gave accepted yoghurt quality. Azzam [6] reported that the source of iron used for fortification had no significant effect or smoothness of yoghurt samples when fresh and during storage period.

The foregoing results suggest possibility of making good quality yoghurt by fortifying yoghurt milk with Fe-WP or Fe-CN or ferrous lactate respectively at a level of 20 mg/kg milk, the resultant yoghurt was not differing than the control and without any inhibition on the bacterial count and any defects in the organoleptic properties.

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