Fortification of Ice Milk with Iron

El-Kholy, A. M., A. Gouda, M. Osman and W. A. Ghareeb
Dairy Department, Faculty of Agriculture, Suez Canal University, Ismailia 41522, Egypt

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Abdtract: The incidence of iron-deficiency anemia suggests that fortification of milk and milk products with iron may be a method of supplementing the diet. Five iron sources were selected, ammonium ferric sulphate, ammonium ferrous sulphate, ferrous lactate, iron-casein complex (Fe-CN) and iron-whey protein complex (Fe-WP). Ice milk mix was fortified with the selected iron salts at a level of 20 mg iron/Kg mix. The properties of the mixes and quality of ice milks were evaluated. The specific gravity of ice milk mix containing two protein binding iron source (Fe-CN and Fe-WP) were significantly higher ($P<0.05$) than others. Addition of different iron salts did not affect the weight per gallon, the pH value and freezing point. Fortification of ice milk mix with ferrous lactate, Fe-CN and Fe-WP increase the viscosity, where fortification with ammonium ferric sulphate, ammonium ferrous sulphate or ferrous lactate increased fat oxidation in comparison to unfortified ice milk mix. Concerning the frozen ice milk, different iron salts had no effect on the overrun, specific gravity or weight per gallon. During storage ice milk fortified with different iron sources had higher values in TBA except ice milk fortified with Fe-WP. The melting rate of ice milk was not affected by the source of iron used. the score of flavour decreased gradually along the storage period up to 45 days. The lowest score for flavour was noticed in ice milk fortified with ammonium ferrous sulphate and ammonium ferric sulphate respectively, while produced a much smoother body and texture than the other iron salts. Fortifying ice milk with iron was accepted for organoleptic properties especially that fotified with Fe-CN and Fe-WP.

Keywords: Iron fortification- -Milk -Ice milk.

INTRODUCTION

Milk and dairy products are widely consumed, providing high quality proteins, vitamins, and minerals except dietary iron. Therefore, fortification of the milk or dairy products with iron has been considered as potential approach for delivering this nutritionally important mineral in required quantities to the consumer, which is a major nutritional problem worldwide (Zhang and Mahoney, 1989). Lack of iron in dairy products decreases the iron density of diets when the proportion of dairy products in the diets increases (Farley *et al.*, 1987). Some literatures reported that the recommended daily intakes (RDI) of dietary iron for normal infants are 1 mg iron per kg of weight per day and for children and male & female adolescents 10, 12 and 15 mg per day respectively. For women during reproductive years 15 mg per day, for adult men and postmenopausal women only 10 mg per day (Baynes & Bothweel, 1990).

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. The sensory quality of iron – fortified dairy products has been shown to be affected by the type of iron source used, the amount of iron added, and the properties of the dairy product being fortified (Zhang and Mahoney, 1989).

Sugiarto and Singh, (2009) reported that when iron is added to milk or milk products, it binds to milk proteins. This binding can have a strong influence on the chemical reactivity of the iron, which consequently is likely to alter the properities of product.

Ice milk is one of the dairy products with a high dietary value. Directions towards ice milk are increased during the last few years as it is favourable by the different ages and it has a refreshing effect as well as its low fatty content in comparison with ice cream (El-Atawy *et al.*, 1990).

Therefore, the objective of this study was to investigate the effect of fortification of ice milk with some iron compounds on ice milk properties and quality during freeze storage.

MATERIALS AND METHODS

Materials:

Bufflaloe's milk (9% SNF and 6% fat) was obtained from a private farm in Ismailia Governorate. Skim milk powder (96% TS, product of Dairy America^{TM}) USA, was obtained from the local market. Commercial grade crystalline sugar (sucrose) was obtained from the local market. Sodium Carboxymethyl Cellulose (CMC) was obtained from Misr Food Additives – MIFAD, Egypt. Ammonium ferric sulphate, ammonium ferrous sulphates 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, 1988) 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 then freezed and stored in plastic bottle until use. 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 twice with lactic acid solution. The Fe-WP complex was freezed and stored in plastic bottles until use (Zhang and Mahoney, 1989).

Preparation of ice milk:

Ice milk mixes were standardized to contain 4% fat, 12% solids not fat, 15% sugar and 0.16% CMC. Ice milk mixes were prepared using the ingredients in

(Table 1). The whole mix was divided to 6 portions (2 kg each). The first one was served as control (T1), the other portions were fortified by iron at a level of 20 mg iron/kg mix using ammonium ferric sulphate (T_2), ammonium ferrous sulphate (T_3). ferrous lactate (T_4), Fe-casein complex (T_5) and Fe-Whey Protein complex (T_6). The mixes were heated at 75°C /5 min, then cooled, aged at 4°C for 24 hours and whipped in an ice milk freezing machine (Taylor-Male, Model 156, Italy). The resultant ice milk was packaged in100 ml cups and placed in a freezing cabinet at-18°C for hardening and storage (Marshall and Arbuckle, 1996). Processing was carried out in triplicate. Ice milk was analyzed when fresh and after 15, 30 and 45 days.

Table (1): The ingredients used in making Ice milk mix.

Ingredients	Quantity Kg	Fat %	SNF %	Sucrose %	CMC %	T.S %
Buffalo's milk (6% Fat, 9%SNF)	66.7	4	6	-------	------	10
Skim milk powder (96% SNF)	6.25	-----	6	-------	-------	6
Sugar	15	-----	-----	15	------	15
CMC	0.16	-----	-----	-------	0.16	0.16
Water	11.9	-----	-----	-------	-------	-------
	100	**4**	**12**	**15**	**0.16**	**31.16**

Methods of analysis:

Total solid, fat content and titratable acidity were determined according to AOAC (1990). The pH values were measured using Jenway pH meter (Jenway limited, England). The specific gravity of the ice milk mix was determined using the method described by Winton, (1958) at 20°C. The Viscosity measurments were carried out by Hoppler viscometer type BH2 No. 17377 at 20°C. The mix was treated to remove the apparent viscosity according to Bhanumurthi *et al.* (1972). Freezing point was determined according to the method recommended for milk by the FAO (1977). The weight per gallon of both mix and ice milk in kilograms was determined according to Burke, (1947) by multiplying the specific gravity of the mix and frozen ice milk by the factor 4.5461. The overrun was determined according to Marshall and Arbuckle, (1996). The melting rate of ice milk was determined according to Tharp *et al.* (1997). Thiobarbaturic acid was estimated as given by Pearson, (1976).

Sensory evaluation of the ice milk were carried out by a regular score panel by members of Dairy Department, Faculty of Agriculture, Suez Canal University. The ice milk samples were taken out of the hardening cabinet. Scoring was carried out according to Marshall and Arbuckle, (1996) where, flavour (45 points), body and texture (30 points), and appearance & colour (25 points).

Statistical Analysis:

All measurements were done in triplicate and analysis of variance with one (treatments) and two factorial (treatments and storage period) were conducted by the procedure of General Linear MODEL (GLM) according to Snedcor and Cochran, (1967) 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

Mix Properties:

Specific gravity and weight per gallon:

The effect of adding iron salts on specific gravity of ice milk mixes are shown in Table (2). The specific gravity of ice milk mixes containing two proteins binding iron source (T5 and T6) were significantly ($p<0.05$) higher than other treatments, this may be due to the protein bind to iron which increased the total solids of the mixes. From the same Table the weight per gallon is closely related to specific gravity there was no significant ($p<0.05$) differences as a result of using different iron salts compounds.

Acidity % and pH values:

The normal titratable acidity % of mixes varies with the percentage of SNF contained. The acidity % and pH values are related to composition of the mix, an increase in SNF raises acidity and lowers the pH. Table (2) indicates the acidity of the mixes treated by iron salts. It was observed that the addition of different iron salts increased the acidity of the mixes. On the other hand control treatment (TI) have significantly ($P<0.05$) lower acidity value than all other iron treatments. This may be due to the effect of chemical properties of iron salts.

The pH values of all mixes was within the normal range. Addition of different iron salts did not affect the pH and no significant ($p<0.05$) differences between all treatments.

Mix viscosity:

Table (2) shows the effect of adding different iron salts on the viscosity of the mixes before and after aging. The attained results revealed that the viscosity of T 4, T5 and T 6 were significantly ($P<0.05$) higher than the other treatments, while the control (T 1) was significantly the lowest one. After aging the results revealed that aging caused increase in apparent viscosity in all samples, these results are agreed with those reported by (Cottrel *et al.*, 1980) who said that aging the mixes affected the viscosity.

Freezing point:

The freezing point of ice cream is dependent on the concentration of the soluble constituents and varies with the composition (Marshall and Arbuckle, 1996). It was noticed from Table (2) that using different source of iron had no significant ($P<0.05$) effect on the freezing point and the values ranged between -2.2 and -2.3°C.

Thiobarbituric acid (TBA):

To monitor oxidative deterioration of supplemented ice milk, thiobarbaturic acid (TBA) assay was used. Fortification of ice milk mix with ammonium ferric sulphate, ammonium ferrous sulphate or ferrous lactate (T2, T3 and T4) respectively increased significantly oxidation in comparison to control (Table 2). On the other hand, fortification by Fe-CN or Fe-WP gave results very close and not significantly different than the control (T1). Hekmat and McMahon, (1997) found that iron bound to milk protein probably reduce its ability to participate in iron catalyzed hydroxyl radical formation and peroxidation.

Ice milk properties:

Specific gravity and weight per gallon:

It could be notice from Table (3) that no significant ($P<0.05$) differences in the specific gravity or weight per gallon in ice milk fortified with different iron salts. Specific gravity is inversely proportional to changes occurring in the overrun Janggiou and Moosavi (2004) and Abbas (2006).

Overrun values:

Table (3) shows that there is slight non significant differences among treatments for overrun which ranged between 61.67 to 64 %. These slight differences in overrun values may be related to an increase in apparent viscosity of the ice milk during freezing as more air was incorporated. These results revealed that using of different iron salts had no significant ($P<0.05$) effect on the overrun.

Thiobarbaturic acid (TBA)

Table (4) revealed that fortification of ice milk mix with different sources of iron salt increased oxidized flavor and lipid peroxidation in the resultant ice milk as measured by the TBA test. During 45 days of freeze storage, there were slight increases in oxidation. There were no significant ($P<0.05$) difference between the control ice milk (T1) and ice milk fortified with Fe-WP (T6) during the storage period and these may be due to iron is already bound to milk protein Fe-WP (T6) which reduce its ability to participate in iron catalyzed hydroxyl radical formation and peroxidation (Hekmat and McMahon, 1997; Kawak and Yang, 2002; Azzam, 2009). While, there were a significant ($P<0.05$) differences between ice milk fortified with ammonium ferric sulphate which had the highest TBA value comparing with the other treatments. However, Edmondson *et al.* (1971) reported that ammonium Ferrous sulphate gave more oxidized flavor than other iron salts.

Melting rate:

Table (5) represents the melting rate (%) of ice milk during 45 days of storage. For all ice milk treatments the melting rate (%) was not affected by the source of iron used in these study, the differences between treatments were not significant ($P<0.05$) throughout the 60 min of the test. The melting rate decreased gradually during the storage period and these could be attributed to the prolonging storage time at -18°C which decrease the latent heat of the ice milk, so it needs more time to melt and accordingly improve the melting quality. The storage time significantly ($P<0.05$) affect the melting rate and all time intervals of the test.

Sensory evaluation:

Sensory evaluation scores for ice milk treatments are listed in Table (6). The differences in flavour approached significant ($P<0.05$) between the control ice milk (T1) and all iron salts treatments. However, the score of flavour was stable up to 15 days then decreased gradually up to 45 days of storage. It could be noticed that the flavour of ice milk fortified with Fe-CN (T5) and Fe-WP (T6) was quite similar and no significant ($P<0.05$) differences between them. The lowest score for flavor was noticed in sample fortified with ammonium ferrous sulphate (T3). The effect of storage on flavor quality was not significant up to 15 days of storage, thereafter the effect was significant. The major finding was that ice milk fortified with ammonium ferric sulphate (T2), ammonium ferrous sulphate (T3) produced a much smoother body and texture than did the others. Through the first 15 days of storage no significant ($p<0.05$) differences in the body and texture of ice milk thereafter it improve significantly up to 45 days of storage. The appearance of control (T1) and all other iron treatments was very close and no significant ($p<0.05$) differences could be noticed. The colour of all treatments was accepted and ranged between white and light yellow. the appearance was slightly decreased after 15 days of storage.

In conclusion, the obtained results revealed that fortifying ice milk with iron was accepted for organoleptic properties especially that fotified with Fe-CN and Fe-WP.

Table (2): Effect of adding different iron salts on some properties of ice milk mixes (average of three replicates)

Properties	Ice milk (mix)					
	T_1	T_2	T_3	T_4	T_5	T_6
specific gravity (gm/cm^3)	1.109 ^{b}	1.109 ^{b}	1.109 ^{b}	1.108 ^{b}	1.111 ^{a}	1.111 ^{a}
Weight /gallon (Kg/gallon)	5.043 ^{a}	5.043 ^{a}	5.042 ^{a}	5.041 ^{a}	5.048^a	5.048^a
Acidity %	0.213 ^{c}	0.23 ^{ab}	0.23 ^{bc}	0.237^{ab}	0.233 ^{a}	0.233^a
pH value	6.43^a	6.393^a	6.417 ^{a}	6.417 ^{a}	6.39 ^{a}	6.393^a
Viscosity (cp) fresh	51.33 ^{c}	54.00 ^{b}	52.00 ^{bc}	55.00 ^{a}	55.33 ^{a}	55.00 ^{a}
Viscosity (cp) After aging	69.67 ^{b}	74.67 ^{a}	64.67 ^{c}	69.67 ^{b}	64 ^{c}	64.33 ^{c}
Freezing point (-°C)	2.3 ^{a}	2.2 ^{a}	2.3 ^{a}	2.3 ^{a}	2.3 ^{a}	2.3 ^{a}
TBA (absorbance at 538 nm)	0.038b^c	0.054 ^{a}	0.054 ^{a}	0.043 ^{b}	0.036 ^{c}	0.035 ^{c}

T1: Control, T2: Ammonium ferric sulphate, T3: Ammonium ferrous sulphate, T4: Ferrous lactate, T5: Fe-CN and T6: Fe – WP.
a, b and c: means with the same letter among the treatments are not significantly different ($p<0.05$).

Table (3): Effect of different iron salts on some properties of frozen ice milk (average of three replicates)

Properties	Ice milk					
	T_1	T_2	T_3	T_4	T_5	T_6
Specific gravity (gm/cm³)	0.682 ᵃ	0.685 ᵃ	0.683 ᵃ	0.679 ᵃ	0.684 ᵃ	0.677ᵃ
Overrun %	62.670 ᵃ	61.670 ᵃ	62.330 ᵃ	63.670 ᵃ	63.000 ᵃ	64.000 ᵃ
Weight /gallon (kg/gallon)	3.101 ᵃ	3.386 ᵃ	3.105 ᵃ	3.084 ᵃ	3.104 ᵃ	3.078ᵃ

See legend Table (2)
a: means with the same letter among the treatments are not significantly different (p<0.05).

Table (4): Effect of using different iron salts on TBA (absorbance at 538 nm) of frozen ice milks during storage period at -18°C

Treatments	Storage period (day)				Mean*
	Fresh	15	30	45	
T 1	0.045	0.054	0.051	0.047	0.049 C
T 2	0.051	0.063	0.065	0.061	0.060 A
T 3	0.049	0.062	0.061	0.054	0.057 B
T 4	0.049	0.061	0.060	0.053	0.056 B
T 5	0.049	0.053	0.057	0.056	0.055 B
T 6	0.047	0.057	0.052	0.048	0.051 C
Mean*	0.048 c	0.059 a	0.058 a	0.053 b	

See legend Table (2)
* A, B & C and a, b & c: means with the same letter among the treatments and storage period respectively are not significantly different (p<0.05).

Table (5): Effect of using different iron salts on melting rate (%) of frozen ice milk within 60 min during storage period at -18°C

Treatments	Storage period (days)				Mean*
	Fresh	15	30	45	
		15 min			
T1	1.37	1.30	0.17	0.13	0.74 A
T2	1.57	0.90	0.50	0.13	0.78 A
T 3	1.53	0.73	0.00	0.67	0.73 A
T4	1.87	0.80	0.00	0.00	0.67 A
T5	0.50	0.33	0.00	0.00	0.21 B
T 6	1.67	1.33	0.00	0.10	0.65 A
Mean*	1.33 a	0.9 b	0.11c	0.17 c	
		30 min			
T1	13.10	9.71	4.34	2.30	7.36 A
T2	13.80	8.03	6.13	1.97	7.48 A
T 3	18.17	8.03	4.17	2.67	8.26 A
T4	19.23	8.47	5.40	2.77	8.97 A
T5	16.37	8.03	5.97	2.00	8.10 A
T 6	21.50	7.53	4.50	2.57	9.03 A
Mean*	17.03 a	8.31 b	5.10 c	2.38 d	
		45 min			
T1	37.33	27.47	16.50	13.50	23.70 A
T2	37.80	24.53	18.17	13.60	23.53 A
T 3	37.13	26.87	18.07	13.10	23.80 A
T4	37.90	25.77	13.40	14.53	22.90 A
T5	36.13	27.17	16.23	15.33	23.72 A
T 6	36.73	25.67	17.47	13.67	23.38 A
Mean*	37.17 a	26.24 b	16.64 c	13.96 d	
		60 min			
T1	51.20	46.93	43.20	41.37	45.68 B
T2	50.17	47.00	46.27	43.53	46.74 AB
T 3	54.40	48.80	47.01	46.10	49.10 AB
T4	58.01	51.13	43.83	44.43	49.37 A
T5	55.10	49.50	46.57	45.13	49.10 AB
T 6	57.63	47.33	45.90	43.00	48.47 AB
Mean*	54.43 a	48.45 b	45.47 c	49.93 c	

See legend Table (2)
* A & B and a, b, c & d: 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 the sensory properties of frozen ice milk during storage period at -18°C.

Mean*	Storage period (day)				Mean*
	Fresh	15	30	45	
		Flavour (45 points)			
T1	43.67	43.33	42.00	42.00	42.75 A
T2	42.33	41.33	39.67	39.67	40.75 CD
T 3	41.33	41.33	39.67	39.67	40.50 D
T4	41.67	41.33	40.67	39.67	40.83 CD
T5	42.67	42.67	41.00	40.33	41.67 B
T 6	42.33	42.33	41.00	39.67	41.33 BC
Mean*	42.33 a	42.05 a	40.66 b	40.16 b	
		Body& Texture (30 points)			
T1	26.67	27.00	27.67	28.33	27.41 B
T2	27.33	27.67	28.67	28.67	28.08 A
T 3	27.67	27.67	27.67	28.67	27.92 AB
T4	26.83	27.17	27.83	28.17	27.50 B
T5	27.00	27.67	27.67	28.33	27.67 B
T 6	27.00	28.00	27.67	28.33	27.75 B
Mean*	27.10 b	27.53 b	27.86 ab	28.42 a	
		Appearance and colour (25 points).			
T 1	24.50	24.30	24.00	24.00	24.20 A
T 2	24.30	24.30	24.00	24.00	24.15 A
T 3	24.30	24.30	24.00	24.00	24.15A
T 4	24.30	24.30	24.00	24.00	24.15 A
T 5	24.20	24.20	24.00	24.00	24.10 A
T 6	24.20	24.20	24.00	24.00	24.10 A
Mean*	24.31 a	24.28 a	24.00 a	24.00 a	

See legend Table (2)
* A, B, C & D and a & b : means with the same letter among the treatments and storage period respectively are not significantly different ($p<0.05$).

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مثلوجات لبنية مدعمة بالحديد

أميرة محمد الخولي – أمين جودة محمد – مجدى عبد المنعم عثمان – وفاء عبد الرحيم غريب

قسم الألبان – كلية الزراعة – جامعة قناة السويس – ٤١٥٢٢ الإسماعيلية - مصر

يمكن تدعيم اللبن ومنتجاته بصور مختلفة من عنصر الحديد وذلك لعلاج الأنيميا الناتجة عن نقص الحديد. وفى هذه الدراسة تم أختيار خمسة صور مختلفة من الحديد : كبريتات امونيوم الحديديك- كبريتات امونيوم الحديدوز - لاكتات الحديدوز - معقد الحديد مع الكازين - معقد الحديد مع بروتينات الشرش. تم تدعيم مخاليط المثلجات اللبنية بالحديد عند مستوى ٢٠ملجم حديد لكل كجم مخلوط باستخدام الخمس أملاح الحديد السابقة و أظهرت النتائج المتحصل عليها أن كثافة مخاليط المعاملات والتي دعمت بمعقدات الحديد مع الكازين أومعقد الحديد مع بروتين الشرش كانت هي الأعلى بالنسبة لباقي المعاملات. ولم يكن هناك أى أختلافات بين المعاملات المختلفة بالنسبة للوزن لكل جالون - قيم الـpH نقط تجمد المخاليط. تدعيم المخاليط بأملاح الحديد سبب زيادة اللزوجة الظاهرية للمخاليط الناتجة من المعاملات المختلفة خاصة المخاليط المدعمة بـ لاكتات الحديدوزومعقد الحديد مع الكازين و معقد الحديد مع بروتينات الشرش ، على حين تدعيم المخاليط بكبريتات اموتيوم الحديديك و كبريتات امونيوم الحديدوز و لاكتات الحديدوز أدى إلى زيادة نواتج أكسدة الدهن عند تقديرها باستخدام TBA بالمقارنة بالمخلوط غير المدعم بالحديد.

على الجانب الأخر وجد أن الريع و الوزن النوعي والوزن لكل جالون للمثلوج اللبنى لم يكن للتدعيم بالحديد من مصادره المختلفة تأثيرا معنويا عليهما. أوضحت قراءات TBA زيادة بعد ٤٥ يوم من التخزين في المجمد فيما عدا المعاملة التي استخدم فيها معقد الحديد مع بروتينات الشرش. معدل إنصهار المثلوج علي درجه حرارة الغرفة كان يتناقص تدريجيا بزيادة فتره التخزين علي -١٨° م ولم يتأثر هذا المعدل بمصدر الحديد المستخدم للتدعيم.

بالنسبة للخواص الحسية ، وجد أن تدعيم المثلجات اللبنية بكل من كبريتات امونيوم الحديدوز و كبريتات امونيوم الحديديك أعطى أقل الدرجات بالنسبة للطعم و النكهة. كانت الدرجات المعطاة للطعم والنكهة تقل تدريجيا بإطالة فترة التخزين. لون ومظهر المثلوج الناتج من المعاملات المختلفة مماثلا لتجربة المقارنة ولم يكن هناك أى اختلافات معنوية بين المعاملات المختلفة وكان للتخزين تأثيرا طبيعيا غير معنوياً.