Evaluation of the Effect of Feeding Rats by Iron Fortified Processed Cheese on Calcium and Iron Absorption

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Abstract: This study aims to evaluate the effect of feeding rats with processed cheese fortified with iron in two concentrations and to what extent iron status in the body is improved and calcium absorption is altered. Two different iron concentrations were used depending on the Recommended Dietary Intake of Nutrients for Rats which is 3.5 mg elemental iron per 100 g diet. Three and five times of that dose which were equivalent to 10.5 and 17.5 mg elemental iron per 100 g diet, respectively were used. Processed cheese was manufactured at the Pilot Unit, National Research Centre and fortified with these two iron concentrations during processing. Then, it was dried and added to the normal balanced semi-synthetic diet as 35% dry matter to each of the control diet and the two treatments. A feeding experiment was done on rats of both sexes and lasted for four weeks. Six groups were included in this experiment as three male groups, one control and two treatments, and three similar female groups. The food intake of each of the groups of treatments was more or less similar to that of the control. The body weight gain of the female group receiving the first treatment was significantly changed compared to the corresponding control. After the end of the feeding period blood hemoglobin, serum iron, total iron binding capacity (TIBC), serum total calcium and serum ionized calcium were analyzed. Blood hemoglobin was slightly increased compared to the control in all treated groups but this increase was not significant. Serum iron was increased non-significantly compared to the control, while TIBC was decreased in almost all groups non-significantly compared to the control. A very slight decrease was noticed in serum total and ionized calcium in all treated groups, but this decrease was not significant. It can be concluded from this study that, fortification of processed cheese with iron does not affect negatively on the quality of this cheese. It improves iron status in the body, although non-significantly, when fed to experimental animals. Also, it has no significant effect on calcium absorption.
[Maha, H. Mahmoud; Hayam, M. Abbas; A. G. Mohamed and Hala, M. Bayoumi. **Evaluation of the Effect of feeding rats by iron fortified processed cheese on calcium and iron absorption**. Journal of American Science 2011;7(9): 340-348].(ISSN: 1545-1003). http://www.americanscience.org

Key words: Iron fortification, processed cheese, calcium**,** iron**,** absorption.

1. Introduction

Iron is essential to most life forms and to normal human physiology. In humans, iron is an essential component of proteins involved in oxygen transport (Dallman, 1986) also, it is essential for the regulation of cell growth and differentiation (Andrews, 1999). That, deficiency of iron limits oxygen delivery to cells, resulting in fatigue, poor work performance, and decreased immunity (Hass and Brownlie, 2001 & Bhaskaram, 2001). Fe deficiency is the most common nutritional disorder world wide affecting people of all ages in both industrialized and developing countries (Lopez-Aliaga et al., 2009). Although the prevalence of iron deficiency anemia is much lower in industrialized societies where meat consumption is high and many foods are fortified with inorganic iron, yet, a small but significant proportion of women and young children still suffer from iron deficiency in western societies. In the USA, 9% of toddlers aged 1-2 years and 9-11% of adolescent girls and women of child-bearing age are iron deficient (Lynch, 2000). Nutritional iron deficiency arises when physiological requirement cannot be met by iron absorption from the diet. The recommended daily intake of iron for infants, children and adults ranges between 7 and 18 mg/day and reaches to 27 mg/day in pregnant women (Institute for medicine, 2001). Iron intake can cover body's requirement but deficiency always occurs due to limitation for absorption of iron from certain dietary components (Lynch, 2000). Calcium from supplements and dairy foods may inhibit iron absorption, but it is very difficult to distinguish between the effects of calcium on iron absorption versus other inhibitory factors such as phytate (Institute for medicine, 2001). Because of the actual trend toward food supplements, iron fortification may solve the problem of iron deficiency anemia by increasing dietary iron intake (Zhang and Mahoney, 1989). Food fortification is generally considered as the best long-term strategy for reducing the prevalence of iron deficiency in most developing countries (Davidsson et al., 1994). It is important

to choose the suitable vehicle or product that can be fortified with iron.

Dairy products are widely consumed, providing high quality of proteins and vitamins but contain almost no iron. Lack of iron in dairy products decreases the iron density of diets when the proportion of dairy products in the diets increases (Farely et al., 1987). So, it is logical that trying to fortify dairy products with iron may increase dietary iron density of the people who consume large amounts of dairy products (Zhang and Mahoney, 1989).

Processed cheese is one of the dairy products that having high consumption pattern by large sectors of population, specially the children. It is produced by blending cheeses of different types and maturity, with melting salts. This mixture is being ground and heated under vacuum with constant stirring until a homogeneous mass is obtained (Chamher and Daurilles, 2000). Fortifying this favorable product of the high consumption pattern with iron may insure ingestion of the desired amount of iron by large sector of population if this iron has a suitable bioavailability and its absorption will not be hindered by any of the components of the processed cheese.

The present study aims to investigate the effect of fortifying the processed cheese with iron as ferrous sulphate on improving iron status in the body and to know to what extent iron absorption may be affected by cheese protein. Also, to establish to what extent the calcium and iron may hinder the absorption of each other. This was done by feeding experimental animals with the iron fortified processed cheese and following the utilization pattern of both calcium and iron by detecting them in the blood of animals after the end of the feeding period.

2. Materials and methods

Materials

Most of the ingredients used for preparation of the diet were obtained from the local market. Ingredients used for formulation of vitamin and salt mixtures were obtained from Fluka (Germany) and BDH (England) Chemical Companies. Constituents of the processed cheese were purchased as follows; Ras cheese (one month old), obtained from Arabic Food Industerial Co. (Domety) 6th October City, Egypt. Matured Cheddar cheese (8 months old) and Kasomel emulsifying salt K-3294 (RhonePoulenc Chimie, France) were obtained from International Dairy & Food Co. (Green Land), 10th Ramadan City, Egypt. Also, Low heat skim milk powder and butter were obtained from Irish Dairy Borad, Gratten House, Dublin, Ireland.

Animals used in the biological experiment were obtained from the Centeral Animal House, National Research Centre, Egypt. The kit used for determination of hemoglobin was obtained from Biodiagnostic Company, Egypt. Kits used for determination of iron, iron binding capacity and total calcium were obtained from Stanbio Laboratories, Italy. Kits used for determination of ionized calcium was obtained from Olymbus Combany, Ireland.

Methods

Manufacture of the processed cheese

Processed cheese was manufactured, according to the method of Meyer (1973) from young Ras and matured Cheddar cheeses as a base blend as shown in table (1). Cheese were weighed, minced, ground and placed into the processing batch type kettle of 10 kg capacities, a pilot machine at the National Research Centre. Calculated balanced amounts of emulsifying salt (2.5%), butter, skim milk powder and water were simultaneously added. The composition of each batch of processed cheese treatments was adjusted to contain, as nearly as possible, 55% moisture and 50% fat in dry matter in the final product, according to the Egyptian Standardization System (1988). The blend was cooked with controlled agitation for 8 min at 85-90 C^o using direct injection steam at pressure of 1.5 bar. The hot product of processed cheese was manually filled into 150 cc sterilized glass jar and also covered with aluminum foil and rapidly cooled at 7 C^o. Then the fresh cheese was dried in an air circulating oven regulated at a temperature of 60 C^o till complete dryness (total solids of the dry form was 45% i.e. each 100 g fresh processed cheese yields 45 g dried processed cheese) and used for the feeding experiment.

Fortification of processed cheese with iron

Ferrous sulphate was the salt of choice as a source for iron for fortification as it provides high iron bioavailability. It is more bioavailable than other forms of iron (Perez-Exposito et al., 2005). In addition to this, it is of low cost that it can be accessible to the whole population. Two different doses of ferrous sulphate were used based on the Recommended Dietary Intake of Nutrients for Rats (Semler, 1992) which is 35 mg elemental iron/kg diet. The two doses used were three times (105 mg iron /kg diet) and five times (175 mg iron/kg diet) of that dose.

By calculating the iron content of ferrous sulphate, an amount of 675 mg ferrous sulphate was added to one kg fresh cheese as the first treatment and the second concentration of ferrous sulphate used was 1126 mg/ kg fresh processed cheese provided that the percent of

cheese added to the diet was 35% dried processed cheese as stated by Zhang and Mahoney (1989). Ferrous sulphate was dissolved in added water of processed cheese.

Chemical composition of processed cheese

The chemical composition of the fresh processed cheese was analyzed as shown in tables (3). Total fat content was measured according to the method of AACC (1987). Total protein was analyzed as described by Ling, (1963). The moisture and ash contents were estimated according to the method in AOAC (1990). Carbohydrate content was determined according to the method of Nielsen (1998).

Physical properities

The processed cheese firmness was measured using a penetrometer as described by Gupta and Reuter (1993). Meltability of the processed cheese was measured as described by Olson and Price, 1958. Oil separation was determined according to the method of Thomas (1973). The obtained data are shown in table (4).

Organoleptic assessment

The organoleptic properties of the processed cheese were evaluated by panel test by the staff members at the Dairy Science Dept., National Research Centre, according to the scheme of Meyer (1973). The results obtained were tabulated as shown in table (5).

Formulation of the diet

All of the diets used including the control diet and the two diets containing the two concentrations of iron were prepared according to Reeves et al., (1993). Three diets were prepared (table 2) as follows:

First diet; a control diet containing 35% dried processed cheese (not fortified with iron) provided that the source of iron in the salt mixture was replaced by ferrous sulphate to be similar to that used in the treatment. The amount used was 175 mg ferrous sulphate, which is equivalent to 35 mg iron per Kg diet as stated by Semler (1992). The composition of the control diet is shown in table (2).

Second diet; was formulated to contain 35% dry processed cheese fortified with iron (675 mg ferrous sulphate / Kg fresh processed cheese) so that each kg diet contains 525 mg ferrous sulphate that is equivalent to 105 mg elemental iron/ kg diet which is 3 times the Recommended Dietary Intake of Nutrients for Rats by (Semler, 1992) (table 2). Other constituents of the diet as shown in table (2) were adjusted according to the chemical composition of the cheese (table 1).

Third diet; was formulated to contain 35% dry processed cheese fortified with iron (1126 mg ferrous sulphate / Kg fresh processed cheese) so that each kg diet contains 875 mg ferrous sulphate that is equivalent to 175 mg elemental iron/ kg diet which is 5 times the Recommended Dietary Intake of Nutrients for Rats by (Semler, 1992) (table 2). Other constituents of the diet as shown in table (2) were adjusted according to the chemical composition of the cheese (table 1).

Biological evaluation of the iron fortified cheese

An animal experiment was conducted to evaluate the biological value of the processed cheese fortified with iron as follows;

Thirty six white albino rats (Sprague Dawley strain) of both sexes with a body weight ranging from 111 to 199 g were obtained from Central Animal House, National Research Center. Six groups (3 male groups and 3 female groups) were formed, each comprising 6 rats as follows;

Group 1: Control male; was given control diet.
Group 2:Control female; was given control diet.
Group 3: Males given treatment 1.
Group 4: Females given treatment 1.
Group5: Males given treatment 2.
Group6: Females given treatment 2.

Food and water were allowed ad-libtum to each rat. Body weight was followed twice a week. Food intake of each rat was recorded daily. The animal experiment lasted for 4 weeks. At the end of this period, body weight gain and food intake were recorded. Food efficiency ratio (FER) for each group was calculated according to the following equation:

FER = body weight gain/total food intake.

At the end of the experiment, rats were fasted overnight. In the morning. non-heparinized and heparinized blood samples were obtained by open heart puncture under slight diethyl ether anesthesia. Heparinized blood was used for estimation of hemoglobin concentration, while, serum was separated from non- heparinized blood samples by centrifugation at 3000 r.p.m and stored at– 40 °C until being used for determination of the different biochemical parameters.

Blood hemoglobin was determined according to Betke and Savelsberg (1950). Serum iron and iron binding capacity were estimated according to Burits and Ashford (1994). Serum total calcium was determined according to the method of Lehman & Henry, (1984). Ionized calcium was calculated from the

following equation as described by Sava et al., (2005);

Ionized Ca (mg/dl) = ((((6*Ca) - (((0.19*T.P) + Alb/3))) / (6+((0.19*T.P)+ Alb)))/4

where, T.P is total protein and Alb is albumin.

Free or ionized calcium is a useful index than total calcium and provides the better indication of calcium status because, Ca++, and not total calcium, is biologically active and tightly regulated by calcium binding hormones.

Since serum total protein and serum albumin are required for the calculation of ionized calcium as illustrated in the previous equation, determination of both of them was a must. Both serum total protein and albumin were determined using a kit obtained from Olympus, Ireland. Serum total protein was estimated according to Lin et al., (1999), while serum albumin was assessed as described by Dumas et al., (1971). Both of them were measured using the automated system; Olympus, Japan.

Results were analyzed statistically using the computerized program SPPS, version "17". The one way ANOVA test was done. Significance was considered at a level of 0.05.

Male groups that received the two iron concentrations were compared to the control of male rats, while the female ones were compared to the female control group.

3. Results

1. Chemical, physical and sensory assessment of the prepared iron fortified processed cheese

Chemical composition

Table (3) represents the chemical composition of processed cheese for the control blend, treatment 1 and treatment 2. Results show that there is slight negligible difference between treatments and control.

Physical properties

The physical properties of processed cheese were done depending on measuring the penetration, oil separation, meltability and color. Table (5) displays no considerable difference between treatments in physical properties. On the other hand, color attributes including lightness (L) and yellowness (b) shows more or less no difference between control and treatment formulas, while, whiteness (a) shows a considerable change between formulas, whereas by increasing iron level, whitness decreased, but in slight amount as a result of the small iron quantities added.

Organoleptic properities

Organoleptic properities (sensory evaluation) are represented in table (5). Appearance is among the most important attribute influencing customer choice, texture and flavor also play a relevant role on the perception of quality of cheese products. From the attributes selected for external evaluation only chewiness, gumminess, oil separation and overall preference showed no differences between formulas and control. In case of the attributes used for breakdown properties evaluation, there is a significant difference between formulas and a significant difference between formula 2 and the control. Surface appearance and firmness showd no significant differences between formula 1 and the control, while, there are significant differences between formula 2 and control for the same properties.

2. Biological evaluation of the prepared iron fortified processed cheese

Food intake, food efficiency ratio and body weight gain

The food intake (g), the body weight gain and the food efficiency ratio for rats in all groups are recorded in table (6). As shown in this table, there was no significant change between all groups in the food intake. Only, a significant decrease was noticed in FER in case of the female group that received the first iron concentration (treatment 1), its value was 0.080 ± 0.008 compared to a value of 0.119 ± 0.003 for the female control.

Body weight gain showed no significant change in all groups compared to the corresponding control, except for the group of female rats of treatment 1 which showed a significant decrease when compared to the female control. Their values were 22.50 ± 2.17 and 32.17 ± 1.92 for treatment 1 and control, respectively.

Blood hemoglobin concentration

Blood hemoglobin concentration is shown in table (7). As shown in the table, feeding animals on either of the two diets containing iron concentrations caused a slight increase in the hemoglobin concentration in blood of rats of treatment 1 and treatment 2 compared to the corresponding control, but this increase was not significant.

Serum iron and total iron binding capacity

Table (8) shows the values of serum iron, total iron binding capacity and percentage saturation of all groups compared to the control. There was an increase in the serum iron either in case of male groups compared to their male control or the female groups when compared to the female control group but this increase was not significant. On the other hand there was a

non-significant decease in TIBC compared to the control. The percent saturation was increased in all groups that received iron fortified processed cheese. This increase was non-significant in most of the groups, except the female group that received the higher iron concentration; treatment 2, there was a significant increase in this group when compared to the female control. The values were 43.07 ± 2.24 and 34.01 ± 1.76 for female rats receiving treatment 2 and female control group, respectively.

Serum total calcium and ionized calcium

Serum total calcium and ionized calcium are shown in table (9). As shown in this table there was a slight negligible decrease in both total and ionized calcium for all groups that was non-significant.

4. Discussion

Worldwide, iron deficiency and the consequences of anemia with major health complications is the commonest nutrition disorder (Lynch, 2000 and Srdjan et al, 2007). Iron fortification may solve the problem of iron deficiency anemia by increasing dietary iron intake (Zhang and Mahoney, 1989). Food fortification is generally considered as the best long-term strategy for reducing the prevalence of iron deficiency in most developing countries (Davidsson et al., 1994).

The present study was a trail to improve iron status in the body by fortification of a product commonly used by a large sector of population which is the processed cheese with ferrous sulphate by concentrations that do not cause any undesirable effects on calcium absorption.

In this study, the physical properties of the processed cheese were more or less not affected by fortification with iron suggesting that iron dose not affect negatively on most of the physical properties of the processed cheese.

For sensory evaluation, there is almost no significant change for formula 1, when compared to control cheese with no iron fortification in the studied properties, while a very slightly significant change occurred for formula 2 compared to control, suggesting that iron fortification with the two concentrations used has no prominent effect on the sensory evaluation.

It was necessary to evaluate the effect of fortifying the processed cheese with iron in respect to food intake and growth represented by body weight gain. This is to insure that iron fortification of the processed cheese dose not affect negatively on the growth.

The food intake of animals either male or female did not show any significant change from the control. With respect to the gain in body weight, there was a significant decrease in the body weight gain in case of female rats that received the first iron concentration which also showed a significant decrease in the food efficiency ratio, a phenomenon which has no reasonable explanation.

As shown from the data obtained, serum iron was increased, but non-significantly, in all groups receiving the iron fortified processed cheese either male or female so the TIBC was decreased in all groups, suggesting that iron status in the body was improved by ingestion of the iron fortified cheese. Two factors were expected to lower iron absorption in cheese, or any other dairy product, protein and calcium. Milk protein was attributed to the category of inhibitors of iron absorption by Morris, (1983), based on one study in which Cook and Monsen, (1976), reported that substitution of beef by milk protein in a typical American diet reduced absorption of extrinsic labeled Fe^{59} from 5.5 to 1.1%. On the other hand, Theuer, et al., (1973), found that heat processed milk-based infant formula with the iron supplement resulted in greater iron bioavailability in anemic rats.

In the present study, the absorption of iron was not affected by the amount of calcium in the cheese. In this respect, Lopez-Aliaga et al., (2009), found that calcium-supplemented goat milk does not interfere with iron absorption in rats with anemia induced by dietary iron depletion. This seems to support our findings.

On the other hand, a non-significant slight decrease in serum total and ionized calcium occurred suggesting that calcium absorption was not affected by iron fortification with the iron concentration used. One study that was carried out in this respect by Davidsson et al., (1994), mentioned that fortification of bread with NaFeEDTA did not affect either zinc or calcium absorption.

It can be concluded from this study that, fortification of processed cheese with iron does not affect negatively on the quality of this cheese. It improves iron status in the body, although non-significantly, when fed to experimental animals. Also, it has no significant effect on calcium absorption.

In the light of the previous considerations, it is recommended that iron fortified processed cheese should be included in the diet of the general population especially for people suffering from iron deficiency anemia.

Table (1): Composition of fresh processed cheese (g/100g) and fresh processed cheese fortified with two concentrations of iron; 0.0675 g & 0.1126 g ferrous sulphate per 100 g fresh processed cheese for treatment 1 & treatment 2, respectively.

Amount Ingredients	Processed Cheese	Treatment 1	Treatment 2
Cheddar cheese	12.80	12.80	12.80
Ras cheese	38.44	38.44	38.44
Skim-milk powder	5.12	5.12	5.12
Butter	10.26	10.26	10.26
Emulsifying salts	2.50	2.50	2.50
Fe SO_4	0	0.0675	0.1126
Water	30.88	30.88	30.88
Total	100	100	100

Table (2): Composition of the control and experimental diets (g/100g diet) after being adjusted according to the composition of the cheese.

Ingredients	Control	Treatment 1	Treatment 2
Dried processed cheese*	35	35	35
Casien*	6.5	6.5	6.5
Sucrose	10	10	10
Oil	0	0	0
Salt mixture*	3.5	3.5	3.5
Vitamin mixture*	1	1	1
Choline chloride	0.06	0.06	0.06
Corn starch	43.94	43.94	43.944

* Protein content of the casein was estimated as 80%.
* Salt mixture, vitamin mixture and diet composition were according to Reeves et al., 1993.
* Zero, 0.0525 and 0.0875 g ferrous sulphate were included in the 35 g dried processed cheese in control, treatment 1 and treatment 2, respectively.

Table (3): Chemical composition (%) of non-fortified and iron fortified processed cheeses.

	Control	Treatment 1	Treatment 2
Total solids	44.69	44.64	44.16
Fat	24.00	24.10	24.03
Total protein	13.8	13.71	13.69
Ash	5.01	5.06	5.11
Lactose	5.01	5.00	5.02

Table (4) Physical properties of processed cheese.

	Control	Treatment 1	Treatment 2
Penterometer value (mm)	172	170	171
Oil Separation (%)	33.33	31.66	32.66
Meltability	110	112	108
Color parameters:			
L	86.85	86.12	86.05
a	-1.62	-1.71	-1.85
b	25.00	25.00	25.40

Table (5) Sensory evaluation of processed cheese.

	Control	Treatment 1	Treatment 2
Surface appearance	4.66^{a}	4.60^{a}	4.55^{b}
Firmness	2.50^{b}	2.50^{b}	2.45^{a}
Spreading	4.00^{a}	4.00^{a}	4.16^{b}
Stickiness	1.00^{a}	1.25^{c}	1.16^{b}
Smoothness	1.33^{b}	1.16^{a}	1.10^{c}
Breakdown properties	4.16^{b}	4.10^{b}	4.00^{a}
Chewiness	5.0^{NS}	5.0^{NS}	5.0^{NS}
Gumminess	1.0^{NS}	1.0^{NS}	1.0^{NS}
Oil separation	1.0^{NS}	1.0^{NS}	1.0^{NS}
Flavor	4.5^{NS}	4.16^{NS}	4.18^{NS}
Overall preference	4.5^{NS}	4.5^{NS}	4.5^{NS}

Data in the same row with the same letters are not significance ($p>0.05$)

Table (6): Food intake, food efficiency ratio (FER) and body weight gain of each of male and female rats fed on the two treatments compared to the control.

Test / Group	Food intake (g)		B. wt. gain (g)		FER	
	male	female	male	female	male	female
Control mean ± SE	339.83 ± 6.72[a]	268.83 ± 10.36[a]	58.00 ± 7.44[a]	32.17 ± 1.92[b]	0.171 ± 0.020[a]	0.119 ± 0.003[a]
Treatment1 mean ± SE	320.83 ± 6.65[a]	284.00 ± 8.13[a]	57.67 ± 7.03[a]	22.50 ± 2.17[a]	0.179 ± 0.020[a]	0.080 ± 0.008[b]
Treatment2 mean ± SE	337.00 ± 6.22[a]	297.50 ± 12.27[a]	49.33 ± 9.96[a]	33.67 ± 2.81[b]	0.148 ± 0.030[a]	0.114 ± 0.010[a]

*Values that share the same letter at the same column are not significant.
*Values that share different letters at the same column are significant.
*The mean difference is significant at the 0.05 level.

Table (7): Hemoglobin concentration of each of male and female rats fed on the two treatments compared to the control.

Test / Group	Hb (g/dl)	
	male	Female
Control mean ± SE	14.67 ± 0.53[a]	13.72 ± 0.53[a]
Treatment1 mean ± SE	15.33 ± 0.27[a]	13.90 ± 0.57[a]
Treatment2 mean ± SE	15.65 ± 0.37[a]	14.57 ± 0.41[a]

*Values that share the same letter at the same column are not significant.
*Values that share different letters at the same column are significant.
*The mean difference is significant at the 0.05 level

Table (8): Serum iron and total iron binding capacity (TIBC) of each of male and female rats fed on the two treatments compared to the control.

Test / Group	s.Iron (µg/dl)		TIBC (µg/dl)		% Saturation	
	male	female	male	female	male	female
Control mean ± SE	156.87 ± 8.36[a]	154.77 ± 5.73[a]	479.08 ± 49.05[a]	457.29 ± 12.43[a]	35.06±3.49[a]	34.01±1.76[a]
Treatment1 mean ± SE	166.39 ± 4.68[a]	167.99 ± 1.31[a]	474.87 ± 24.50[a]	413.59 ± 26.43[a]	35.39±1.57[a]	39.16±2.81[ab]
Treatment2 mean ± SE	172.83 ± 5.75[a]	170.10 ± 6.14[a]	438.77 ± 12.93[a]	399.20 ± 21.07[a]	39.57±1.73[a]	43.07±2.24[b]

*Values that share the same letter at the same column are not significant.
*Values that share different letters at the same column are significant.
*The mean difference is significant at the 0.05 level.

Table (9): Serum total calcium and ionized calcium concentration of each of male and female rats fed on the two treatments compared to the control.

Test / Group	s.Total Calcium (mg/dl)		s.Ionized calcium (mg/dl)	
	male	female	male	female
Control mean ± SE	9.09 ± 0.7[a]	9.98 ± 0.83[a]	4.93 ± 0.08[a]	4.96 ± 0.14[a]
Treatment1 mean ± SE	8.19 ± 0.55[a]	9.66 ± 0.3.4[a]	4.86 ± 0.06[a]	4.90 ± 0.51[a]
Treatment2 mean ± SE	8.81 ± 0.33[a]	8.86 ± 0.46[a]	4.78 ± 0.16[a]	4.73 ± 0.21[a]

*Values that share the same letter at the same column are not significant.
*Values that share different letters at the same column are significant.
*The mean difference is significant at the 0.05 level.

6. Reference

AACC, (1987), Method 46-11, Approved Methods of the AACC, American Association of Cereal Chemists, Inc., St Pau, Minn.

Andrews, N. C., (1999), "Disorders of iron metabolism.", N. Engl. J. Med.; 341: 1986-95.

AOAC, (1990), Official Methods of Analysis, (15th Ed.), Association Official Analytical Chemists, Arlington VA, USA, pp.: 840-850.

Betke, K. and Savelsberg, W., (1950), "Stage Photometry of hemoglobin using cyanohemoglobin.", Biochem. Z.; 320: 431 -439.

Bhaskaram, P., (2001), "Immunobiology of mild micronutrient deficiencies.", Br. J. Nutr.; 85: 575-80.

Burits, C. A. and Ashford, E., (Eds.), (1994), Tietz Text Book of Clinical Chemistry, (2nd Ed.), Sounders, pp.: 2195.

Chambre, M. & Daurelles, J., (2000), "Processed cheese.", In: Cheese making from Science to quality Assurance, (3rd Ed.), Eck, A & Gillis, J. C., (Eds.), Veterinaer, Danmarks, pp.: 641-657.

Cook, J. D. and Monsen, E. R., (1976), "Food iron absorption in human subjects.III . Comparison of the effect of animal proteins on non-heme iron absorption.", Am. J. Clin. Nutr.; 29: 859.

Dallman, P.R., (1986), "Biochemical basis for the manifestations of iron deficiency.", Annu. Rev. Nutr.; 6: 13-40.

Davidsson, L., Kastenmayer, P. and Hurrell, R., (1994), "Sodium iron EDTA [NaFe(III)EDTA] as a food fortificant: the effect on the absorption and retention of zinc and ca in women.", Am. J. Clin. Nutr.; 60(2): 231-237.

Dumas, B. T., Watson, W. A. and Biggs, H. G., (1971), "Albumin standards & the measurement of serum albumin with bromocresol green.", Clin. Chem. Acta; 31: 87-96.

Egyptian Standards, (1988), "Processed cheese. Part II., Spreadable processed cheese.", Egyptian Organization for Standardization and Quality Control, Cairo, pp.:3-5.

Farley, M. A., Smith, P. D. S., Mahoney, A. W., West, D. W. and Post, J. R., (1987), "Adult dietary characteristics affecting iron intake: A comparison based on iron density.", J. Am. Diet. Assoc.; 87: 184.

Gupt, V. K. & Reuter, H., (1993), "Firmness and melting quality of processed cheese foods with added whey protein concentrates.", Lait; 73: 381-388.

Haas, J. D. & Brownlie, T., (2001), "Iron deficiency and reduced work capacity: a critical review of the research to determine a causal relationship.", J. Nutr; 131: 6915-65.

Institute of Medicine, (2001), "Food and Nutrition Board. Dietary Reference intakes for vitamin A, vitamin K, Arsenic, Boron, Chromium, Copper, iodine, Manganese, Nickel, Silicon, Vanadium and Zinc.", Washington, D.C., National Academy Press.

Lehman, H. P. & Henry, J. B., (1984), In: Clinical Diagnosis and Management by Laboratory Methods, (17th Ed.), Henry, J. B., (Ed.), Saunders, Philadelphia, pp.: 1431 & 1438.

Lin, J., Idee, J. M., Port, M. Diai, A., Berthominier, C, Robert M., et al., (1999), "Interferrence of magnetic resonance imaging contrast agents with the serum calcium measurement technique using calorimetric reagents.", J. Pharm. Biomed. Analysis; 931- 943.

Ling, E. F., (ed.), (1963), A Text Book of Dairy Chemistry, Vol. 2, Practical, (3rd), Chapman and Hall Ltd., London, pp. 58-65,

Lopez-Aliaga, I., Diaz-Castro, J, Nestares, T., Alferez, M. J. M. and Campos M. S., (2009), "Calcium-supplemented goat milk dose not interfere with iron absorption in rats with anemia induced by dietary iron depletion.", Food Chemistry; 113: 839-841.

Lynch, S. R., (2000), "The effect of calcium on iron absorption.", Nutrition Research Reviews; 13: 141-158.

Meyer, A., (Ed.), (1973), In: Processed Cheese Manufacture, (1st Ed.), Food Trade Press Ltd., London, UK, pp.: 98-277.

Morris, E. R., (1983), "An overview of current information on bioavailability of dietary iron to humans.", Fed. Proc.; 42: 1716.

Nielsen, Suzanne S., (Ed.), (1998), In: Food Analysis (2nd Ed.), Aspen Publishers, Inc., Maryland, pp.: 167-215.

Olson, N. F. & Price, W. V., (1958), "A melting test for pasteurized processed cheese spread.", J. Dairy Sci.; 41: 991-999.

Perez-Exposito, A. B., Villapando, S., Rivera J. A., Griffin, I. J. and Abrams, S. A., (2005), "Ferrous sulfate is more bioavailable among preschoolers than other forms of iron in a milk-based weaning food distributed by PROGRESA; a national program in Mexico.", J. Nutr.; 35(1): 64-69.

Reeves, P. G.; Nielsen, F. H. and Fahey, G. C., (1993), "AIN-93 Purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on reformulation of the

AIN-76A rodent diet”., J. Nutr.; 123: 1939-1951.

Sava, L., Pillai, S., More, U. and Sontakke, A., (2005), "Serum calcium measurement: Total versus free (ionized) calcium.", Indian Journal of Clinical Biochemistry; 20(2): 158-161.

Semler, D. F., (1992), " The Rat; Toxicology.", C. in Animal Models in Toxicology by Gad, S. C. and Chengelis, C. P. (Eds.), Marcel Dekker, INC, Ch. 2, pp: 21-164.

Srdjan Denic, M.D., Mukesh, M. and Agarwal, M.D., (2007), 'Nutritional iron deficiency: an evolutionary perspective.", Nutrition Reviews; 23: 603-614.

Stoltzfus, R. J., (2001), "Defining iron deficiency anemia in public health terms: reexamining the nature and magnitude of the public health problem.", J. Nutr.; 131: 565S-567S.

Theuer, R. C., Martin, W. H., Wallander, J. F. and Sarett, H. P., (1973), "Effect of processing on availability of iron salts in liquid infant formula products. Experimental milk-based formulas.", J. Agric. Food Chem.; 21: 482.

Thomas, M. A., (1973), "The Use of hard milk fat fraction in processed cheese.", Aust. J. Dairy Technol.; 28: 77-80.

Zhang, D. and Mahoney, A. W., (1989), "Bioavailability of iron-milk-protein complexes and fortified cheddar cheese.", J. Dairy Sci.; 72: 2845-2855.

8/12/2011