BIOLOGICAL EVALUATION OF MUFFINS FORTIFIED WITH MICROENCAPSULATED IRON SALTS

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ABSTRACT

To avoid the development of color and flavor changes, rancidity and any unacceptable change in food fortified with iron salts. microencapsulated iron salts were used to protect the fortified food from these dislike characteristics. Therefore, the iron salts (ferrous fumarate, Fe Na EDTA, ferrous glouconate, ferrous citrate, ferrous carbonates and anhydrous ferrous sulphate) were microencapsulated with palm stearin, lecithin and ascorbic acid. For testing the efficiency of iron salts microencapsules, wheat flour (72 % extraction) was fortified with different microencapsules iron salts and the wheat flour fortified samples used to produce muffins. The chemical composition, Texture Profile Analysis (TPA), weight loss, sensory and biological evaluation of muffins prepared by wheat flour fortified with different microencapsules iron salts were estimated. The results indicated that there was no effect for wheat flour fortified with different microencapsules of iron salts on chemical composition, Texture Profile Analysis (TPA), weight loss, sensory evaluation of prepared muffins except the iron content compared to muffins prepared by unfortified wheat flour (MWF). It was increased in the fortified muffins. Depletion and repletion of iron biological experiments were designed. All male *albino* rats were fed on Fe deficient basal diet for six weeks. After the depletion period, the rats were divided randomly into 7 groups. Group 1: Fed on iron deficient basal diet and group 2: Fed on muffins prepared by unfortified wheat flour (MWF) and five groups fed on muffins prepared by wheat flour fortified with different microencapsules of iron salts for 30 days. Growth of rats, apparent digestibility coefficient (ADC), relative organs weight ratio, serum lipid profile, hematological evaluation and Fe absorption were measured. There was a significant increase in body weight, ADC, relative liver, kidney and heart weights ratio, hematological parameters, serum iron, total iron-binding capacity (TIBC), Fe kidney and Fe spleen of rats fed on muffins prepared by wheat flour fortified with different iron salts microencapsules compared to rats fed on Fe deficient basal diet and MWF. No significant difference was found in serum lipid profile among all tested rat groups. These results indicated that microencapsulation of iron salts had a positive effect to treat the dislike characteristics produced from fortified products with iron and had no effect on Fe absorbed and bioavailability.

Key words: bioavailability- iron absorbed- iron salts- microencapsulation- muffins.

1. INTRODUCTION

Anemia is considered as one of the most common index of nutritional deficiency worldwide and is caused by iron deficiency store or iron deficiency erythroanpoiesis. Several authors have reported that iron deficiency anemia (IDA) is mainly caused by some food constituents that may contribute to inhibition of iron absorption, hence contribute to the high prevalence of IDA (Lin *et al.,* 2003). Iron is the most difficult mineral to add to foods and ensure adequate absorption.

The main problem is that the water soluble iron compounds (*i.e.* ferrous sulfate, ferrous lactate and ferrous gluconate), which are the most bioavailable, often lead to the development of unacceptable color and flavor changes in the food vehicle. When water-soluble compounds are added to cereal flours, for example, they often cause rancidity, and in low-grade salt, they rapidly lead to color formation. Insoluble compounds,

such as elemental iron powders, on the other hand, do not cause sensory changes but may be so poorly absorbed as to be of little or no nutritional benefit.

Moreover, palm stearin is the solid fraction of palm oil that is produced by partial crystallization at controlled temperature (Lim, 2010). It is more variable in composition than palm olein, the liquid fraction of palm oil, especially in terms of its solid fat content, and therefore has more variable physical characteristics. Like crude palm fruit oil, palm stearin contains carotenoids, but physically refined palm oils do not, as they are removed or destroyed in the refining process. It is a useful source of natural hard vegetable fat for food applications. Palm stearin consists of mostly glyceryltripalmitate, with most of the rest of the fat content being glyceryl dipalmitate monooleate, (Kuo and Gardner, 2002). In terms of fatty acid composition, a typical soft palm stearin might contain almost 50% palmitic acid and 35% oleic acid.

Microencapsulation is a process where thin films or polymer coats are applied to small solid particles or droplets, of liquids. The food industry employs microencapsulation to protect sensitive food components, prevent nutritional loss, preserve flavors and aromas and mask undesirable flavors. Microencapsules can be designed to release core material by mechanical fracture, temperature changes pH manipulation or a combination of such factors. Stability of microencapsules can be optimized through choice of coating material, encapsulation technique and amount of core. Materials used to encapsulate water soluble compounds include hardened fats and wax. In successful fortification programme, both the fortificant and the vehicle should be acceptable to the target population (Bovell-Benjamin and Guinard, 2003). For example, the sprinkle-sized particles of ferrous fumarate are coated with a mono or diglyceride (hydrogenated soy lipid) and this thin coating protects the iron from the food (and food from the iron) and also masks the taste of the iron. The contents of the packet are sprinkled on the food that is served to the child. The iron will not react with the food altering its appearance or taste because it is encapsulated. The coating will dissolve in the stomach, releasing the iron salt, to be absorbed along with iron contained in the foods that constituted the meal. The availability of the added iron for absorption will be affected by inhibitors and enhancers of iron absorption that might be present in a meal fed to infants and toddlers (Wegmuller et al.,2003). Knowledge of the bioavailability of iron supplements is therefore necessary for a sound approach to establishing strategies for meeting the needs of the absorbed iron in them.

Muffin is a sweet, high-calorie baked product which is highly appreciated by consumers due to its good taste and soft texture. Muffin batter is a complex fat-in-water emulsion composed of an egg, sucrose, water and fat mixture as the continuous phase and bubbles as the discontinuous phase in which flour particles are dispersed (Martinez-Cervera et al.,2012). Therefore, muffins are considered a suitable product to examine the success of fortified wheat flour with different microcapsulated of Fe sources and insurance about the quality of product for sensory evaluation, and bioavailability of iron.

2. MATERIALS AND METHODS
2.1. Materials
- Refined, bleached and deodorized (RBD) palm stearin sample was obtained from Gulf Company for Industrial Oils Co., Suez, Egypt.
- Ferrous fumarate, Fe Na EDTA, ferrous gluconate, ferrous carbonates, anhydrous ferrous sulphate and ascorbic acid were obtained from Sigma-Aldrich Company, St. Louis, USA.
- Lecithin was obtained from soybean pilot plant, Food Technology Research Institute, Agric. Res. Center, Giza, Egypt.
- Wheat flour (72 % extraction), sugar, egg, milk, sunflower oil, salt, bicarbonate of soda, vanillin were purchased from the local market, Dokki, Giza, Egypt.
- The analytical kits were obtained from Randox Laboratories Ltd., Diamond Road, Crumlin. Co., Antrim, United Kingdom, BT294QY.

2.2. Methods
2.2.1. Production of microencapsules
The coating material used for the encapsulation process consisted of palm stearin (0.005% of Butylated Hydroxy Toluene, BHT added) with a melting point of 63 °C. The lecithin (1%) was added to the molten palm stearin together with the Fe to reduce the viscosity of microencapsules. The ratio between palm stearin: Fe substrate was of 3.5:1.5 (g/g), 50 ml distilled water was added. Moreover, the ascorbic acid was added (1:1 molar ratio of Fe substance: ascorbic acid, Premkumar and Bowlus, 2003). The mixture was homogenized at 1250 rpm/min for 20 min. Spray dryer granulator PGL-B was used to produce

different Fe microencapsules.The microencapsules were stored at -5 ℃ in polyethylene till use.

2.2.2.Fortifying wheat flour with microencapsules of different iron salts

The Fe microencapsules were added to the wheat flour after determination of the iron content by atomic absorption spectrophotometry (AAS) of each microencapsule to obtain 100 μg Fe/g flour, (Yeung *et al.,*2005) and the fortified flour was used to prepare muffins.

2.2.3. Manufacture of muffins

Six muffin formulations were prepared. One was used as a control treatment (wheat flour 72 % unfortified with Fe) and the other five were prepared by replacing wheat flour with fortified flour with different microencapsulated Fe. The quantities of the ingredients were: 100 g Wheat flour 72%, 27 g egg yolk, 54 g egg white, 50 g milk, 100 g sugar, 46 g sunflower oil, 4 g bicarbonate of soda, 3 g citric acid, 1.5 g salt and 0.5 g vanillin according to Martinez-Cervera *et al.*(2011). After baking, the muffins were left to cool at room temperature for 1 h on a rack in order to avoid moisture condensing on their undersurface.

2.2.3.1. Gross chemical composition of muffins

Protein, ether extract, ash, and crude fiber contents were determined (A.O.A.C., 2000). Total carbohydrates were calculated by difference.

2.2.3.2. Texture Profile Analysis (TPA) and weight loss of muffins

Crumb texture was determined by a universal testing machine (Cometech, type B, Taiwan). An aluminium 25 mm diameter cylindrical probe was used in a Texture Profile Analysis (TPA) double compression test to penetrate to 50% depth, at 1 mm/s speed test. Cohesiveness, gumminess, chewiness, springiness and resilience were calculated according to Boume (2002).

The muffins were weighed before (W3) and after baking and cooling (W4) and the weight loss was calculated, Martinez-Cervera *et al.* (2012). Each formulation was prepared twice, on different days, and seven muffins from each batch (fourteen determinations) were measured.

2.2.3.3. Sensory evaluation

The samples of muffins were organoleptically evaluated according to Martinez-Cervera *et al.* (2012). Ten consumers rated the appearance, color, texture, sweetness, flavor and overall acceptance of each muffin sample.

2.2.3.4. Bioassay of muffins

Forty two male *albino* rats weighing 105 ± 5 g. were housed at Ophthalmology Research Institute, Giza, Egypt. All animals were kept under normal healthy conditions and fed on basal diet (Eder and Kirchgessner, 1996) for one week. Water and diet were provided *ad libitum*. Then, the animals were fed on Fe deficient basal diet with low Fe content (5 mg/Kg diet, Diaz-Castro *et al.,*2011) for 6 weeks. After depletion period, the rats were reweighed and divided randomly into 7 groups each one consist of 6 rats and fed on as follows (for 30 days):

Group (1): Fed on iron deficient basal diet.

Group (2): Fed on muffin prepared by unfortified wheat flour (MWF).

Group (3): Fed on muffin prepared by wheat flour fortified with ferrous fumarate microencapsules (MWF+M ferrous fumarate) .

Group (4): Fed on muffin prepared by wheat flour fortified with Fe Na EDTA microencapsules (MWF+M Fe Na EDTA).

Group (5): Fed on muffin prepared by wheat flour fortified with ferrous gluconate microencapsules (MWF+M ferrous gluconate).

Group (6): Fed on muffin prepared by wheat flour fortified with ferrous carbonates microencapsules (MWF+M ferrous carbonates).

Group (7): Fed on muffin prepared by wheat flour fortified with anhydrous ferrous sulphate microencapsules (MWF+M ferrous sulphate).

2.2.3.4.1. Growth of rats

The rats were weighed at zero time, after depletion period and during the experiment. The apparent digestibility coefficient (ADC) was calculated from the data on Fe intake and Fe fecal (on dry weight basis), according to the equation:

ADC=[(Fe intake – Fe fecal) x 100] /Fe intake, (Alferez *et al.,*2006).

At the end of the experimental period, the rats were weighed and killed by diethyl ether. The carcasses were dissected. Liver, kidney, spleen, heart and lung were separated by careful dissection, washed using saline solution and weighed. The organs weight ratio was calculated as follows (Weight of organ / total body weight at the end of the experiment x 100).

2.2.3.4.2. Biochemical assay

Blood samples were collected from the animals. Heparin was used as an anticoagulant. The serum was separated after centrifugation for 10 min at 3000 rpm and kept frozen at –20 ℃ for biochemical assays:

Serum triglycerides, total cholesterol, HDL-cholesterol and LDL-cholesterol were determined using the methods described by Fossati and Prencipe (1982),Wastson (1960), Assmann (1979) and Wieland and Seidel (1983), respectively. VLDL-cholesterol was calculated according to

Wallach (1992) using the following equation:
VLDL – cholesterol = serum triglycerides / 5

2.2.3.4.3. Hematological evaluation

Blood samples were received in cleaned tubes contained ethylene diamine tetra acetic acid (EDTA) as anticoagulant. Leucocytes count (WBC_s), red blood cells (RBC_s), blood haemoglobin (Hg) and haematocrite value (PVC) were estimated by Wintrobe (1967), Dacie and Lewis (1975), Leong *et al.*(2003) and Burch and Siegel (1971), respectively.

2.2.3.4.4. Fe absorption

Serum Fe concentrations and the total iron-binding capacity (TIBC) were determined according to the methods described by Feigin (1977) and the International Committee for Standardization in hematology (1978), respectively.

The rate of transferrin saturation was subsequently calculated using the following equation:

Transferrin saturation (%) = [Serum Fe concentration (mg/dl) /TIBC (mg/dl)] × 100

The Fe content of rat organs and feces were determined by atomic absorption Spectrophotometer. The samples were previously mineralized by a wet ash method as described by Parker *et al.* (1967).

2.2.4. Statistical analysis

Each parameter was analyzed separately by using one-way analysis of variance (ANOVA). For determining differences between groups, the Duncan test was used. All *p* values of ≤ 0.05 were considered to be significant (Bouveresse *et al.,* 2011).

3. RESULTS

According to the chemical analysis of muffins, the data in Table (1) clear that the percentages of protein, fat, crude fiber, ash, and total carbohydrate were similar approximately on dry weight basis. The muffins prepared by unfortified wheat flour (MWF) were considered the lowest content of iron. Moreover, there was a fairly difference in protein content. The Texture Profile Analysis (TPA) parameters were estimated and the results in Table (2) illustrate that there was a little difference in cohesiveness of all tested muffin samples. Moreover, wheat flour fortified with different microencapsules of Fe salts had no effect on muffin gumminess, chewiness, springiness and resilience. The values for weight loss were fairly different in tested muffin samples. The weight loss ranged from 13.75 to 16.48 g. The data in Table (3) show that the sensory evaluation

of different tested muffins had no significant effect on sensorial properties. It was observed that microencapsules of iron had a good effect to prevent changes of sensorial properties when the food was fortified with iron.

The data presented in Table (4) illustrate the initial weight, weight after depletion period, final weight and body weight gain. At the end of the experimental period, the final weight of tested rat groups increased. There was no significant difference in the final weight among rats fed on tested muffins prepared by wheat flour fortified with different encapsulated iron. There was a fairly increase in final body weight of rats fed on iron deficient basal diet or MWF. The Fe intake, Fe fecal and Fe absorbed were significantly different among tested rat groups. Fe intake and Fe fecal were the highest in rats fed on MWF+M ferrous gluconate. Moreover, the digestive utilization of iron was assessed by the apparent digestibility coefficient (ADC), as an index of the absorbed to ingested iron ratio. The results indicated that, there was no significant difference in ADC among rats fed on muffins prepared by wheat flour fortified with ferrous fumarate or $FeSo_4$ microencapsules. There was a significant decrease in ADC in rats fed on MWF+M ferrous gluconate or MWF+M ferrous carbonates. It could be noticed that the lowest ADC was observed in rats fed on basal diet or unfortified muffins.

The relative weight ratio of interior organs was affected with the iron absorption as shown in Table (5). There was no significant difference in relative weight ratio of kidney and heart in tested rat groups except rats fed on iron deficient basal diet or MWF. Relative weight ratio of liver and spleen had significant difference among tested rat groups fed on muffins prepared by wheat flour fortified with different microencapsules iron.

The data in Table (6) show that there was no significant difference in serum triglycerides, total cholesterol and HDL-cholesterol and VLDL-cholesterol among all tested rat groups. The serum LDL-cholesterol had a little significant difference. These results indicated that palm stearin has no effect in lipid profile of the tested rats.

The data in Table (7) show the haematological parameters of the experimental rat groups. There was no significant difference in blood WBC_s, RBC_s, haemoglobin, PVC% and PLT among rat groups fed on muffins prepared by wheat flour fortified with different encapsulated iron. There was a significant decrease in items of blood picture of rat groups fed on iron deficient basal diet or muffins without fortified iron.

Table (1): Chemical composition of muffins prepared by wheat flour fortified with different microencapsules of iron salts and iron content on dry weight basis

	Protein %	Fat %	Crude fiber %	Ash%	Total Carbohydrate%	Caloric value (Kcal/100 g.)	Fe content (mg/100g.)
1	10.06	17.51	0.28	1.14	71.06	482.07	0.58
2	10.32	17.25	0.24	1.38	70.81	478.77	6.33
3	9.45	16.92	0.24	1.29	72.10	478.48	6.51
4	9.81	17.47	0.23	1.31	71.18	481.19	6.56
5	10.12	17.31	0.21	1.35	71.01	480.31	6.43
6	10.09	16.85	0.25	1.38	71.43	477.69	6.38

1=MWF (Muffin with unfortified wheat flour) 2= MWF+M ferrous fumarate 3= MWF+M Fe Na EDTA
4= MWF+M ferrous gluconate 5= MWF+M ferrous carbonates 6= MWF+M ferrous sulphate

Table (2): Texture Profile Analysis (TPA) and weight loss of muffins prepared by wheat flour fortified with different microencapsules of iron salts

	Cohesiveness	Gumminess	Chewiness	Springiness	Resilience	Weight loss (g.)
1	0.71	3.83	3.16	0.82	0.58	16.48
2	0.68	4.17	3.34	0.74	0.51	14.59
3	0.69	3.67	3.50	0.75	0.56	14.09
4	0.56	3.54	3.14	0.69	0.58	13.75
5	0.72	3.33	4.84	0.77	0.50	15.28
6	0.69	4.08	3.13	0.77	0.52	15.11

1=MWF (Muffin with unfortified wheat flour) 2= MWF+M ferrous fumarate 3= MWF+M Fe Na EDTA
4= MWF+M ferrous gluconate 5= MWF+M ferrous carbonates 6= MWF+M ferrous sulphate

Table (3): Sensory evaluation of muffins prepared by wheat flour fortified with different microencapsules of iron salts

	Appearance	Color	Texture	Flavor	Sweetness	General acceptance
1	9.20±0.40 [ab]	8.93±0.50 [a]	9.00±0.40 [a]	8.87±0.64 [a]	8.73±0.23 [a]	44.73±1.33 [a]
2	8.73±0.11 [abc]	9.00±0.53 [a]	8.47±0.31 [a]	8.40±0.72 [a]	8.67±0.41 [a]	43.27±0.23 [a]
3	9.27±0.42 [a]	8.80±0.20 [a]	8.67±0.64 [a]	8.87±0.42 [a]	8.67±0.31 [a]	44.27±0.14 [a]
4	8.53±0.11 [c]	8.93±0.58 [a]	8.93±0.31 [a]	8.07±0.42 [a]	8.73±0.31 [a]	44.13±1.30 [a]
5	8.67±0.31 [bc]	9.00±0.72 [a]	8.67±0.31 [a]	8.27±0.70 [a]	8.67±0.31 [a]	43.27±1.50 [a]
6	8.43±0.31 [c]	8.67±0.64 [a]	8.80±0.53 [a]	8.60±0.35 [a]	8.87±0.31 [a]	43.53±1.92 [a]
LSD	0.5370	0.9851	0.7732	0.3548	0.6442	2.4565

Any two means have the same letter in the same column did not significantly different at $P \le 0.05$
1=MWF (Muffin with unfortified wheat flour) 2= MWF+M ferrous fumarate 3= MWF+M Fe Na EDTA
4= MWF+M ferrous gluconate 5= MWF+M ferrous carbonates 6= MWF+M ferrous sulphate

Table (4): Weight gain, daily feed intake and nutritive utilization of Fe of rats fed on muffins prepared by wheat flour fortified with different microencapsules of iron salts.

Rat groups	Initial weight(g.)	Weight after depletion	Final weight (g.)	Body weight gain (g)	Feed intake (g./day)	Fe intake (µg/day)	Fe fecal (µg/day)	Fe absorbed*	ADC**
1	105.27±3.16[a]	120.74 ±3.27[a]	139.28±4.16[b]	18.54±1.01 [b]	13.14±0.58[b]	68.35±3.02[c]	41.54±1.88 [e]	26.80±1.41 [c]	39.21± 0.92 [c]
2	105.68±5.30 [a]	120.71±5.54[a]	141.27±3.21[b]	20.23±1.90 [b]	12.31±1.19[b]	71.44±6.94 [c]	43.90±4.94 [e]	27.53±23.75 [c]	38.54± 3.07 [c]
3	105.21±2.51 [a]	120.20±2.26[a]	175.71±3.21[a]	55.51±2.63 [a]	15.94±0.32[a]	1009.02±20.09[ab]	270.16±26.28[cd]	738.86±26.85 [ab]	73.23±2.50 [a]
4	105.15±5.73 [a]	120.56±4.78[a]	181.00±3.25[a]	60.44±6.31 [a]	15.31±1.49[a]	996.02±95.50 [ab]	297.79±20.45[bc]	698.89±82.46 [ab]	70.03±1.95 [ab]
5	106.10±3.81 [a]	120.46±2.57[a]	177.58±4.94[a]	57.12±2.66 [a]	16.65±1.32[a]	1092.02±86.64 [a]	377.37±24.04 [a]	714.65±103.32 [ab]	65.23±4.36 [b]
6	105.30±4.34 [a]	120.10±2.59[a]	175.87±9.03[a]	55.78±11.31[a]	15.04±0.58[a]	967.29±37.36 [b]	324.14±24.32 [b]	643.15±60.95 [b]	66.39±3.85 [b]
7	105.36±2.61 [a]	121.53±2.99[a]	174.42±2.45[a]	52.88±4.44 [a]	16.04±0.67[a]	1023±42.75 [ab]	258.10±14.85 [d]	765.04±56.92 [a]	74.71±2.48 [a]
LSD (*P*≤0.05)	25.288	6.336	8.678	9.734	1.508	88.777	35.611	99.336	5.139

Any two means have the same letter in the same column did not significantly different at *P* ≤ 0.05
1- Iron deficient basal diet   2=MWF (Muffin with unfortified wheat flour)   3= MWF+M ferrous fumarate   4= MWF+M Fe Na EDTA
5= MWF+M ferrous gluconate   6= MWF+M ferrous carbonates   7= MWF+M ferrous sulphate
*Fe absorbed= Fe intake – Fe fecal   **ADC (apparent digestibility coefficient)

Table (5):Weights and relative ratio of rat organs fed on muffins prepared by wheat flour fortified with different microencapsules of iron salts.

Rat groups	Liver		Kidney		Spleen		Heart	
	Weight (g.)	Relative ratio	Weight (g.)	Relative ratio	Weight (g.)	Relative ratio	Weight (g.)	Relative ratio
1	3.50±0.09[c]	0.023±0.002[c]	0.84±0.04[b]	0.006±0.0065[b]	0.376±0.04 [c]	0.003±0.0029[c]	0.51±0.041[b]	0.004±0.0038[b]
2	3.62±0.41[c]	0.026±0.003[c]	0.87±0.05[b]	0.006±0.0069[b]	0.400±0.03[c]	0.004±0.0030[bc]	0.53±0.041[b]	0.004±0.0039[b]
3	6.90±0.20[a]	0.039±0.001[a]	1.47±0.06[a]	0.008±0.0086[a]	0.691±0.05 [a]	0.004±0.0042[a]	0.85±0.089[a]	0.005±0.0053[a]
4	6.79±0.64[a]	0.037±0.004[ab]	1.51±0.10[a]	0.008±0.0087[a]	0.663±0.04[a]	0.004±0.0039[a]	0.83±0.054[a]	0.005±0.0049[a]
5	5.97±0.06[b]	0.033±0.001[b]	1.42±0.11[a]	0.007±0.0083[a]	0.563±0.08[b]	0.003±0.0036[b]	0.80±0.033[a]	0.005±0.0048[a]
6	6.31±0.57[ab]	0.035±0.003[ab]	1.38±0.03[a]	0.007±0.0083[a]	0.664±0.01[a]	0.004±0.0039[a]	0.80±0.081[a]	0.005±0.0045[a]
7	6.70±0.17[a]	0.038±0.001[a]	1.49±0.10[a]	0.008±0.0091[a]	0.684±0.04[a]	0.004±0.0041[a]	0.81±0.017[a]	0.005±0.0047[a]
LSD (*P* ≤0.05)	0.6686	0.0043	0.1349	0.0008	0.0811	0.00004	0.0986	0.00004

Any two means have the same letter in the same column did not significantly different at *P* ≤ 0.05
1- Iron deficient basal diet   2=MWF (Muffin with unfortified wheat flour)   3= MWF+M ferrous fumarate   4= MWF+M Fe Na EDTA
5= MWF+M ferrous gluconate   6= MWF+M ferrous carbonates   7= MWF+M ferrous sulphate

Table (6): Lipid profile of rats fed on muffins prepared by wheat flour fortified with different microencapsules of iron salts

Rat groups	Triglycerides (mg/dl)	T.cholesterol (mg/dl)	HDL-cholesterol (mg/dl)	LDL-cholesterol (mg/dl)	VLDL-cholesterol (mg/dl)
1	97.70±7.28 [a]	62.83±3.84 [a]	25.19±2.31 [a]	17.69±0.47 [ab]	19.54±1.45 [a]
2	95.99±5.74 [a]	61.99±2.94 [a]	24.93±1.28 [a]	17.88±0.54 [ab]	19.20±1.15 [a]
3	100.19±6.80 [a]	64.81±4.28 [a]	25.77±1.72 [a]	19.00±1.29 [ab]	20.04±1.36 [a]
4	97.26±3.70 [a]	63.30±2.88 [a]	25.71±1.15 [a]	18.12±1.03 [ab]	19.45±0.74 [a]
5	98.22±8.11 [a]	61.67±3.81 [a]	23.93±0.70 [a]	17.37±1.25 [b]	19.64±1.62 [a]
6	97.27±7.83 [a]	62.58±4.04 [a]	24.94±1.53 [a]	18.18±1.24 [ab]	19.46±1.56 [a]
7	97.60±5.83 [a]	63.10±2.70 [a]	23.92±0.51 [a]	19.63±1.66 [a]	19.54±1.14 [a]
LSD (*P* <0.05)	11.600	6.215	2.510	2.003	2.3119

Any two means have the same letter in the same column did not significantly different at *P* ≤ 0.05
1- Iron deficient basal diet   2=MWF (Muffin with unfortified wheat flour)   3= MWF+M ferrous fumarate   4= MWF+M Fe Na EDTA
5= MWF+M ferrous gluconate   6= MWF+M ferrous carbonates   7= MWF+M ferrous sulphate

Table (7): Haematological parameters of rats fed on muffins prepared by wheat flour fortified with different microencapsules of iron salts

Rat groups	WBC x10³/ul	RBC$_s$x10⁶/ul	Hemoglobin g/dl	PVC%	PLT x10³/ul
1	6.37±0.60 [b]	4.52±0.52 [b]	7.22±0.31 [b]	19.90±1.87 [b]	143±3.61 [b]
2	6.86±0.85 [b]	4.44±0.41 [b]	7.43±0.59 [b]	19.88±1.32 [b]	149±5.86 [b]
3	11.96±0.59 [a]	8.00±0.56 [a]	13.88±1.19 [a]	40.22±2.67 [a]	396±28.88 [a]
4	11.97±0.79 [a]	8.15±0.42 [a]	12.60±0.96 [a]	38.08±4.03 [a]	264±19.42 [a]
5	11.62±0.78 [a]	7.61±0.57 [a]	12.53±1.01 [a]	38.17±2.10 [a]	393±15.18 [a]
6	11.40±0.61 [a]	7.93±0.44 [a]	12.70±0.89 [a]	37.40±1.72 [a]	391±35.50 [a]
7	12.38±0.57 [a]	7.63±0.84 [a]	13.79±1.44 [a]	39.71±4.32 [a]	404±9.45 [a]
LSD (*P* <0.05)	1.217	0.973	1.693	4.893	37.372

Any two means have the same letter in the same column did not significantly different at *P* ≤ 0.05
1- Iron deficient basal diet   2=MWF (Muffin with unfortified wheat flour)   3= MWF+M ferrous fumarate   4= MWF+M Fe Na EDTA   5= MWF+M
ferrous gluconate   6= MWF+M ferrous carbonates   7= MWF+M ferrous sulphate

Table (8): Iron absorption of muffins prepared by wheat flour fortified with different microencapsules of iron salts

Rat groups	Serum iron(µ/dl)	TIBC (µ/dl)	Transferin Sat. (%)	Fe liver (µg/g)	Fe kidney (µg/g)	Fe bone (µg/g)	Fe heart (µg/g)	Fe spleen (µg/g)
1	6.66±0.31 [b]	21.33±0.75 [b]	31.30±2.52 [c]	80.99±3.98 [e]	40.49±2.34 [c]	137.51±8.88 [d]	40.54±3.47 [d]	211.59±4.14 [c]
2	6.34±0.74 [b]	20.00±0.43 [b]	31.64±3.08 [c]	93.61±1.29 [d]	58.08±3.61 [b]	165.47±7.77 [c]	60.04±2.46 [c]	258.87±4.53 [b]
3	12.95±0.53 [a]	25.14±1.08 [a]	51.57±2.59 [a]	172.10±3.03 [a]	88.90±4.17 [a]	288.28±8.17 [a]	115.38±4.27 [ab]	464.84±4.18 [a]
4	13.16±0.34 [a]	26.34±0.63 [a]	49.93±2.47 [ab]	168.43±2.90 [a]	86.57±2.74 [a]	278.38±5.78 [ab]	106.04±4.71 [b]	461.53±8.75 [a]
5	12.06±1.07 [a]	25.64±2.43 [a]	47.07±1.31 [b]	161.29±3.07 [b]	84.53±1.50 [a]	271.00±6.41 [b]	118.41±7.74 [a]	460.63±6.46 [a]
6	12.23±0.50 [a]	26.37±0.85 [a]	46.38±0.65 [b]	155.66±2.35 [c]	84.10±2.52 [a]	265.37±5.80 [b]	108.43±6.67 [ab]	463.23±2.28 [a]
7	12.36±0.25 [a]	24.88±1.20 [a]	49.73±2.22 [ab]	169.09±4.25 [a]	88.87±3.07 [a]	288.48±9.17 [a]	109.51±6.10 [ab]	463.30±6.16 [a]
LSD (*P* <0.05)	1.044	2.136	3.954	5.464	5.198	13.210	9.363	9.739

Any two means have the same letter in the same column did not significantly different at *P* ≤ 0.05
1- Iron deficient basal diet   2=MWF (Muffin with unfortified wheat flour)   3= MWF+M ferrous fumarate   4= MWF+M Fe Na EDTA
5= MWF+M ferrous gluconate   6= MWF+M ferrous carbonates   7= MWF+M ferrous

Table (8) illustrates that rat groups fed on muffins prepared by wheat flour fortified with different iron microencapsules had no significant effect on serum iron, TIBC, Fe kidney and Fe spleen. There was a fairly significant difference in transferin saturation, Fe liver, Fe bone and Fe heart of the tested rat groups fed on muffins prepared from wheat flour fortified with different iron microencapsules. Generally, rats fed on iron deficient basal diet or MWF had significant lower values in all measured parameters. The results point out that MWF+M ferrous fumarate had the highest value of transferin saturation followed by MWF+M Fe Na EDTA and MWF+M ferrous sulphate.

4. DISCUSSION

The obtained results indicated that gross chemical composition showed no difference among tested muffins except for the iron content. It was increased in muffins prepared from wheat flour with different microencapsules of iron salts compared with the MWF. Martinez-Cervera *et al.*(2011) cited that these ingredients of muffins were suitable to obtain muffins with good characters. Muffins are characterised by a typical porous structure and high volume which confer a spongy texture. To obtain such a final structure, a stable batter lodging many tiny air bubbles is required. The Texture Profile Analysis (TPA) parameters were not affected with fortified wheat flour with microencapsules of Fe sources. Chewiness is a secondary texture parameter that is associated with difficulty in chewing the sample and forming a bolus before swallowing (Baixauli *et al.,*2008). Moreover, Kiskini *et al.* (2012) showed that microencapsulated forms are not expected to bring about any negative effects on physical and sensory quality of gluten free bread. Sensory evaluation, one of the most important parameters to be taken into account in food fortification is the no change of the sensorial properties in fortified food. Several iron compounds with high bioavailability, in general, produce modifications on the taste and color of the fortified foods or they might produce rancidity because of fat oxidation (Yao *et al.,*2010). In this case, it could be noticed that the sensorial properties of the fortified muffins were not affected after the addition of the microencapsules of Fe. The main target of microencapsulation process is to prevent any harmful effects on sensory parameters from the fortified materials (Diosady *et al.,*2002). Therefore, microencapsules of Fe had a good effect in sensory properties of fortified muffins.

Regarding the body weight gain and feed intake, it could be noticed that addition of Fe microencapsules in the muffins improved body weight and feed intake. There was a fairly increase in body weight of rats fed on iron deficient basal diet or MWF. Iron deficiency was accompanied with a decrease in weight gain and feed intake in rats. This result is in agreement with Strube *et al.,*2002, Hernandez *et al.,*2003 and Soliman *et al.,*2010. In contrast, the present data show that no significant difference was noticed in body weight gain of rats fed on different muffins prepared by wheat flour fortified with different microencapsules of iron. The supplement of muffins with iron improved the weight gain of anemic rats. These results are in agreement with those reported by Salgueiro *et al.,*2005 and Lobo *et al.,*2011). As described by Windisch (2002), the regulation of iron homeostasis occurs mainly at the digestive level, with hardly any renal excretion of this mineral taking place. Therefore, taking these facts into consideration, iron absorption would be a term synonymous with bioavailability. These results demonstrate that the muffins prepared by wheat flour fortified with microencapsules of ferrous fumarate, $FeSo_4$ and MWF+M Fe Na EDTA had the highest ADC. Iron compounds have different effects on Fe utilization. Hernandez *et al.* (2003) reported that iron compounds must be selected to fortify wheat flour when intended for public nutrition programs. Iron utilization was greater in rats fed on wheat flour supplemented with ferrous sulfate, followed by fumarate and citrate than in rats fed on reduced iron. Moreover, Hernandez *et al.*(2006) cited that iron bioavailability indicators were best in rats fed corn tortillas fortified with ferrous sulfate and NaFe(III)EDTA than in those fed unfortified corn tortillas.

The data in Table (5) illustrate that there was significantly higher relative weight ratios in liver, kidney and heart of rats fed on different muffins prepared by wheat flour fortified with different microencapsules of iron than rats fed on iron deficient basal diet or MWF. It was clear that iron maintained the normal weight of organs. Roodenburg *et al.*(1995) stated that weights of liver, spleen, heart and kidney were significantly higher in groups of rats fed on diets with iron, vitamin A and vitamin C fortification. Meanwhile, organ weights were lower in rat groups that received no dietary vitamin A, vitamin C and any source of iron (Kelleher and Lonnerdal, 2005).

Palm stearin (PS) is the solid fraction obtained by controlled temperature fractionation, and the liquid fraction is known as palm olein. PS can be used as a source of fully natural hard component in the manufacture of edible fat products, such as margarine and shortening. The data in Table (6) clearly indicate that there were no significant difference in parameters of serum lipid profile among all tested rat groups. This proves that palm stearin had no effect on the serum triglycerides, total cholesterol and HDL-cholesterol and VLDL-cholesterol in the tested rats. It may be related to palm stearin that could be considered a negligible competent compared to the whole weight of muffin ingredient.

Formation of hemoglobin happens to be the chief function of iron. Not only that, being a part of hemoglobin, it gives the dark red shade to the blood and also aids in transporting oxygen to the body cells (Knutson and Wessling-Resnick 2003). All the haematological parameters studied, WBC_s, RBC_s, haemoglobin, PVC and PLT were within normal limits for the rats fed on muffins prepared by wheat flour fortified with different encapsulated iron at the end of the experimental period. After consuming iron deficient basal diet or muffins without fortified iron the rats were anaemic and the haematological parameters decreased under the normal limits. These results are in agreement with those reported by Alferez *et al.,*2006 and Lobo *et al.,*2011.

The source of iron is either from food supply (heme) or free iron from other sources. Kinds of iron are processed by the gut (stomach and intestine) where they are converted to a form of iron readily used by the body. Finally, the iron winds up in the intestinal epithelial cells, ready for export to red blood cells, muscle tissue and organs (Vulpe *et al.,* 1999). The data in Table (8) clarify that fortification of wheat flour with the microcapsules of different iron sources had a positive effect to improve the absorbability of iron and treat the effect of depletion period of iron. Fe concentrations decreased in the serum and all the organs of anemic rats were analyzed, compared with tested rat groups fed on muffins prepared by wheat flour fortified with different encapsulated iron (diets supplied with iron). Similar findings were reported by other authors (Reeves *et al.,*2005; Alferez *et al.,*2006). Moreover, Urbano *et al.* (2007) cited that the Fe content in heart, kidney or femur did not maintain any correlation with the intestinal absorption of this mineral.

In summary, new microencapsulation techniques continuously emerge and the growing market for functional foods is the major driving force behind innovation in food technology field (Fang *et al.,*2005). Spray drying, freeze drying, air-suspension coating, and extrusion are considered methods to produce microcapsules (Gharsallaoui *et al.,*2007). Spray drying was chosen for the production of the microencapsules because of the desirable coating characteristics produced by the technique and because spray drying is currently the least expensive encapsulation technology (Gouin, 2004). Palm stearin was chosen as the coating material because of its high melting point (63°C), which may allow the sprayed capsules to resist high temperatures encountered during manufacture, storage, transport and because of its hydrophobic properties, which prevent the entrance of water and reduce reactions between different core of microcapsule component, (Dary and Mora, 2002).

Several factors in the diet can influence the mineral bioavailability, the magnitude of which depends on inhibitors and promoters in a meal, and hence on the food matrix (Gibson, 2007).The presence of ascorbic acid in the microencapsules helps to increase absorbability and bioavailability of iron. Lee *et al.* (2003) showed that serum iron content and transferring saturation increased dramatically when subjects consumed milk containing both encapsulated iron and encapsulated ascorbic acid compared with those when consumed uncapsulated iron or encapsulated iron without ascorbic acid. Moreover, ascorbic acid is capable of reducing Fe (III) to Fe (II), (Wienk *et al.,*1999) and it is used as an absorption enhancer (Hurrell, 2002). Moreover, the source of iron had an effect on bioavailability of iron. All iron supplements are not the same. Ferrous iron is much better absorbed than ferric iron (Davidsson *et al.,* 2000). The most common form of iron supplement is ferrous sulfate, but it is known to produce intestinal side effects such as constipation, nausea, and bloating in many users. Some forms of ferrous sulfate are enteric-coated to delay tablet dissolving and prevent some side effects, but enteric-coated iron may not be absorbed as well as iron from standard supplements. Ferrous iron is the form that is mostly used for correction of iron deficiency. About 3–5% of the iron present in alimentary canal in ferrous form is absorbed (Kurtoglu *et al.,* 2003). Other forms of iron supplements, such as ferrous fumarate, ferrous gluconate, and iron glycine amino acid chelate (Fox *et al.,* 1998) are readily absorbed and less likely to cause intestinal side effects. In order to overcome the problems

(side effects) created from using tradition treatment of anemia, they tried to use iron chelated amino acids. Therefore, Salgueiro *et al.*(2005) illustrated that biological behavior of ferrous gluconate does not differ significantly from the reference standard (iron sulphate). Meanwhile, NaFeEDTA is a suitable fortificant for improving iron-deficiency status in developing countries (Igarashi *et al.,*2006). It is noteworthy the foods (infant formula, cereal, sugar and fish sauce) were consumed with an enhancer of iron absorption (EDTA) added to overcome absorption inhibitors (Hurrell, 2002), but the fortification occurred unacceptable change in foods.

Generally, microencapsules of iron have a fat coating which protects them from chemically reacting with unsaturated fats in the flour or meal, and be degraded by lipases in the gut so that the ferrous salt is available for absorption. The microencapsules of iron may have a large particle size causing them to be removed from flour during final sifting. But preliminary studies have shown them to be well absorbed, even with high extraction flour, and costs may drop if their use becomes more prevalent.

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التقييم البيولوجى للكيك (الموفينية) المدعم بالحديد فى الصوره الكبسوليه

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ملخص

كان الهدف من الدراسه هو محاوله التخلص من التغيرات فى النكهه و اللون و التزنخ و غيرها من التغيرات غير المرغوبه الناتجه عن تدعيم الأغذيه بأملاح الحديد. لذلك تم أعداد أملاح الحديد العاليه فى الاتاحه الحيويه فى صوره كبسوليه لحمايه الغذاء المدعم من هذه الصفات غير المرغوبه. تم تغطيه أملاح الحديد وهى

</div>

(ferrous fumarate, Fe Na EDTA, ferrous glouconate, ferrous citrate, ferrous carbonates and ferrous sulphate anhydrous)

بأستيارين النخيل فى وجود الليسيسين و حمض الأسكوربيك. و حتى يتم إختبار أملاح الحديد المكبسله فقد تم تدعيم الدقيق (استخلاص 72%) بأملاح الحديد المكبسله المختلفه و استخدام الدقيق المدعم فى أعداد الكيك   . أوضحت النتائج عدم وجود تأثير على التركيب الكيميائى و ال Texture Profile Analysis (TPA) وفقد الوزن و الخصائص الحسيه للكيك المعد من الدقيق المدعم بأملاح الحديد المكبسله مقارنه بالكيك المعد من الدقيق غير المدعم. عدا محتوى الحديد الذى زاد فى الكيك المعد من الدقيق المدعم بأملاح الحديد المكبسله. اوضح التقييم البيولوجى حدوث زياده معنويه فى أوزان أجسام الفئران؛ و ال apparent digestibility coefficient (ADC) و الوزن النسبى لأوزان الكبد و الكليه و الطحال و ال hematological parameters والسيرم فى الحديد و ال- binding capacity total iron(TIBC) والحديد فى الكليه و الطحال مقارنه بالفئران المغذاه بالكيك المعد من الدقيق غير المدعم و الفئران المغذاه على وجبه خاليه من الحديد. لم يكن هناك اختلاف فى لبيدات السيرم بين مجاميع الفئران المختلفه. تدل النتائج على أن عمليه الكبسله لأملاح الحديد لها أثر ايجابى فى معالجه الصفات غير المرغوبه للمنتجات المدعمه بالحديد و لا يكون لها أثر على إمتصاص الحديد.