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Iron Bioavailability of Wheat Biscuit Supplemented by Fenugreek Seed Flour

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Abstract: Biscuits produced from wheat flour replaced by different levels (5, 10, 15 and 20%) of soaked or germinated fenugreek seed flours (FSF) were evaluated for sensory characteristics and some minerals content. Investigation of bioavailability for FSF-biscuits iron was the major target of this study. The sensory results showed that a maximum 10% FSF can be incorporated to prepare acceptable quality biscuits. Results of chemical analysis indicated that incorporation of FSF into biscuits formula obviously increased ($P<0.05$) Fe, Ca and Zn contents with increasing FSF level as compared to wheat biscuit (control). Addition of soaked or germinated FSF to wheat flour decreased markedly phytic acid content of produced biscuits by ranges between 20 to 44%. Regarding the biological trials, rat groups were made anemic through feeding on Fe-deficient diet for 10 days duration followed by 20 days feeding on wheat biscuit and FSF-biscuit diets. The results indicated that rats fed on FSF-biscuit diets showed a good hematological response. Whereas, rats exhibited extremely higher ($P<0.05$) values of blood hemoglobin (Hb), hematocrite (Hct), serum iron, liver weights and liver minerals content (Fe, Ca and Zn) as compared to rats fed on wheat biscuit diet. The biscuit diet containing 10% germinated FSF recorded the highest values of studied blood criteria among all tested diets. It could be concluded that, using of 10% germinated FSF into wheat biscuit formula considerably improved bioavailability of FSF-biscuit iron.

Key words: Biscuit · Fenugreek seeds · Sensory characteristics · Iron bioavailability

INTRODUCTION

Iron deficiency is the most common nutritional disorder in the world. It is estimated that as many as 3.5 billion people could be affected , leading to an important public health problem in both developed and developing countries [1,2]. According to WHO, around 0.8 million deaths can be attributed to iron deficiency each year. In terms of the loss of healthy life, expressed in disability-adjusted life years (DALYs), iron-deficiency anemia results in 25 million DALYs lost [3]. Subclinical iron deficiency resulting in functional disadvantages is as widespread as iron deficiency with anemia [4], growth retardation, low birth weight, increased prenatal mortality, increased maternal morbidity and mortality [5]. About one-fifth of prenatal mortality and one-tenth of maternal mortality in developing countries is attributable to iron deficiency [6]. Iron-deficiency anemia is also a serious public health problem in all countries comprising the Eastern Mediterranean Region, Middle East and North Africa Region [7].

The quality of biscuits depends on quantity and quality of ingredients, especially the flour. It was found that mixing two or more different materials help to solve the deficiency problem of cereals as low nutritional value by used legumes as high nutritive source [8]. Fenugreek seeds have high contents of I ron (21 mg/100g), calcium (182 mg/100g), zinc (4.9 mg/100g) and other ingredients [9]. Additionally, it also possesses hypocholesterolemic and hypoglycemic properties [10]. Hence, development and consumption of such therapeutic bakery products would help to raise the nutritional status of population [11]. Information on incorporation of fenugreek seed flour in bakery products is scanty. Therefore, this study was designed to study the effect of replacement wheat flour by 5, 10, 15 and 20% of both soaked and germinated fenugreek seed flours on the sensory characteristics and some minerals content of produced biscuits. Also, evaluation of iron bioavailability for final products through rat feeding trials was another target.

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MATERIALS AND METHODS

Materials: Fenugreek seeds were obtained from Agriculture Research Center, Giza, Egypt. Wheat flour (72% extraction) and other ingredients were obtained from the local market. Ingredients used in processing of biscuits included 65.1% wheat flour or blends, 21.4% sugar, 9.3% shortening (palm oil), 0.93% skimmed milk powder, 1.86% high fructose, 0.37% sodium bicarbonate, 1.02% ammonium bicarbonate, 0.02% vanilla and required amount of water.

Methods

Experimental Treatments:

Preparation of Fenugreek Seed flour (FSF): Fenugreek seeds were cleaned and freed of broken seeds, dust and other foreign materials and divided into two equal parts: the first part was soaked, while the second part was germinated as following:

Soaking: The seeds were soaked in tap water for 12 h at 37°C. with a seeds: water ratio of 1:5 (w/v) was used. The unimpeded water was discarded and the soaked seeds were rinsed twice by distilled water.

Germination: The soaked seeds were germinated in sterile Petri dishes lined with wet filter papers for 48 h at 37°C, with frequent watering. The sprouts were rinsed by distilled water.

The soaked and germinated seeds were dried in an oven at 55-60°C for 10-12 hr. The dried seeds were ground to particles passing through 20 mesh sieve which is similar to the size of wheat flour as described by Shalini and Sudesh [8].

Preparation of Wheat Flour-fenugreek Seed Flour Blends: Wheat flour was supplemented by 5, 10, 15 or 20% of either soaked or germinated FSF. The flour mixtures were individually blended and homogenized, packed in polyethylene bags, tightly closed and stored at room temperature until utilized.

Processing of Biscuits: Fat and sucrose were firstly creamed by using the mechanical mixer for 10 min. bicarbonate and ammonium bicarbonate were dissolved in a part of water and added to the prepared creamed mixture, then high fructose was added. As creaming process was continued, flour, skimmed milk powder and vanilla were added and stirred well together. The full prepared dough was laminated, sheeted, extruded, molded and formed to the required form. The formed biscuits were baked at 230°C for 7 min. according to A.A.C.C. [12]. After cooling for 30 min. biscuits were packaged in cellophane and subjected for sensory evaluation.

Analytical Methods

Sensory Evaluation of Biscuits: The organoleptic characteristics of wheat biscuit (control) and FSF-biscuits were evaluated by using a taste panel, consisting of 20 judges. The panelists were asked to evaluate color, appearance, taste, flavor, texture and overall acceptability. The ratings were on a 9-point hedonic scale, ranging from 9 as like extremely to 1 as dislike extremely as outlined by Austin and Ram [13].

Chemical Analysis: Iron, calcium and zinc contents were determined in tested biscuits and rat livers by atomic absorption (Perkin-Elmer-Crop, Norwalk, model 560) according to A.O.A.C. [14]. Phytic acid of biscuits was extracted and estimated as described by Camir and Clydesdale [15].

Iron Bioavailability Determination: White weanling male rats (with weighing average 70 g) were housed individually in mesh-bottom stainless steel cages in a controlled environment. To improve iron absorption, all rats were first made anemic through feeding on Fe-deficient diet, prepared as reported by Ranhotra and Gelroth [16], for 10 days . Blood hemoglobin (Hb contents dropped from around13 to 17g/dl At this point, rats were randomly assigned to test diets (six rats for each diet) for iron repletion (Hb regeneration) studies which lasted 20 days. Body weight and diet intakes were recorded. Blood criteria measurements were made at 5 days intervals for 20 days. Diet and distilled deionized water were supplied ad libitum throughout the study. On day 20, blood was withdrawn by heart puncture from anesthetized rats which were sacrificed while livers were removed and frozen until using for determination of Fe, Ca and Zn contents by atomic spectrophotometry.

Blood Hemoglobin (Hb) Determination: Blood samples were withdrawn from the eye orbital plexus by heparinized capillary tubes. 0.02 ml blood sample was placed in a test tube containing 5 ml Drabkins solution. The tube was left at room temperature for 10 min. The developed color was calorimetrically measured using spectrophotometer (Spekol 11 No. 849101) at 548 nm as described by Betk and Savelsberg [17].

Iron Content of Hb: It was calculated as described by Hernandez *et al.* [18] as follows:

$$\text{Fe Hb (mg)} = \text{body weight (g)} \times \text{Hb (g/L)} \times 6.7 \times 0.335/10000$$

Hematocrite %: Blood samples were taken with a micro capillary tube and centrifuged at 5000 r.p.m. for 5 min. The volume of blood cells was measured using a graded scale.

Serum Iron Content: Serum iron of rats was determined by spectrophotometer (Spekol 11 No. 849101) as described in A.O.A.C. [14].

Statistical Analysis: The original sensory panel data and other results were statistically analyzed using analysis of variance (ANOVA) and least significance difference (LSD) at a significance of probability 5 % [19].

RESULTS

Sensory Characteristics of Biscuits Containing FSF: Biscuits supplemented by different levels of soaked or germinated FSF were sensory evaluated and compared with wheat biscuit as shown in Table 1. Data indicated that there were no significant differences among control sample and biscuits containing 5 or 10% FSF in all sensory properties. But biscuits containing 15 or 20% FSF were significantly different ($P<0.05$) in all properties and were unacceptable to panelists as compared to the other samples. Therefore, it was excluded from this study. Whereas overall acceptability scores for these samples were ranged from 4.69 to 5.72, against 8.37 of control biscuit.

Mineral and Phytic Acid Contents of Biscuits Containing FSF: Fe, Ca and Zn contents of control and FSF-biscuits are presented in Table 2. Wheat biscuit had low Fe (4.65 mg/100g), Ca (36.23 mg/100g) and Zn (2.24mg/100g) contents. These contents obviously increased ($P<0.05$) with increasing FSF level in wheat flour. Biscuit containing 10% germinated FSF exhibited higher contents of Fe (7.81), Ca (47.89) and Zn (3.19 mg/100g) than other biscuit samples. On the other hand, data in Table 2 indicated that the wheat biscuit had 175 mg/100g phytic acid and this decreased markedly ($P<0.05$) with rise FSF levels. The maximum decrease (44%) in phytic acid content was found in biscuit containing 10% germinated FSF, while minimum decrease (20%) was found in biscuit containing 5% soaked FSF as shown in Fig. 1.

Table 1: Sensory characteristics* of biscuits supplemented by soaked or germinated fenugreek seed flours

Substitution level (%)	Organoleptic properties					
	Color	Appearance	Flavor	Taste	Texture	Overall acceptability
Control	8.00^{a}	8.12^{a}	8.50^{a}	8.89^{a}	8.22^{a}	8.37^{a}
W : SF						
95 : 5	7.72^{a}	7.89^{a}	7.95^{a}	7.98^{a}	7.61^{a}	7.79^{a}
90 : 10	7.50^{a}	7.68^{a}	7.62^{a}	7.54^{a}	7.45^{a}	7.63^{a}
85 : 15	6.32^{b}	6.35^{b}	5.23^{b}	4.32^{b}	5.82^{b}	5.58^{b}
80 : 20	5.18^{c}	5.68^{c}	4.51^{c}	3.21^{c}	4.58^{c}	4.69^{c}
W : GF						
95 : 5	7.89^{a}	7.97^{a}	7.98^{a}	8.12^{a}	7.69^{a}	7.86^{a}
90 : 10	7.66^{a}	7.73^{a}	7.71^{a}	7.67^{a}	7.53^{a}	7.69^{a}
85 : 15	6.45^{b}	6.42^{b}	5.34^{b}	4.57^{b}	5.90^{b}	5.72^{b}
80 : 20	5.31^{c}	5.55^{c}	4.78^{c}	3.29^{c}	4.67^{c}	4.76^{c}

*Scores were: 9 = like extremely to 1 = dislike extremely

W: wheat flour - SF: soaked fenugreek - GF: germinated fenugreek

^{a},^{b}and^{c} means in the same column with different superscripts are different significantly (p● 0.05)

Table 2: Mineral and phytic acid content of biscuits supplemented by soaked or germinated fenugreek seed flours

Mineral content (mg/100g)	Control	Substitution level (%): SF 5%	SF 10%	GF 5%	GF 10%
Iron	4.65^{b}	7.15^{a}	7.44^{a}	7.42^{a}	7.81^{a}
Calcium	36.23^{c}	41.37^{b}	43.96^{b}	44.24^{b}	47.89^{a}
Zinc	2.24^{c}	2.67^{b}	2.93^{b}	2.81^{b}	3.19^{a}
Phytic acid	175^{a}	140^{b}	128^{b}	112^{c}	98^{c}

^{a},^{b}and^{c} means in the same raw with different superscripts are different significantly (p● 0.05)

Table 3: Hemoglobin regeneration% in rats fed on Fe-deficient and Fe-intrinsic diets during two feeding periods*

	Rats hemoglobin (g/dl)						
	Initial	Fe-deficient diets	Fe-intrinsic diets				
Substitution level (%)	Zero day	Day 10	Day 15	Day 20	Day 25	Day 30	Hb regeneration (%)
Control	13.12[a]	7.09	10.45[c]	10.59[c]	11.21[c]	11.35[c]	60.08
W:SF							
95:5	12.87[c]	7.10	11.39[c]	12.36[c]	13.89[b]	14.21[b]	100.14
90:10	12.91[c]	7.13	11.92[c]	12.51[c]	13.95[b]	14.96[a]	109.82
W:GF							
95:5	13.00[c]	7.17	11.97[c]	12.52[c]	14.11[b]	15.21[a]	112.13
90:10	12.95[c]	7.11	12.21[c]	12.93[c]	14.27[b]	15.56[a]	118.85

*Feeding periods were 10 days of fed on Fe-deficient diet and 20 days on biscuit (Fe-intrinsic) diets

[a],[b]and[c] means in the same raw with different superscripts are different significantly (p● 0.05)

Table 4: Bioavailability% of intrinsic iron in anemic rats fed on different biscuit diets

Substitution level (%)	Diet intake (g)	Fe in diet (mg/100 g)	Fe-intake (mg)	Fe-Hb gain (mg)	Fe-intrinsic bioavailability* (%)
Control	235	4.92[c]	11.56[c]	2.94[c]	25.43
W:SF					
95:5	225	5.19[c]	11.67[c]	3.29[c]	28.19
90:10	239	5.34[b]	12.76[a]	4.79[b]	37.54
W:GF					
95:5	216	5.39[b]	11.64[c]	4.81[b]	41.32
90:10	210	5.78[a]	12.13[b]	5.68[a]	46.83

*Intrinsic iron bioavailability % = [gain in Hb iron (mg) / iron intake (mg) x 100]

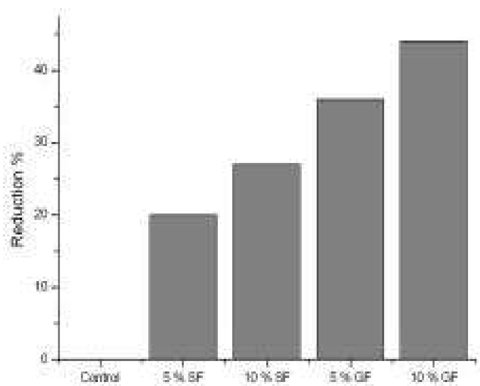

Fig. 1: Reduction % of phytic acid in different biscuit samples

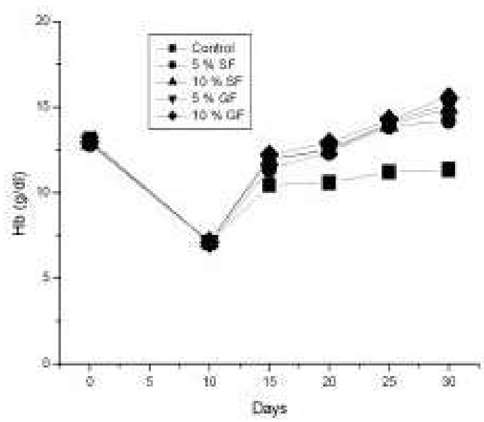

Fig. 2: Changes in blood Hb contents of rats fed on Fe-deficient and biscuit diets

Bioavailability of FSF-biscuits Iron: Results presented in Table 3 and illustrated in Fig. 2 shows rat blood hemoglobin (Hb) levels initially and after feeding on Fe-deficient diet followed by biscuit diets. After feeding on Fe-deficient diet, Hb contents dropped from around 13 to 7.1g/dl in all rats. Hb content was obviously increased ($P<0.05$) to contents s ranged between 14.21 to 15.56 g/dl after feeding the anemic rats on FSF-biscuit diets, while it was 11.35 g/dl in rats fed on wheat biscuit diet with Hb regeneration of 60.08%. While, the maximum Hb regeneration% was achieved by rats fed on biscuit diet containing 10% germinated FSF (118.85 %).

The per cent bioavailability of intrinsic iron in biscuit diets are presented in Table 4. Rats fed on biscuit diet containing10% germinated FSF proved to have maximum Fe-bioavailability (46.83%). While, the lower Fe-bioavailability (25.43%) was observed in rats fed on wheat biscuit diet. Rat groups fed on the other biscuit diets gave Fe-bioavailability between these two per cents.

Table 5 and Fig. 3 shows blood hematocrite % of rat groups fed on test diets. Data indicate the decrease of Hct values after feeding on Fe-deficient diet and it increased after feeding on biscuit diets by different levels.

Table 5: Blood Hematocrite % in rats fed on Fe-deficient and Fe-intrinsic diets during two feeding periods

	Rats hematocrite (%)					
	Initial	Fe-deficient diets	Fe-intrinsic diets			
Substitution level (%)	Zero day	Day 10	Day 15	Day 20	Day 25	Day 30
Control	40	30	32	35	38	44
W:SF						
95:5	39	32	36	41	46	50
90:10	40	31	37	45	49	52
W:GF						
95:5	41	31	37	44	49	54
90:10	40	32	39	49	53	57

Table 6: Serum iron of rats fed on Fe-deficient and Fe-intrinsic diets during two feeding periods

	Serum iron (mg/dl)						
	Initial	Fe-deficient diets	Fe-intrinsic diets				
Substitution level (%)	Zero day	Day 10	Day 15	Day 20	Day 25	Day 30	Regeneration (%)
Control	10.0	5.6	6.5[c]	6.8[c]	7.9[c]	8.7[c]	55.4
W:SF							
95:5	10.0	5.4	7.5[b]	8.4[b]	8.8[b]	9.9[b]	83.3
90:10	10.2	5.5	8.2[a]	8.8[a]	9.4[a]	10.5[a]	90.9
W:GF							
95:5	10.1	5.5	7.7[b]	8.5[b]	8.9[b]	10.2[a]	85.5
90:10	10.2	5.3	8.3[a]	9.0[a]	9.6[a]	10.6[a]	100

[a],[b] and[c] means in the same column with different superscripts are different significantly (po 0.05)

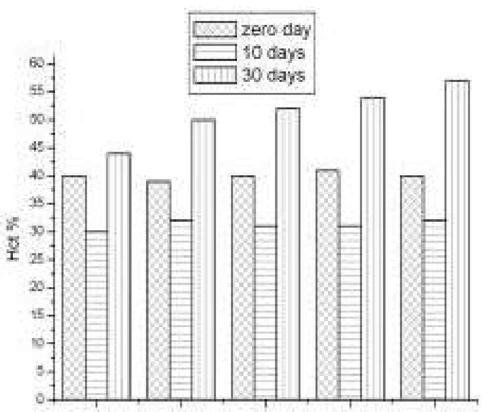

Fig. 3: Blood hematocrite % of rats fed on Fe-deficient and biscuit diets

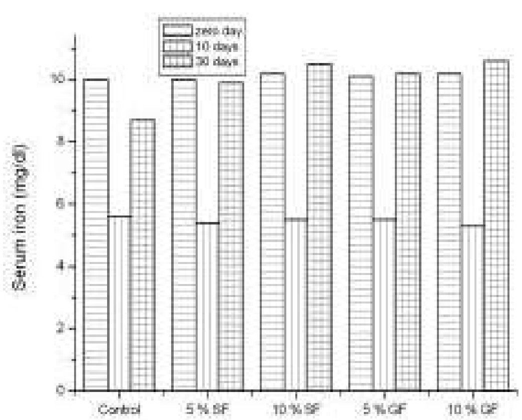

Fig. 4: Serum iron (mg/dl) of rats fed on Fe-deficient and biscuit diets

The maximum Hct% was occurred in rats fed on biscuit diet containing 10% germinated FSF (57%), while was 44% in rats fed on wheat biscuit diet.

Serum iron concentrations of anemic rats fed on different biscuit diets are illustrated in Table 6 and Fig. 4. Feeding anemic rats on FSF-biscuit diets exhibited extremely higher ($P<0.05$) values of serum iron than rats fed on wheat biscuit diet. On the other hand, serum iron regeneration% rats fed on control diet was 55.4%, while were ranged between 83.3 - 100% for rats fed on FSF-biscuit diets. The higher value was noticed in rats fed on biscuit diet containing 10% germinated FSF.

The effect of iron deficiency on rats liver weights and minerals content are illustrated in Table 7 and Fig. 6. Feeding rats on Fe-deficient diet resulted in evident loss of liver weights ranged between 27.7-32.9%.

Table 7: Liver weights and minerals content of rats fed on Fe-deficient and Fe-intrinsic diets during two feeding periods

Substitution level (%)	Stages*	Liver wt. (g)	Liver minerals content (mg /100g) Fe	Ca	Zn
	Initial	2.56	2.86	8.59	5.45
Control	Anemic	1.85	1.79	6.49	2.23
	Fe-intrinsic	4.32^{c}	5.62^{c}	11.37^{c}	5.56^{c}
W:SF					
	Initial	2.59	2.79	8.61	5.63
95:5	Anemic	1.87	1.75	6.52	2.32
	Fe-intrinsic	5.29^{b}	7.49^{b}	14.20^{b}	5.98^{b}
90:10	Initial	2.62	2.89	8.72	5.71
	Anemic	1.78	1.98	6.45	2.21
	Fe-intrinsic	5.36^{a}	7.81^{a}	15.29^{a}	6.54^{b}
W:GF					
	Initial	2.52	2.61	8.59	5.61
95:5	Anemic	1.69	1.91	6.72	2.65
	Fe-intrinsic	5.39^{a}	7.92^{a}	15.19^{a}	6.23^{b}
	Initial	2.55	2.79	8.71	5.53
90:10	Anemic	1.78	1.89	6.67	2.25
	Fe-intrinsic	5.55^{a}	8.23^{a}	16.31^{a}	7.35^{a}

*Stages refer to rats at initial (zero time), anemic (10 days Fe-deficient) and Fe-intrinsic (20 days biscuit diets)

^{a},^{b}and^{c} means in the same column with different superscripts are different significantly (p● 0.05)

The ultimate liver weights after feeding on different biscuit diets exceeded (P<0.05) the initial weights by varying per cents. The increases varied between 104 and 118% in rats fed on FSF-biscuit diets, while was 69% in rats fed on wheat biscuit diet.

Iron content in livers of rat groups fed on Fe-deficient diet showed sharp declines as shown in Table 7. Reaccumulation of liver iron occurred after feeding on different biscuit diets and exceeded (P<0.05) the initial concentrations. The highest exceeding value (127%) was noted in rats fed on biscuit diet containing 10% germinated FSF, while the minimum level (62%) was shown in rats fed on wheat biscuit diet. The decreases in Ca and Zn contents of anemic rat livers and its increases after fed on different biscuit diets were accompanied by parallel changes in livers iron content.

DISCUSSION

`In the recent years, there is a trend to use novel sources in the bakery products to decrease the amount of imported wheat by using local and cheap sources. Thus, fenugreek seed flour has high nutritional properties as monitored by the nutritional quality of processed products following its addition.

Sensory evaluation of biscuits has revealed that biscuits supplemented by 5 or 10% soaked or germinated FSF had the highest acceptance level for all sensory characteristics, no significant differences could be detected among these samples and control biscuit. Whereas, replacement of wheat flour by 15 or 20% FSF significantly impaired the taste of biscuits due to the bitter taste of fenugreek.

Chemical analysis showed evident increase in Fe, Ca and Zn contents of FSF-biscuits as compared to wheat biscuit, this was in coincide with the result of Shalini and Sudesh [8]. Phytic acid contents of produced biscuits were decreased by ranges between 20 to 44%, this might be due to phytate leaching or phytate hydrolysis during soaking and germination processes by phytase and phosphatase enzymes [20].

Rat feeding trials indicated that the wheat flour iron in control diet was proved to be bioavailable and resulted in hemoglobin (Hb) regeneration of 60.08%, this per cent changed to higher values in subsequent substitutions of wheat flour by 5 or 10% FSF. The variation in Hb regeneration levels between rat groups fed on different biscuit diets was related to the high differences in iron content of diets. Aykut and Baysal [21] found that hemoglobin and serum iron levels were increased as Fe intake increased.

Regarding the bioavailability% of biscuits iron, it could be noticed that maximum value (46.83%) was found in rats fed on biscuit diet containing10% germinated FSF, while the lower per cent (25.43%) was observed in rats fed on wheat biscuit diet. These results suggested the important role of dietary indispensable amino acids in absorption and utilization of iron. El-Guindi *et al.* [22] reported that certain amino acids (especially cycteine, histidine and lysine) improve iron absorption.

Blood hematocrite % in anemic rats fed on biscuit diet containing 10% germinated FSF was the highest (57%) as compared to rats fed on wheat biscuit diet (44%). One explanation for variation in Hct% is the differences in balance of protein amino acid composition for fed diets [22].

Serum iron was dropped to half its initial content in all anemic rat groups. Regeneration% after feeding on different biscuit diets exceeded the initial values with range between 55.4% for rats fed on wheat biscuit diet to 100% for rats fed on biscuit diet containing 10% germinated FSF. These high increases in serum iron regeneration as compared to control were related to the higher dietary Fe content of FSF-biscuit diets. Generally, these results might be interpreting the role of serum iron (as a Fe-mobile phase) in regeneration of the studied blood criteria [23].

Reduction of liver weights was noticed in all rat groups fed on Fe-deficient diet. Regaining of liver weights after feeding on FSF-biscuit diets exceeded the initial weights by 104 to 118%, while was 69% for rats fed on wheat biscuit diet, this might be due to the clear bioavailability of FSF-biscuit iron from side and effect of dietary protein quality on iron bioavailability from other side. On the other hand, respective decreases and increases in livers content of Fe, Ca and Zn accompanied changes in liver weights. These results indicate that interactions of calcium - iron and zinc - iron [24].

It could be concluded that, using of 10% germinated FSF into wheat biscuit formula improved their nutritional quality criteria as reduced phytic acid content and enhanced iron bioavailability of FSF-biscuit.

REFERENCES

1. ACC/SCN, 2000. Fourth report on the world nutrition situation. Geneva: ACC/SCN in Collaboration with IFPRI.
2. UNICEF/UNU/WHO/MI, 1999. Preventing iron deficiency in women and children. Technical consensus on key issues, technical workshop, UNICEF, New York; 7-9 October 1998. Boston: International Nutrition Foundation and Micronutrient Initiative.
3. Allen, L.H., B. Benoist, O. Dary and R. Hurrell, 2006. Guidelines on food fortification with micronutrients. World Health Organization and Food and Agriculture Organization of the United Nations, Geneva.
4. WHO, 1998. Regional Office for the Eastern Mediterranean, Fortification of flour with iron in countries of the Eastern Mediterranean, Middle East and North Africa, WHO-EM/NUT/202/E/G. Alexandria.
5. Allen, L.H., 1997. Pregnancy and iron deficiency: effects of the newborns-unsolved issues. Nutr. Rev., 55: 91-101.
6. WHO, 2002. Report, of WHO Reducing risks, promoting healthy life. World Health Org., Geneva.
7. Regional Committee for the Eastern Mediterranean, 2001. Progress report on flour fortification in the Eastern Mediterranean Region. RC48/INF. DOC. 6. 48 th Sessions. Riyadh, Saudi Arabia: WHO.
8. Shalini, H. and J. Sudesh, 2005a. Organoleptic and nutritional evaluation of wheat biscuits supplemented with untreated and treated fenugreek flour. Food Chemistry, 90: 427-435.
9. El-Shimi, N.M., A.A. Dami and M. Ragab, 2003. Changes in some nutrients of fenugreek seeds during germination. Egyptian J. Applied Sci., 18: 172-186.
10. Neeraja, A. and P. Rajyalakshmi, 1996. Hypoglycemic effect of processed fenugreek seeds in humans. J. Food Sci. and Tech., 33: 427-430.
11. Shalini, H. and J. Sudesh, 2005b. Effect of fenugreek flour blending on physical, organoleptic and chemical characteristics of wheat bread. Nutrition and Food Sci., 35: 229-242.
12. A.A.C.C., 1984. Approved Methods of Analysis. St. Paul, Minnesota: The American Association of Cereal Chemists.
13. Austin, A. and A. Ram, 1971. Studies on chapatti making quality of wheat. Indian Council of Agricultural Research, New Delhi., Technical Bulletin, 31: 96-101.
14. A.O.A.C., 1995. Official Methods of Analysis. Washington, DC: Association of Official Analytical Chemists.

15. Camir, A.L. and F.M. Clydesdale, 1982. Analysis of phytic acid in Foods by HPLC. J. Food Sci., 47: 275.
16. Ranhotra, G.S. and J. Gelroth, 1979. Bioavailability of iron in high cellulose bread. Cereal Chem., 56: 165.
17. Betk, K. and W. Savelsberg, 1950. Stufenphotometer is cyanhomoglobin. Biochem. Zeitchr., 320: 431 by Corresponding.
18. Hernandez, M., V. Sousa, A. Morena, S. Villapando and A.M. Lopez, 2003. Iron bioavailability and utilization in rats are lower from lime-treated corn flour than from wheat flour when they are fortified with different sources of iron. J. Nutr., 133: 154.
19. Steel, R.G. and J.H. Torrie, 1980. Principles and procedure of statistics analysis. Biometrical approach. 2 nd ed. MC Graw-Hill Book Co. INC New York, N.Y.
20. Beal, L. and T. Mehta, 1985. Zinc and phytate distribution in peas influence of heat treatment, germination, pH, substrate and phosphorus on pea phytate and phytase. J. of Food Sci., 50: 96.
21. Aykut, M. and A. Baysal, 1978. Absorption of dietary iron in women and the affecting factors. Hacettepe. Bull. Med. Surg., 11: 66.
22. El-Guindi, M., S.R. Lunch and J.D. Cook, 1988. Iron absorption form fortified flat breads. Br. J. Nutr., 59: 205.
23. Thannoun, A.M., A.W. Mahoney, M. Buchowski and D.G. Hendricks, 1988. Heme and non-heme iron absorption from meat and meat loaf by anemic and healthy rats. Nutr. Report Inter., 37: 487.
24. Abrams, S.A., I.J. Griffinm, P. Davila and L. Liang, 2001. Calcium fortification of breakfast cereals enhances calcium absorption in children without affecting iron absorption. J. Pediatric, 139: 522-526.