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Minerals Bioavailability of Wheat Biscuit Supplemented by Quinoa Flour

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ABSTRACT

Biscuits produced from wheat flour replaced by different levels (10, 20, 30 and 40%) of detoxified quinoa meal flour (QMF) were evaluated for sensory characteristics and some minerals content. Investigation of bioavailability for QMF-biscuit minerals was the major target of this study. The results of chemical analysis indicated that incorporation of QMF into biscuits formula was obviously increased the contents of protein, fiber, ash, Fe, Ca and Zn with increasing QMF level as compared to wheat biscuit (control). Also, the present results revealed that biscuits processed from wheat flour supplemented by 10, 20 and 30% of QMF exhibited good sensory properties. While, the produced biscuit batch was contain 40% QMF significantly ($P < 0.05$) varied and had less judging scores for the tested organoleptic quality properties and less acceptability as compared with the other samples. 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 QMF-biscuit diets. The results indicated that the rats fed on QMF-biscuit diets showed a good hematological response. Whereas the 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 40% QMF recorded the highest values of studied blood criteria between all tested diets. It could be concluded that, using of QMF at different levels into wheat biscuit formula improved their nutritional quality criteria and enhanced minerals bioavailability of produced biscuits.

Key words: Biscuit - Quinoa seeds - Sensory characteristics – Mineral bioavailability

Introduction

Mineral deficiencies (especially iron, calcium and zinc) have a negative effect on human health and may lead to conditions such as iron deficiency anemia, rickets, osteoporosis and diseases of the immune system (Bock, 2000 and Frontela *et al.,* 2011). WHO estimates that anemia affects over 2.5 billion people worldwide. WHO indicated that iron deficiency anemia is a significant problem throughout the world ranging from 1% in the industrialized countries to 56% in developing countries (De Benoist *et al.,* 2008 and Mclean *et al.,* 2008). The most affected individuals is preschool-age children (35%), followed by pregnant women (30%), non-pregnant women (18%), school-age children (11%) and 6% for people older than 60 years of age (Massot and Vanderpas 2003; and Erhabor *et al.,* 2013).

According to WHO, around 0.8 million deaths can be attributed to iron deficiency each year (WHO, 2001 and Allen *et al.,* 2006). Subclinical iron deficiency resulting in functional disadvantages is as widespread as iron deficiency with anemia (WHO, 1998), growth retardation, low birth weight, increased prenatal mortality, increased maternal morbidity and mortality (Allen, 1997). Iron-deficiency anemia is also a serious public health problem in all countries comprising the Eastern Mediterranean Region, Middle East and North Africa Region (De Benoist *et al.,* 2008).

The biscuits quality 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 other seeds as high nutritive source (Shalini and Sudesh, 2005a).

According to the National Academy of Sciences of the United States, quinoa is considered "golden grain" because of its high nutritional value, which is taken into account by NASA to integrate the diet of astronauts (Vilche *et al.,* 2003; and Carrasco and Soto, 2010).

Quinoa seeds are an excellent raw material for healthy and tasty foods. They are considered easy to digest because gluten free and are unusually complete food because they possess a well-balanced set of essential amino acids for humans and a good sources of protein (12 - 18 g/100 g on dry weight), fiber, vitamins (such as C, E and B complex) and important minerals (such as Fe, Ca, K, Mg, P and Zn), in accordance to what reported by Ogungbenle (2003), Jancurová *et al.,* (2009) and Vega-Gálvez *et al.,* (2010). In general, the minerals content of quinoa is about three times more than other cereals. Bock (2000) and Frontela *et al.,* (2011) indicated that the

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calcium content of quinoa is from 100 to 340 mg/100 g of dry matter, iron from 26 to 32 mg/100g and zinc from 4.8 to 6.1 mg/100 g.

In addition, the whole seeds contains a large variety of antioxidant compounds, such as carotenoids (Eberhardt *et al.,* 2000 and Dini *et al.,* 2010) and flavonoids (Dini *et al.,* 2004), all of which are protective against a variety of diseases, particularly cancer, allergy and inflammatory diseases; and reduce the risk of cardiovascular diseases (Scalbert *et al.,* 2005 and Alvarez-Jubetea *et al.,* 2010), diabetes and Alzheimer's disease (Rimm *et al.,* 1996 and Nsimba *et al.,* 2008). Unfortunately, quinoa seeds contain bitter-tasting constituents (chiefly water-soluble saponins) located in the outer layers of the seed coat, making it essentially unpalatable. Therefore, most commercial quinoa seeds, have been processed to remove their coating by washing or milling so to eliminate bitter compounds before consumption (Popenoe *et al.,* 1989).

Unfortunately, there is also several antinutritional substances have been found in quinoa, such as saponins, phytic acid, tannins and protease inhibitors; which can have a negative effect on metabolic reactions (Improta and Kellems, 2001; Aguirre *et al.,* 2004; Khattak *et al.,* 2007 and Rosero1 *et al.,* 2013).

Generally, development and consumption of such therapeutic bakery products would help to raise the nutritional status of population (Shalini and Sudesh, 2005b). Information on incorporation of quinoa seeds flour in biscuits is scanty. Therefore, this study was designed to evaluate the effect of replacement wheat flour by 10, 20, 30 and 40% of quinoa seeds flour on the nutritional and sensory characteristics of produced biscuits. Also, evaluation of some minerals bioavailability for final products through rat feeding trials was another target.

Materials and Methods

Materials:

Quinoa seeds (*Chenopodium quinoa* Willd.) 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, 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 Quinoa Meal Flour (QMF) and Detoxification Treatment:

Quinoa meal flour was prepared according to the methods described by Chauhan *et al.,* (1999). Quinoa seeds were cleaned and freed of broken seeds, dust and other foreign materials. These seeds were soaked in mineral salt solution for 2 h at room temperature to remove toxic materials according to Varriano-Marston and De Francisco (1984). After that the detoxified seeds were soaked again in tap water for one hour then dried in oven at 50 ^{O}C for 12 h (Zhu *et al.,* 2002). The quinoa seeds were ground to fine powder in an electric grinder stainless steel using a Laboratorial disc mill and sifted through a 60 mesh. The flour so obtained was defatted for 24 h with hexane in a 10% (w/v) suspension with continuous stirring, air-dried at room temperature, packed in polyethylene bags and stored at 4±1 °C until used.

Preparation of Wheat Flour/Quinoa Meal Flour (WF/QMF) Blends:

Wheat flour was supplemented by 10, 20, 30 and 40% of QMF. 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 sugar were firstly creamed by using the mechanical mixer for 10 min. Sodium bicarbonate and ammonium bicarbonate were dissolved in 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^{O}C for 7 min. according to A.A.C.C. (1995). After cooling for 30 min. biscuits were packaged in cellophane and subjected for sensory evaluation.

Analytical Methods:

Chemical Analysis:

Saponins were determined according to Hiai *et al.,* (1976). Phytate content was determined by the method of Hauag and Lantzsch (1983). Tannins were determined by the method described by Makkar *et al.,* (1993). Protease inhibitors were determined according to Smith *et al.,* (1980). While, iron, calcium and zinc contents were determined in produced biscuits and rat livers by atomic absorption (Perkin-Elmer-Crop, Norwalk, model 560) according to A.O.A.C. (2005).

Sensory Evaluation of Biscuits:

The sensory evaluation of control biscuit (100% wheat flour) and biscuits containing QMF at different concentrations was performed by 20 panelists from the staff members of Food Science and Technology Department, Faculty of Agriculture, Cairo, AL-Azhar University. The panelists were asked to evaluate color, appearance, oder, taste, texture and overall acceptability. During the panel test, rinse the panelist's mouth by water to remove any traces of residual food. The ratings were on a 9-point hedonic scale, ranging from 9 as like extremely to 1 as dislike extremely as outlined by Ihekoronye and Ngoddy (1985).

Rats Experimental:

White weanling male rats (weighing on average 90 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 (1979), for 10 days. Blood hemoglobin (Hb) contents dropped from around 13 to 7 g/dl. After wards, rat groups 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. 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.

A- 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 (1950).

B- Iron Content of Blood Hemoglobin:

It was calculated as described by Hernandez *et al.,* (2003) as follows:

Fe Hb (mg) = body weight (g) × Hb (g/L) × 6.7 × 0.335/10000

C- Hematocrite (%):

Blood samples were taken with a micro capillary tube and centrifuged by using centrifuge apparatus (model 2041, Q. 336x225, UK) at 5000 r.p.m. for 5 min. The volume of blood cells was measured using a graded scale.

D- Serum Iron Content:

Serum iron of rats was determined by spectrophotometer (Spekol 11 No. 849101) as described in A.O.A.C. (1995).

Statistical Analysis:

The obtained data of sensory evaluation of produced biscuit batches and other results were statistically analyzed by using SPSS (Version 16.0 software Inc., Chicago, USA) of completely randomized design as described by Gomez and Gomez (1984). Treatment means were compared using the least significant differences (LSD) at 0.05 of probability level.

Results and Discussion

Detoxification Treatment of Quinoa Meal:

Table (1) show that raw quinoa seeds contained considerable contents of saponins, phytate, tannins and protease inhibitors at ratio of 4.68 g/100g, 1.17 g/100g, 1.67 mg/100g and 3.21 mg/100g, respectively. These results are in agreement with those of Stuardo and San Martin (2008) who reported that the content of saponins varies in quinoa from 0.1 to 5%. Also, Kozioł (1992) indicated that the phytic acid content for five different varieties of quinoa was ranged from 10.5 to 13.5 mg/g. While, Aguirre *et al.,* (2004) found that the quinoa contain small amounts (lees than 50 ppm) of trypsin inhibitors which are much lower than those in commonly consumed grains and hence do not pose any serious concern.

Table 1: Detoxification treatment of quinoa meal:

Component / Material	Saponins (g/100gm)	Phytate (g/100gm)	Tannins (mg/100g)	Protease inhibitors (mg/100g)
Raw seeds	4.68	1.17	1.67	3.21
Treated meal	0.04	0.30	0.24	N.D

N.D: not detected

Also, the same table shows that the detoxification treatment completely eliminated protease inhibitors as well as about 99.1, 74.3 and 85.6% of saponins, phytate and tannins, respectively. Reduction of contents of saponins, tannins and protease inhibitors may be due to the physic-chemical changes in their configuration or enzymatic activity during soaking leading to their diffusion into the soaking media (Deshpande *et al.,* 1982). Also, the decrease of phytate may be due to leaching or phytate hydrolysis during soaking by phytase and phosphatase enzymes (Beal and Mehta, 1985).

Chemical Composition of Biscuits Containing QMF:

The chemical composition results for biscuits made from wheat flour and its blends with 10, 20, 30 and 40% QMF were tabulated as in Table (2).

Table 2: Gross chemical composition (on dry weight basis) and some mineral contents of biscuits supplemented by QMF at different levels:

Substitution level (%)	Gross chemical components (%)					Minerals content (mg/100g)		
	Protein	Fat	Fiber	Ash	Carbohydrates	Fe	Ca	Zn
Control	8.82	21.65	1.73	1.58	66.22	4.42	32.23	2.18
WF : QMF								
90 : 10	8.89	22.71	1.77	1.66	64.97	5.98	49.35	3.53
80 : 20	9.16	23.20	1.82	1.89	63.93	7.71	57.86	4.02
70 : 30	9.35	24.38	1.90	2.11	62.26	9.46	63.13	4.75
60 : 40	9.69	24.94	2.00	2.32	61.05	10.22	71.44	5.39

As shown in Table (2), the contents of protein, fat, crude fiber and ash were gradually increased with increasing the incorporation level of QMF as compared with control biscuit. This alteration could be attributed to the addition of quinoa flour as partial substituting of wheat flour in making biscuit; which is rich in protein content and the other components. In this regard, Jancurova *et al.,* (2009) and Atef *et al.,* (2014) indicated that the protein, fat, ash and crude fiber contents of different quinoa flours were higher than those contents of wheat flour. On the other hand, a slight decrease in total carbohydrates content was noticed in the same samples and reached 61.05% in biscuit batch containing 40% QMF, against 66.22% of control sample. These findings are in agreement with the results of Park *et al.,* (2005) and Rosell *et al.,* (2009), who indicated that the carbohydrates content decreased with the increasing the incorporation level of the quinoa flour added to wheat flour used for making bread.

Also, from the same table, it could be also observed that the control biscuit contained Fe, Ca and Zn at levels of 4.42, 32.23 and 2.18 mg/100g, on dry weight basis; respectively. In addition that produced biscuit batches contents from the former minerals were increased with increasing the incorporation level of QMF into the biscuit formula. Where, biscuit containing 40% QMF exhibited higher contents of Fe (10.22), Ca (71.44) and Zn (5.39 mg/100g) than other biscuit batches. This attributing to the high contents of mineral salts in quinoa flour (Vilche *et al*., 2003 and Alvarez-Jubete *et al.,* 2009); when compared with wheat flour. On the same context, Repo-Carrasco *et al.,* (2003) and Valcárcel-Yamani *et al.,* (2012) indicated that the minerals content of quinoa is about twice or three times more than other cereals.

Sensory Characteristics of Biscuits Containing QMF:

Biscuits supplemented by different levels of QMF were sensory evaluated and compared with control biscuit (100% wheat flour) as shown in Table (3).

Table 3: Sensory characteristics* of biscuits supplemented by QMF at different levels:

Substitution level (%)	Organoleptic properties					Overall acceptability
	Color	Appearance	Oder	Taste	Texture	
Control	8.20^{a}	8.17^{a}	8.48^{a}	8.66^{a}	8.27^{a}	8.38^{a}
WF : QMF						
90 : 10	8.14^{a}	8.09^{a}	8.36^{a}	8.32^{a}	8.10^{a}	8.10^{a}
80 : 20	8.06^{a}	8.03^{a}	8.32^{a}	8.10^{a}	7.94^{a}	7.97^{a}
70 : 30	7.98a	7.94ab	8.18a	7.75^{a}b	7.82b	7.81^{a}b
60 : 40	7.62b	7.58b	7.86b	7.56b	7.53c	7.72b

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

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

From the Table (3), there were no significant differences among control sample and biscuit samples containing 10, 20 and 30% of QMF in all sensory characteristics with exception, the texture of 30% QMF-sample was significantly reduced ($P < 0.05$) when compared with control sample. But the biscuit sample containing 40% QMF was significantly different ($P < 0.05$) in all properties and had less judging scores as compared to the other samples. Whereas overall acceptability score for these sample was 7.72, against 8.38 of control biscuit. According to a study conducted by Lorenz *et al.,* (1993) no statistically significant differences were observed between noodles made with 10% or 30% quinoa flour and the sample control. Also, some

characteristics of dough and bread containing quinoa flour were studied by Park *et al.,* (2005), the substitution of 7.5 to 10 % of quinoa flour for wheat flour significantly ($P < 0.05$) increased the loaf volume of bread.

Generally, substitution levels of up to 30% were acceptable in biscuit samples. The ingredients level and recipes of biscuit preparation may affect the sensory attributes, consumer's preference and overall acceptability (Nazni and Pradeepa, 2010; and Eke-Ejiofor, 2013).

Bioavailability of QMF-biscuits Iron:

Results presented in Table (4) and illustrated in Fig. (1) shows rat blood hemoglobin (Hb) levels initially and after feeding on Fe-deficient diet followed by different biscuit diets. After feeding on Fe-deficient diet, Hb levels dropped from 13.16 to 7.08 g/dl in all rat groups. After 30 day of feeding rats, Hb content was obviously ($P < 0.05$) increased to range from 13.97 to 15.27 g/dl after feeding the anemic rats on QMF-biscuit diets, against 11.30 g/dl for rats fed on wheat biscuit diet with Hb regeneration% of 59.60%. While, the maximum Hb regeneration% was achieved by rats fed on biscuit diet containing 40% QMF (113.87%). The variation in Hb regeneration (%) among rat groups fed on different biscuit diets was related to the high differences in iron content of diets. Aykut and Baysal (1978) found that hemoglobin and serum iron levels were increased as Fe intake increased.

Table 4: Blood hemoglobin level and hemoglobin regeneration% in rats fed on Fe-deficient and Fe-intrinsic diets during two feeding periods*:

Substitution level (%)	Rats hemoglobin (g/dl)						
	Initial	Fe-deficient diets	Fe-intrinsic diets				Hb regeneration (%)
	Zero day	10 Day	15 Day	20 Day	25 Day	30 Day	
Control	13.14^{Aa}	7.08^{Ad}	10.39^{Cc}	10.56^{Cc}	11.16^{Cb}	11.30^{Cb}	59.60
WF : QMF							
90 : 10	12.97^{Ab}	7.11^{Ad}	11.25^{Bc}	12.30^{Bb}	13.65^{Ba}	13.97^{Ba}	96.48
80 : 20	13.01^{Ab}	7.10^{Ad}	11.87^{Bc}	12.50^{Ac}	13.81^{Aa}	14.26^{Ba}	100.85
70 : 30	13.09^{Ab}	7.16^{Ad}	11.99^{Ac}	12.64^{Ac}	13.98^{Aa}	14.84^{Aa}	107.26
60 : 40	13.16^{Ab}	7.14^{Ad}	12.18^{Ac}	12.96^{Ac}	14.16^{Aa}	15.27^{Aa}	113.87

**Feeding periods were 10 days of fed on Fe-deficient diet and 20 days on biscuit (Fe-intrinsic) diets*
Mean values in the same column (as a capital letter) or row (as a small letter) with the same letter are significantly differences at 0.05 level

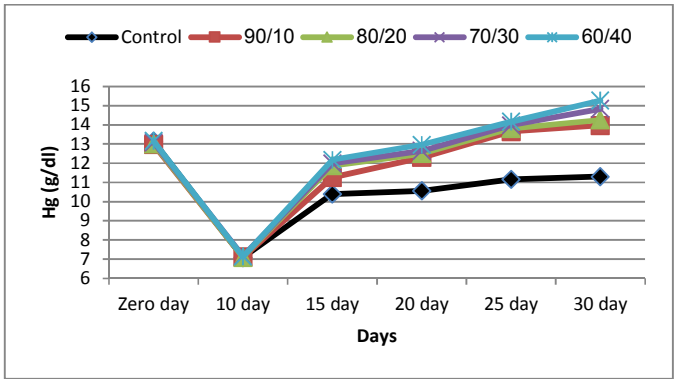

Fig. 1: Changes in blood Hb levels of rats fed on Fe-deficient and different biscuit diets

The bioavailability (%) of intrinsic iron in tested biscuit diets are presented in Table (5). Rats fed on biscuit diet containing 40 % QMF proved to have maximum Fe-bioavailability (48.9%). While, lower Fe-bioavailability (25.3%) was observed in rats fed on wheat biscuit diet. Rat groups fed on the other biscuit diets give Fe-bioavailability (%) from 29.9 to 43.6%.

Table 5: Bioavailability % of intrinsic iron in anemic rats fed on different biscuit diets:

Substitution level (%)	Diet intake(g)	Fe in diet (mg/100g)	Fe-intake (mg)	Fe-Hb gain (mg)	Fe-intrinsic bioavailability*(%)
Control	226	4.86	11.49	2.91	25.3
WF:QMF					
90:10	230	5.19	11.86	3.54	29.9
80:20	232	5.48	12.54	4.80	38.3
70:30	225	5.72	12.89	5.62	43.6
60:40	222	5.94	13.18	6.44	48.9

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

Table (6) and Fig. (2) shows blood hematocrite % of rat groups fed on tested diets. Data indicate the decrease of Hct values after feeding on Fe-deficient diet and it gradually ($P < 0.05$) increased through feeding on different biscuit diets by different levels. The maximum Hct % was observed in rats fed on biscuit diet containing 40% QMF (60%), while was 43% in rats fed on wheat biscuit diet. One explanation for variation in Hct % is the differences in balance of amino acids composition for diets protein (El-Guindi *et al.,* 1988).

Table 6: Blood hematocrite % in rats fed on Fe-deficient and Fe-intrinsic diets during two feeding periods*:

Substitution level (%)	Rats hematocrite (%)					
	Initial	Fe-deficient diets	Fe-intrinsic diets			
	Zero day	10 Day	15 Day	20 Day	25 Day	30 Day
Control	41^{Aa}	31^{Ad}	33^{Cc}	35^{Cc}	39^{Cb}	43^{Ca}
WF : QMF						
90 : 10	41^{Ab}	30^{Ad}	37^{Bc}	42^{Bb}	46^{Ba}	50^{Ba}
80 : 20	39^{Ac}	32^{Ad}	38^{Ac}	44^{Bb}	48^{Bb}	53^{Ba}
70 : 30	40^{Ac}	30^{Ad}	39^{Ac}	46^{Ab}	51^{Ab}	56^{Aa}
60 : 40	41^{Ac}	31^{Ad}	41^{Ac}	49^{Ab}	54^{Ab}	60^{Aa}

**Feeding periods were 10 days of fed on Fe-deficient diet and 20 days on biscuit (Fe-intrinsic) diets*
Mean values in the same column (as a capital letter) or row (as a small letter) with the same letter are significantly differences at 0.05 level.

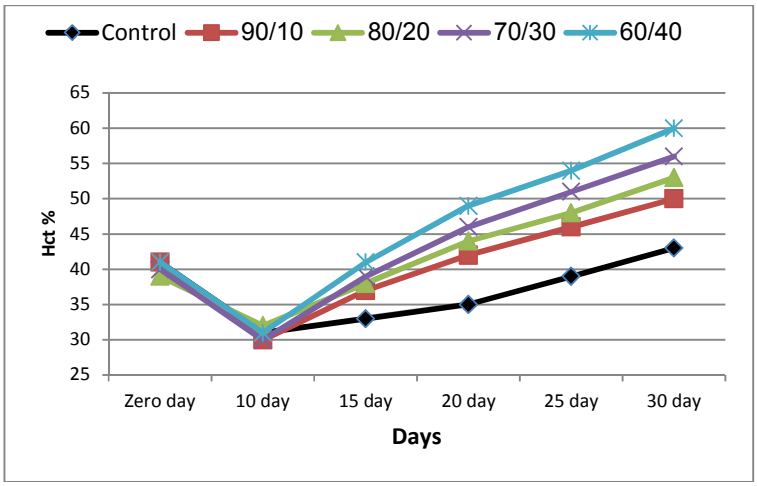

Fig. 2: Blood hematocrite % of rats fed on Fe-deficient and different biscuit diets

Serum iron concentrations of anemic rats fed on different biscuit diets are illustrated in Table (7) and Fig. (3). Feeding anemic rats on QMF-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% for rats fed on control diet was 61.8%, while were ranged from 73.2 to 110.9% for rats fed on QMF-biscuit diets. Whereas the highest percent was noticed in rats fed on biscuit diet containing 40% QMF. These high increases in serum iron regeneration as compared to control were related to the higher dietary Fe content of QMF-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 (Thannoun *et al.,* 1988).

The effect of iron deficiency on livers weight and minerals content of rats are illustrated in Table (8). Feeding rats on Fe-deficient diet resulted in evident ($P < 0.05$) loss of livers weight ranged from 31.9 to 33.5% as shown in Fig. 4. The ultimate liver weights after feeding on tested biscuit diets exceeded ($P < 0.05$) the initial weights by varying percents. The increases varied from 62.5 to 102.4% in rats fed on QMF-biscuit diets, while was 43.8% in rats fed on wheat biscuit diet (Fig. 4), this might be due to the clear bioavailability of QMF-biscuit iron from side and effect of dietary protein quality on iron bioavailability from other side (Palacios *et al.,* 2008 and Frontela *et al.,* 2011).

Iron content in rat livers fed on Fe-deficient diet showed sharp declines as shown in Table (8). Re-accumulation of liver iron occurred after feeding on different biscuit diets and exceeded ($P < 0.05$) the initial contents varying rates. The highest exceeding value (163.7%) was noted in rats fed on biscuit diet containing 40% QMF, while the minimum level (61.2%) was shown in rats fed on wheat biscuit diet. On the other hand, 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. Generally, these results indicate that interactions of calcium - iron and zinc - iron (Abrams *et al.,* 2001).

It could be concluded that, using of QMF at different levels into wheat biscuit formula improved their nutritional quality criteria and enhanced minerals bioavailability of produced biscuits.

Table 7: Serum iron and regeneration% in rats fed on Fe-deficient and Fe-intrinsic diets during two feeding periods*

Substitution level (%)	Serum iron (mg/dl)						
	Initial	Fe-deficient diets	Fe-intrinsic diets				Hb regeneration (%)
	Zero day	10 Day	15 Day	20 Day	25 Day	30 Day	
Control	10.1^{Aa}	5.5^{Ae}	6.4^{Dd}	6.9^{Cc}	7.8^{Cc}	8.9^{Cb}	61.8
WF : QMF							
90 : 10	10.0^{Aa}	5.6^{Ad}	7.6^{Cc}	8.5^{Bb}	8.9^{Bb}	9.7^{Ca}	73.2
80 : 20	10.1^{Aa}	5.4^{Ad}	8.4^{Bc}	8.9^{Bb}	9.5^{Ba}	10.6^{Ba}	96.3
70 : 30	10.2^{Ab}	5.3^{Ad}	8.7^{Ac}	9.2^{Ac}	9.8^{Ab}	10.9^{Ba}	105.7
60 : 40	10.0^{Ab}	5.5^{Ad}	8.9^{Ac}	9.6^{Ab}	10.3^{Ab}	11.6^{Aa}	110.9

**Feeding periods were 10 days of fed on Fe-deficient diet and 20 days on biscuit (Fe-intrinsic) diets.*
Mean values in the same column (as a capital letter) or row (as a small letter) with the same letter.
are significantly differences at 0.05 level.

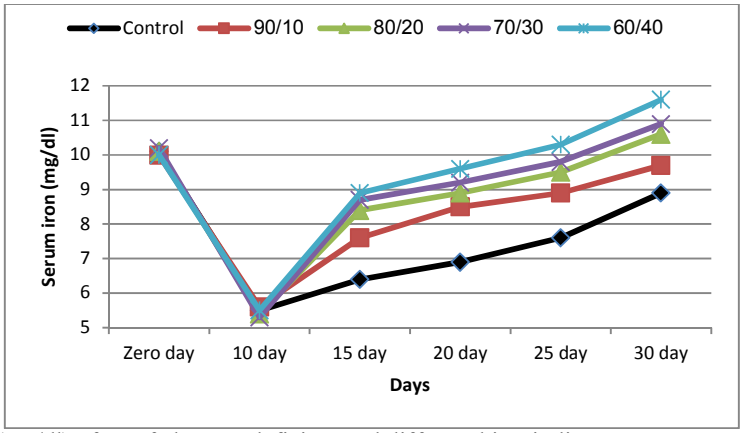

Fig. 3: Serum iron (mg/dl) of rats fed on Fe-deficient and different biscuit diets

Table 8: Livers weight and minerals content of rats fed on Fe-deficient and Fe-intrinsic diets during two feeding periods:

Substitution level (%)	Stage*	Liver wt. (g)	Liver minerals content (mg /100g)		
			Fe	Ca	Zn
Control	Initial	2.97^{c}	2.94^{d}	8.68^{d}	5.58^{c}
	Anemic	1.98^{d}	1.85^{e}	6.54^{e}	2.34^{d}
	Fe-intrinsic	4.27^{b}	4.74^{c}	11.20^{c}	5.64^{b}
90:10	Initial	2.96^{c}	2.88^{d}	8.58^{d}	5.49^{c}
	Anemic	1.97^{d}	1.80^{e}	6.48^{e}	2.30^{d}
	Fe-intrinsic	4.81^{b}	6.48^{b}	12.87^{b}	5.99^{b}
80:20	Initial	2.98^{c}	2.85^{d}	8.66^{d}	5.60^{c}
	Anemic	2.03^{d}	1.90^{e}	6.50^{e}	2.40^{d}
	Fe-intrinsic	5.22^{a}	6.92^{b}	13.92^{a}	6.26^{a}
70:30	Initial	2.94^{c}	2.90^{d}	8.74^{d}	5.56^{c}
	Anemic	1.99^{d}	1.88^{e}	6.70^{e}	2.38^{d}
	Fe-intrinsic	5.76^{a}	7.32^{a}	14.67^{a}	6.67^{a}
60:40	Initial	2.95^{c}	2.92^{d}	8.67^{d}	5.62^{c}
	Anemic	1.98^{d}	1.92^{e}	6.68^{e}	2.44^{d}
	Fe-intrinsic	5.97^{a}	7.70^{a}	15.82^{a}	7.22^{Aa}

**Stages refer to rats at initial (zero time), anemic (10 days Fe-deficient) and Fe-intrinsic (20 days biscuit diets)*
^{a,b,c} and ^{d} means in the same column with different superscripts are different significantly ($p < 0.05$)

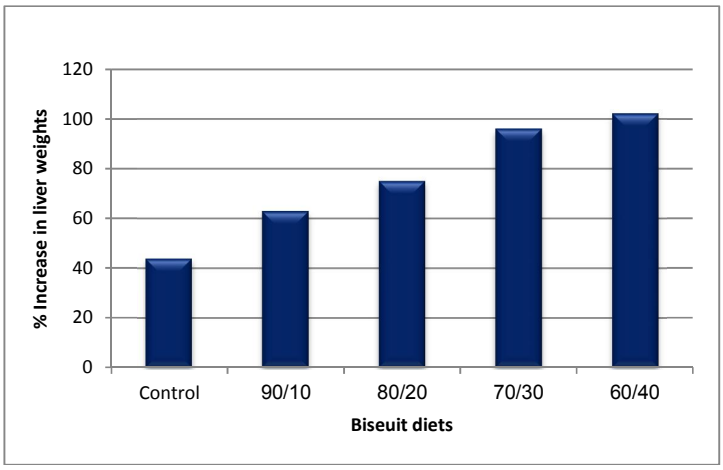

Fig. 4: % Increase in liver weight of rats fed on different biscuit diets for 20 days

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