Iron Bioavailability in White, Brown and Bran Bread on Mice

Dr. Moufid Yassin*
Aya tollah Hasan Yousef**

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□ ABSTRACT □

Evaluation of bioavailability of iron as ferrous sulfate ($FeSO_4$) 100 $mg.kg^{-1}$ from three fortified bread types of 80% extraction wheat flour was assessed on27Balb-cmice. depletion-repletion method was applied, during the depletion period mice were made anemic using low iron diet for 14-days then they were divided into seven groups receiving different diets as follow: (A1: white non-fortified bread, A2: white fortified bread, B1: brown non-fortified bread, B2: brown fortified bread, C1: bran non-fortified bread, C2: bran fortified bread, D: fodder)for 21-days.Wheat flour nutritional profile values were: moisture 12.6%, ash0.58%, protein11.2 mg/100g, fat1.2 mg/100g, and iron 3.4 mg/100g. The study of the hematological and biochemical indices (Red blood cells, Hemoglobin, Hematocrit, Serum iron, total iron binding capacity, Transferrin and Ferritin) of the blood collected at days (1-15-36) of the experimental periods, showed that the live body weight of the experimental animals generally increased with the time period for all mice, except for the non-fortified bran diet. Hematological and biochemical tests decreased at the end of depletion period. The highest values for hematological and biochemical tests at the end of diets were HG: (13.1 g/dl), Serum iron: (52µmol.L-1) and Ferritin: (2.7 ng/m) for the mice fed with bran fortified bread diet, were HCT: (31.2%), TIBC: (361Ug/dl) for the mice fed with brown fortified bread diet, were TRF: (8,6%) for the mice fed with white fortified bread diet, and RBC: 3.4 for the mice fed with brown and bran fortified bread diet. The highest increase among all tests was in hemoglobin and serum iron level for the (white, brown and bran) bread respectively, especially for the fortified ones.

Keyword: Iron, bioavailability, bread, mice, hematological, biochemical tests.

* **Professor – Department of Nutritional and Analytical Chemistry, Faculty of Pharmacy, Tishreen University, Lattakia, Syria.**
** **Postgraduate Student – Department of Nutritional and Analytical Chemistry, Faculty of Pharmacy, Tishreen University, Lattakia, Syria.**

التوافر الحيوي للحديد في الخبز (الأبيض، الأسمر والنخالة) لدى الفئران

د. مفيد ياسين*

أية الله حسن يوسف**

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□ ملخّص □

تمّ تقييم تأثير التوافر الحيوي لكبريتات الحديدي 100 مغ /كغ في ثلاثة أنواع من الخبز المدعم بدءاً من دقيق القمح ذو درجة الاستخلاص 80% على مجموعة مكونة من 27 فأر، وذلك بتطبيق طريقة الحذف–التزويد، حيث غذيّت الفئران بحمية منخفضة الحديد لمدة 14 يوم طول فترة الحذف، بعدها قُسّمت إلى سبعة مجموعات بحسب نوع الحمية الغذائية كالتالي: خبز أبيض غير مدعم (A1)، خبز أبيض مدعم (A2)، خبز أسمر غير مدعم (B1)، خبز أسمر مدعم (B2)، خبز نخالة غير مدعم (C1)، خبز نخالة مدعم (C2)، ومجموعة العلف (D).

قيّم متوسط التركيب الكيميائيّ لدقيق القمح فكانت القيم كالآتي: الرطوبة: 12,6%، الرماد: 0,58%، البروتين: 11.2مغ / 100غ، الدسم 1.2مغ / 100غ، والحديد: 3.4مغ / 100غ. تمت دراسة المؤشرات البيوكيميائية والدموية التالية (كريات الدم الحمراء، الهيموغلوبين، الهيماتوكريت، حديد المصل، السعة الكلية الرابطة للحديد، الترانسفرين والفيرتين) لعينات الدم التي جُمعت في الأيام 1–15–36 من فترة التجربة. لوحظ زيادة في وزن حيوانات التجربة بشكل عام مع مرور الزمن، باستثناء مجموعة خبز النخالة غير المدعم، كما لوحظ تناقص في قيم المؤشرات البيوكيميائية والدموية مع نهاية فترة الحذف حيث سُجلت القيم الأعلى لهذه الاختبارات البيوكيميائية والدموية مع نهاية فترة الحمية والتي كانت كالتالي: الهيموغلوبين:13,1 g/dl، حديد المصل:52 µmol/l والفيرتين: 2.7 ng/m لدى الفئران التي تمت تغذيتها بخبز نخالة مدعم، الهيماتوكريت 31,2% والسعة الكلية الرابطة للحديد: 361 Ug/dl لدى الفئران التي تمت تغذيتها بخبز أسمر مدعم، الترانسفرين 8,6% لدى الفئران التي تمت تغذيتها بخبز أبيض مدعم و كريات الدم الحمراء:$10^6/mm^3$ 3,4 لدى الفئران التي تمت تغذيتها بخبز أسمر ونخالة مدعمين. لوحظ أن أعلى زيادة بين الاختبارات كانت لقيم الهيموغلوبين وحديد المصل لدى الفئران التي غُذّيت باستخدام الخبز (الأبيض، الأسمر والنخالة) على التوالي، وبشكل خاص لدى النوع المدعم منه.

الكلمات المفتاحية: حديد، توافر حيوي، خبز، فئران، دموية، اختبارات بيوكيميائية.

* **أستاذ – قسم الكيمياء التحليلية والغذائية، كلية الصيدلة، جامعة تشرين، اللاذقية، سورية.**

** **طالبة دراسات عليا – قسم الكيمياء التحليلية والغذائية، كلية الصيدلة، جامعة تشرين، اللاذقية، سورية.**

Introduction:

Iron is present in the body in small amounts (0.005% of body weight) plays key roles in many biological functions. Iron is presented in human body in two forms: hemic iron, incorporates in heme structure (approximately 65%) which is part of the hemoglobin, myoglobin and protein enzymes, its intestinal absorption could occur by an endocytic process, by receptor-mediated endocytosis or by heme transporters called heme carrier protein 1 (HCP1) [1], while the non-hemic iron, participates in the composition of some non-hemic enzymes, the uptake of non-hemic iron by enterocytes occurs through a divalent metal transmembrane receptor (DMT1) [2].

Anemia is considered one of the most serious health problems today, affecting all ages. According to WHO [3] (world health organization), it is at present the second most significant problem of public health, only surpassed by energy protein deficiency. For pre-school children and non-pregnant women of reproductive age, the proportion of anemia associated with iron deficiency was 25% and 37%, respectively.

in situation of anemia, iron metabolism is seriously impaired because there is a clear relationship between iron and cell oxidative damage processes, the reason is that generation of oxidants and antioxidants is modified in this situation, so the oxidative/antioxidative balance is also impaired. [4]

Many persons do not tolerate adequately the iron therapy due to difficulties associated with the ingestion of tablets and its adverse gastrointestinal effects, present in half of the patients. Most of the oral iron preparations contain ferrous salts, characterized by a low absorption, which is limited by the ingestion of some food and by the damage in the mucous intestine. [5].

High systemic iron burden is also associated with adverse effects arising from degradation of tissue ferritin and subsequent free radical damage of surrounding tissues [6] which highlights the importance to continue searching for new strategies to palliate and prevent this common nutritional deficiency

Bread is an important food in the daily diet of several populations around the world. It is generally produced from refined white flour that lacks the nutrients, fibers and bioactive components present in the bran, but other ingredients can be added to increase the nutritional value of bread without altering its properties or the eating habits of population. Refining of cereal products leads to an important loss of minerals and fibers and may have important consequences on such disease as obesity [7], type two diabetes [8], cancer [9] and cardiovascular disease (CVD) [10].

Cereals are food with high phytate content [11],[12], which is a strong inhibitor of non-heme iron absorption [13], [14], and its fortification with micronutrients is a strategy used worldwide particularly in less developed nations to fight iron deficiency, aiming to the reinstatement of micronutrient composition affected during processing.

Phytic acid content ranges from 200 to 400 mg/100 g in refined flour and 600–1000 mg/100 g in whole flour [15]. In wheat bran, it ranges from 3116 to 5839 mg/100 g dry weight [16]. During food processing of cereal products or during digestion, InsP6 is capable to fix metal cations and can form stable structures which are not able to diffuse through gastrointestinal wall [17].

One mmol of InsP6 is able to fix up to 6 mmol of bivalent metallic cations (Fe, Ca, Zn). This contributes to an essential demineralization of food in the case of high intake of fibers and cereals. During food fortification, processing and storage, numerous physio-chemical

and enzymatic processes take place which may greatly influence biological value of the products.

Phytic acid and phytate hydrolysis take place at all stages of the bread making process, being influenced by: flour extraction degree, the amount of yeast used, temperature and duration of fermentation, dough *pH* value, water amount in the dough, phytate solubility especially of its salts, additive presence such as ascorbic acid and sodium bicarbonate [18].

Many fortificants are used in wheat flour fortification, ferrous (sulfate, fumarate and citrate), NaFeEDTA and electrolytic iron, according to WHO (World Health Organization) ferrous sulfate (FeSO4) is the most recommended fortificant to be used in iron fortification programmers [19].

The importance and the aim of the study:

- **The importance of the study:**

people in developing countries are particularly vulnerable to ID (iron deficiency) due to a negative iron balance. This effect might be more profound in countries depending on Mediterranean diet which significantly rely on cereals especially wheat grain. Reports in Levant have shown that deteriorating standards of living and the soaring price of food economic blockade have forced many families to decrease their intake of the most nutritious foods. Therefore, the rates of anemia, especially IDA (iron deficiency anemia), are expected to increase due to shortages of food and medications, which highlights the importance of cereals fortification so that we designed this study to asses iron bioavailability in fortified wheat flour bread diets on mice [20][21] [22].

- **The aim of the study:**

1. Studying the bioavailability of iron in (white, brown and bran) bread used locally in Syria on mice by evaluating their hematological and biochemical parameters to investigate the efficiency of fortification strategy on the three bread types.
2. determination of (protein, lipids, ash, moisture and iron) in wheat flour.

Materials and methods:

I. Equipment:

Varian Atomic Absorption, Spectrophotometer model 220, kjheldal apparatus, Soxhlet extractor, air oven, muffle furnace, analytical balance, desiccator, Vortex mixer, Hot plate.

Table (1): methods used in analysis

target	Ash	Moisture	lipid	protein	iron
methods	Muffle furnace (550 C°)	Air oven (105 C°)	Soxhlet (n-hexane)	kjheldal	Flame-AAS

II. Reagents:

standard Iron solutions (1000 mg/l), iron sulfate (II) (FeSO4·7H20),concentrated nitric acid HNO3, concentrated sulfuric acid (H2SO4), selenium, potassium and copper sulfate k2SO4, CuSO4, boric acid (H3BO3), sodium hydroxide (NaOH), concentrated hydrochloric acid HCl, anhydrous sodium sulfate, n-hexane, deionized Water.

III. Production of bread:

The flour supplied by the General Company for Cereal Processing was used. Table sugar (sucrose), table salt and baking yeast were also used from local markets, and their percentages are listed in table (2).

Dough was made for (white, brown and bran) bread starting from wheat flour, while making each type of bread dough was divided into two parts: fortified with 100 mg/kg and non-fortified one, added bran ratio was (0%, 20% and 50%) for (white, brown and bran) respectively.

The recipe of dough preparation was as shown in Table 2. The flour was placed in the mixing bowl. The dehydrated yeast was added to the warm water and then added to the flour and mixed for 1 min followed by 10 min. Then the other ingredients were added and mixed well. The dough was divided into rounded pieces and left to rest under a cover for 10 min. dough pieces were transferred to the oven and baked. The bread loaves were cooled and packed in polyethylene bags for testing.

Table (2): formulation of wheat bran doughs.

	White bread	Brown bread	Bran bread
Wheat	100%	80%	50%
bran	0%	20%	50%
Dry yeast % (of wheat\bran mix)	1%	1%	1%
salt % (of wheat\bran mix)	1%	1%	1%
Sugar % (of wheat\bran mix)	1%	1%	1%
Water % (of wheat\bran mix)	40%	50%	55%

I.Experiment design:

Mice (3 months old) with an approximate initial weight of 20-30g, obtained from Atomic Energy commission (AEC) were housed in cages situated in a well-ventilated room. Mice had free access to food and double distilled water ad libitum, noting that fixed quantities were provided for each group. After one week of adaptation, mice were fed with low iron diet for two weeks (14 days) depletion period, then animals were randomly divided into 7 different groups receiving 7 different diets for three next weeks (21 days) as shown in table 3:

Table 3: diets presented to the groups

GROUP	**1**	**2**	**3**	**4**	**5**	**6**	**7**
DIETS	**A1**	**A2**	**B1**	**B2**	**C1**	**C2**	**D**

A1: white non-fortified bread, A2: white fortified bread, B1: brown non-fortified bread, B2: brown fortified bread, C1: bran non-fortified bread, C2: bran fortified bread, D: fodder

During the experiment at days (1-15 and 36) (day1: the start of depletion period, day 15: the beginning of repletion period until day 35) body weights were recorded and blood samples were collected from the heart (heart puncture) for the purpose of hematological and biochemical analysis.

At the end of the experimental periods, animals were fasted for 16h, anesthetized with intraperitoneal injection of pentobarbital (10 mg/kg) and sacrificed. Blood was drawn via

cardiac puncture; the blood was immediately placed in (EDTA and empty) tubes and sent to the laboratory for analysis.
The animal experiments were carried out in strict accordance with the recommendation of the European Guidelines for the Care and Use of Animals for Research Purposes.

II. Analysis:
Hematological and biochemical:
During diet period blood samples were taken to laboratory for hematological and biochemical tests to evaluate iron bioavailability including the following tests (red blood cells RBC, hemoglobin HG, hematocrit HCT, serum iron Fe, total iron binding capacity TIBC, transferrin TRF and ferritin).

Results and Discussion:

1- nutritional profile of Wheat flour, bran and bread:
Wheat flour, bran and bread types' nutritional profile was assessed in triplicate by analyzing (ash, moisture, proteins, lipids and iron)

Table (4): composition of Wheat flour

Sample	Moisture %	Ash %	Protein g/100g	Fat g/100g	Iron mg/100g
Wheat flour 80%	**12.6**	**0.58**	**11.2**	**1.2**	**3.4**
bran	**2.1**	**5.8**	**15.2**	**4.3**	**16.4**
White bread	**32.4**	**1.23**	**9.11**	**0.98**	**2.53**
Brown bread	**33.2**	**2.1**	**11.97**	**1.77**	**5.71**
Bran bread	**34.1**	**3.74**	**13.48**	**2.59**	**8.7**

The nutritional profile of Wheat flour is in table (4), as it is shown that wheat flour had a moisture, ash, protein, fat and iron content of 12.6%, 0.58%, 11.2 mg/100g, 1.2 mg/100g, 3.4 mg/100 g respectively.

2- hematological and biochemical tests of pre-diet period:
Table (5 and 6) clarifies the results of weight, hematological and biochemical tests at the first- and fifteenth-day during depletion and at the start of repletion period. It is noticed from table (5 and 6) a weight gain for mice while the results for hematological and biochemical tests decreased at the end of depletion weeks, it is might be ought to the low iron diet that we offered to the mice for 14 days.

Table (5): the results of weight recording, hematological and biochemical tests at the first-day of depletion

The first day	Weight	RBC $.10^6/mm^3$	HG g/dl	HCT %	IRON Fe μmol.L-1	TIBC Ug/dl	TRF %	Ferritin (ng/m)
	27.52	**2.95**	**8.9**	**27.5**	**32**	**341**	**7.1**	**3.8**
	26.27	**2.47**	**8.5**	**27.6**	**36**	**339**	**6.5**	**2.2**
	28.25	**2.87**	**9.3**	**26.3**	**29**	**332**	**6.7**	**2.7**

Table (6): the results of weight recording, hematological and biochemical tests at the fifteenth-day of depletion

The fifteenth day	Weight Day 1	Weight Day 14	RBC $.10^6/mm^3$	HG g/dl	HCT %	IRON Fe µmol.L-1	TIBC Ug/dl	TRF %	Ferritin (ng/m)
	28.53	30.18	2.86	7.2	26.57	29	328	6.8	3.2
	27.44	29.29	2.39	7.9	26.36	31	327	6.4	2.0
	28.98	30.58	2.56	6.9	27.28	27	335	6.5	2.1

3- hematological and biochemical tests of diet period:

Table (7): comparison of iron bioavailability with the results of weight recording, hematological and biochemical tests during diet period for the three bread types.

The thirty-six day	Weight before	Weight after	Weight gain	RBC $.10^6/mm^3$	HG g/dl	HCT %	IRON Fe µmol.L-1	TIBC Ug/dl	TRF %	Ferritin (ng/m)
White(u)	20.6	23.2	2.6	2,42	8.9	27.6	36	332	7.1	1.9
white(u)	22.5	23.1	0.6	2.92	9.8	27.3	33	349	6.8	2.1
white(u)	20.9	22.9	2	2.71	9.5	28.1	37	339	6.6	2.3
White(f)	21.38	22.95	1.57	3,1	10.3	27.9	44	332	8.6	2.2
white(f)	20.0	29.82	9.82	2.7	10.9	28.6	40	367	7.2	2.3
white(f)	20.34	25.49	5.15	2.9	10.4	28.5	49	347	7.3	2.2
brown(u)	24.63	26.91	2.28	2.7	10.2	26.9	39	339	7.7	2.3
brown(u)	22.08	25.14	3.06	2.8	9.9	28.7	37	347	7.4	2.1
Brown(u)	23.36	26.03	2.67	3.0	10.4	27.1	38	345	7.2	2.4
brown(f)	23.5	27.1	3.6	3.4	11.9	29.7	49	354	8.1	2.3
brown(f)	22.5	27.8	5.3	3.1	11.7	27.9	45	361	7.6	2.4
brown(f)	23.7	28.0	4.3	3.3	12.1	31.2	46	351	8.3	2.5
bran(u)	24.1	22.6	-1.5	3.1	11.6	28.9	43	342	7.6	2.5
bran(u)	23.8	22.1	-1.7	3.0	11.4	29.2	42	350	7.9	2.4
bran(u)	23.1	21.9	-1.2	2.9	10.8	29.6	41	328	8.2	2.3
bran(f)	28.72	29.43	0.71	3.2	13.6	30.8	49	359	7.9	2.3
bran(f)	28.32	31.52	3.2	3.4	13.1	29.9	52	338	8.3	2.4
Bran(f)	29.16	30.17	1.01	3.1	12.9	29.7	48	349	7.4	2.7
Fodder	26.58	31.28	4.7	2,23	7.9	27.2	32	332	5.9	2.0
Fodder	25.17	30.69	5.52	2.52	8.8	27.3	29	329	6.5	2.1
fodder	25.92	30.35	4.43	2.92	7.5	27.5	31	331	6.4	2.2

Table 7 summarizes the results of comparative bioavailability of iron in seven different diets during the repletion period. The highest values for hematological and biochemical tests were HG:(13.1g/dl), Serum iron: (52µmol.L-1) and Ferritin: (2.7ng/m) for the mice fed with bran fortified bread diet, were HCT: (31.2**%**), TIBC: 361 (Ug/dl) for the mice fed with brown fortified bread diet, were TRF: (8,6**%)** for the mice fed with white fortified bread diet, and RBC: 3.4 for the mice fed with brown and bran fortified bread diet.

As we notice there was light increase in the hematological and biochemical tests, for red blood cells (RBC), total iron binding capacity (TIBC), transferring (TRF), ferritin and

hematocrit (packed cell volume) values, whereas there was high increase in hemoglobin and serum iron level for the (white, brown and bran) bread respectively, especially for the fortified ones. The essential changes of hemoglobin concentration to iron diets have long been the primary indicator for evaluating the efficacy or effectiveness of intervention programs. Since the serum iron refers to ferric ions (Fe3+) bound to serum transferrin and its concentration is highly variable affected by dietary iron intake for this reason serum iron is almost always measured with other iron tests, including ferritin, transferrin, and calculated total iron-binding capacity (TIBC) and transferrin saturation.

Bread making is a multiphase process in which fermentation and baking are the most important phases [23], Gargari et al [24] showed significant decrease (approximately 60%) in phytic acid content of flour during bread making.

Since that high water content in the dough increase the hydrolysis of phytic acid [25], the rate of hydrolysis and the concequent decrease of the phytic acid content depend on several parameters including starter fermentation cultures [26], phytase activity, temperature, Ph, water content, fermentation time and added enzymes [27].

Qazi et al [28] found that the phytic acid content of leavened bread was lower than the control (unleavened) bread of about more than 60%, this may be explained by natural fermentation which provides optium ph conditions for enzymatic degradation of phytic acid [29] that activates phytase enzymes which is naturally present in wheat and yeast [27], moreover tannin content may be reduced in some fermented cereals leading to increased absorption if iron [30].

Subsequently fermentation is one of the most economic and effective measures for reducing the content of anti-nutritional factors .

Transferrin saturation (TSAT) is the value of serum iron divided by the total iron-binding capacity of the available transferrin, measured as a percentage. TSAT calculated average values were (13.4%) for the fortified bread and (11.3%) for the non-fortified bread, and it is noticed from the results that Transferrin saturation is raised in iron overload with fortified diets and failed in iron deficiency with unfortified diets.

Results of paired t-test showed that t-calculated for red blood cells (RBC), total iron binding capacity (TIBC), hemoglobin, serum iron and hematocrit was bigger than t-tabulated which indicates a significant difference between the fortified and non-fortified bread diets and clarifies the effect of bread fortification on these tests unlike transferrin and ferritin tests whose t-calculated was lower than t-tabulated.

We also note from table (7) that weight gain is more obvious for fortified bread compared to unfortified one, knowing that weight gain decreased when bran content has risen with observing that the largest weight gain was for the fodder group knowing that 40 to 70% of BMI variation is due to genetic factors [31] and that we provided fixed quantities of food to each group with the freedom to eat to the point of satiety.

- Tables (8-9-10) shows LSD values for HG, HCT, Serum Iron, TIBC, Ferritin and a weight whereas tables (11-12-13-14) shows Mean, Std Deviation and Std Error values for weight, HG, HCT, Serum Iron, TIBC and Ferritin.

Table (8): LSD values for HG, HCT and Serum Iron

Dependent Variable: HG LSD			Dependent Variable: HCT LSD			Dependent Variable: Serum Iron LSD		
White (f)	End of depletion	.0000	White (f)	End of depletion	.1580	White (f)	End of depletion	.0040
	White (u)	.0050		White (u)	.3040		White (u)	.0000
	Brown (f)	.0410		Brown (f)	.8750		Brown (f)	.1720
	Brown (u)	.0000		Brown (u)	.0070		Brown (u)	.0000
	Bran (f)	.0000		Bran (f)	.0240		Bran (f)	.0030
	Bran (u)	.0000		Bran (u)	.0010		Bran (u)	.0000
	fodder	.0010		fodder	.6030		fodder	.0230
White (u)	End of depletion	.0000	White (u)	End of depletion	.0220	White (u)	End of depletion	.0000
	White (f)	.0050		White (f)	.3040		White (f)	.0000
	Brown (f)	.3030		Brown (f)	.2400		Brown (f)	.0040
	Brown (u)	.0010		Brown (u)	.0610		Brown (u)	.2280
	Bran (f)	.0490		Bran (f)	.1710		Bran (f)	.2280
	Bran (u)	.0000		Bran (u)	.0110		Bran (u)	.0110
	fodder	.0000		fodder	.1310		fodder	.0000
Brown (f)	End of depletion	.0000	Brown (f)	End of depletion	.2050	Brown (f)	End of depletion	.0000
	White (f)	.0410		White (f)	.8750		White (f)	.1720
	white (u)	.3030		white (u)	.2400		white (u)	.0040
	Brown (u)	.0000		Brown (u)	.0050		Brown (u)	.0000
	Bran (f)	.0060		Bran (f)	.0170		Bran (f)	.0470
	Bran (u)	.0000		Bran (u)	.0010		Bran (u)	.0000
	fodder	.0000		fodder	.7150		fodder	.0010
Brown (u)	End of depletion	.0000	Brown (u)	End of depletion	.0000	Brown (u)	End of depletion	.0000
	White (f)	.0000		White (f)	.0070		White (f)	.0000
	White (u)	.0010		White (u)	.0610		White (u)	.2280
	Brown (f)	.0000		Brown (f)	.0050		Brown (f)	.0000
	Bran (f)	.0850		Bran (f)	.5670		Bran (f)	.0230
	Bran (u)	.0020		Bran (u)	.4080		Bran (u)	.1270
	fodder	.0000		fodder	.0020		fodder	.0000
Bran (f)	End of depletion	.0000	Bran (f)	End of depletion	.0010	Bran (f)	End of depletion	.0000
	White (f)	.0000		White (f)	.0240		White (f)	.0030
	White (u)	.0490		White (u)	.1710		White (u)	.2280
	Brown (f)	.0060		Brown (f)	.0170		Brown (f)	.0470
	brown (u)	.0850		brown (u)	.5670		brown (u)	.0230
	Bran (u)	.0000		Bran (u)	.1710		Bran (u)	.0010
	fodder	.0000		fodder	.0080		fodder	.0000
Bran (u)	End of depletion	.0000	Bran (u)	End of depletion	.0000	Bran (u)	End of depletion	.0000
	White (f)	.0000		White (f)	.0010		White (f)	.0000
	White (u)	.0000		White (u)	.0110		White (u)	.0110
	Brown (f)	.0000		Brown (f)	.0010		Brown (f)	.0000
	Brown (u)	.0020		Brown (u)	.4080		Brown (u)	.1270
	Bran (f)	.0000		Bran (f)	.1710		Bran (f)	.0010
	fodder	.0000		fodder	.0000		fodder	.0000
Fodder	End of depletion	.0490	Fodder	End of depletion	.3560	Fodder	End of depletion	.3840
	White (f)	.0010		White (f)	.6030		White (f)	.0230
	White (u)	.0000		White (u)	.1310		White (u)	.0000
	Brown (f)	.0000		Brown (f)	.7150		Brown (f)	.0010
	Brown (u)	.0000		Brown (u)	.0020		Brown (u)	.0000
	Bran (f)	.0000		Bran (f)	.0080		Bran (f)	.0000
	Bran (u)	.0000		Bran (u)	.0000		Bran (u)	.0000

Table (9): LSD values for TIBC, TRF and Ferritin.

Dependent Variable: TIBC LSD			Dependent Variable: TRF LSD			Dependent Variable: Ferritin LSD		
White (f)	End of depletion	.2030	White (f)	End of depletion	.4310	White (f)	End of depletion	0.150
	White (u)	.2670		White (u)	.0180		White (u)	0.554
	Brown (f)	.6330		Brown (f)	.0880		Brown (f)	0.461
	Brown (u)	.0590		Brown (u)	.0030		Brown (u)	0.192
	Bran (f)	1.000		Bran (f)	.0050		Bran (f)	0.192
	Bran (u)	.2670		Bran (u)	.0060		Bran (u)	0.116
	fodder	.2330		fodder	.1050		fodder	1.000
White (u)	End of depletion	.0250	White (u)	End of depletion	.0030	White (u)	End of depletion	0.378
	White (f)	.2670		White (f)	.0180		White (f)	0.554
	Brown (f)	.5160		Brown (f)	.4310		Brown (f)	0.882
	Brown (u)	.3890		Brown (u)	.3770		Brown (u)	0.461
	Bran (f)	.2670		Bran (f)	.5530		Bran (f)	0.461
	Bran (u)	1.000		Bran (u)	.6200		Bran (u)	0.306
	fodder	.0300		fodder	.0010		fodder	0.554
Brown (f)	End of depletion	.0880	Brown (f)	End of depletion	.0180	Brown (f)	End of depletion	0.461
	White (f)	.6330		White (f)	.0880		White (f)	0.461
	white (u)	.5160		white (u)	.4310		white (u)	0.882
	Brown (u)	.1410		Brown (u)	.1050		Brown (u)	0.554
	Bran (f)	.6330		Bran (f)	.1760		Bran (f)	0.554
	Bran (u)	.5160		Bran (u)	.2080		Bran (u)	0.378
	fodder	.1040		fodder	.0030		fodder	0.461
Brown (u)	End of depletion	.0040	Brown (u)	End of depletion	.0010	Brown (u)	End of depletion	0.882
	White (f)	.0590		White (f)	.0030		White (f)	0.192
	White (u)	.3890		White (u)	.3770		White (u)	0.461
	Brown (f)	.1410		Brown (f)	.1050		Brown (f)	0.554
	Bran (f)	.0590		Bran (f)	.7660		Bran (f)	1.000
	Bran (u)	.3890		Bran (u)	.6920		Bran (u)	0.766
	fodder	.0050		fodder	.0000		fodder	0.192
Bran (f)	End of depletion	.2030	Bran (f)	End of depletion	.0010	Bran (f)	End of depletion	0.882
	White (f)	1.000		White (f)	.0050		White (f)	0.192
	White (u)	.2670		White (u)	.5530		White (u)	0.461
	Brown (f)	.6330		Brown (f)	.1760		Brown (f)	0.554
	brown (u)	.0590		brown (u)	.7660		brown (u)	1.000
	Bran (u)	.2670		Bran (u)	.9210		Bran (u)	0.766
	fodder	.2330		fodder	.0000		fodder	0.192
Bran (u)	End of depletion	.0250	Bran (u)	End of depletion	.0010	Bran (u)	End of depletion	0.882
	White (f)	.2670		White (f)	.0060		White (f)	0.116
	White (u)	1.000		White (u)	.6200		White (u)	0.306
	Brown (f)	.5160		Brown (f)	.2080		Brown (f)	0.378
	Brown (u)	.3890		Brown (u)	.6920		Brown (u)	0.766
	Bran (f)	.2670		Bran (f)	.9210		Bran (f)	0.766
	fodder	.0300		fodder	.0000		fodder	0.116
Fodder	End of depletion	.9310	Fodder	End of depletion	.3770	Fodder	End of depletion	0.150
	White (f)	.2330		White (f)	.1050		White (f)	1.000
	White (u)	.0300		White (u)	.0010		White (u)	0.554
	Brown (f)	.1040		Brown (f)	.0030		Brown (f)	0.461
	Brown (u)	.0050		Brown (u)	.0000		Brown (u)	0.192
	Bran (f)	.2330		Bran (f)	.0000		Bran (f)	0.192
	Bran (u)	.0300		Bran (u)	.0000		Bran (u)	0.116

Table (10): LSD values for Weight.

Dependent Variable: Weight LSD					
White (f)	End of depletion	0.000	Brown (u)	End of depletion	0.049
	White (u)	0.016		White (f)	0.001
	Brown (f)	0.018		White (u)	0.186
	Brown (u)	0.001		Brown (f)	0.170
	Bran (f)	0.450		Bran (f)	0.000
	Bran (u)	0.000		Bran (u)	0.026
	fodder	0.000		fodder	0.013
White (u)	End of depletion	0.003	Bran (f)	End of depletion	0.000
	White (f)	0.016		White (f)	0.450
	Brown (f)	0.958		White (u)	0.003
	Brown (u)	0.186		Brown (f)	0.003
	Bran (f)	0.003		brown (u)	0.000
	Bran (u)	0.001		Bran (u)	0.000
	fodder	0.001		fodder	0.000
Brown (f)	End of depletion	0.003	Bran (u)	End of depletion	0.754
	White (f)	0.018		White (f)	0.000
	white (u)	0.958		White (u)	0.001
	Brown (u)	0.170		Brown (f)	0.001
	Bran (f)	0.003		Brown (u)	0.026
	Bran (u)	0.001		Bran (f)	0.000
	fodder	0.001		fodder	0.725
Brown (u)	End of depletion	0.049	Fodder	End of depletion	0.508
	White (f)	0.001		White (f)	0.000
	White (u)	0.186		White (u)	0.001
	Brown (f)	0.170		Brown (f)	0.001
	Bran (f)	0.000		Brown (u)	0.013
	Bran (u)	0.026		Bran (f)	0.000
	fodder	0.013		Bran (u)	0.725

Table (11): Mean, Std. Deviation and Std Error values for Weight and HG.

weight	Mean	Std. Deviation	Std. Error	HG	Mean	Std. Deviation	Std. Error
End of depletion	30.0167	0.66033	0.38124	End of depletion	7.3333	0.51316	0.29627
fodder	30.7733	0.47057	0.27168	fodder	8.0667	0.66583	0.38442
White (f)	23.0667	0.15275	0.08819	White (f)	9.4000	0.45826	0.26458
White (u)	26.0867	3.47365	2.00551	White (u)	10.5333	0.32146	0.18559
Brown (f)	26.0267	0.88500	0.51096	Brown (f)	10.1667	0.25166	0.14530
Brown (u)	27.6333	0.47258	0.27285	Brown (u)	11.9000	0.20000	0.11547
Bran (f)	22.2000	0.36056	0.20817	Bran (f)	11.2667	0.41633	0.24037
Bran (u)	30.3733	1.05973	0.61184	Bran (u)	13.2000	0.36056	0.20817

Table (12): MEAN, Std. Deviation, Std Error values for HCT and SERUM IRONE.

HCT	Mean	Std. Deviation	Std. Error	SERUM IRONE FE	Mean	Std. Deviation	Std. Error
End of depletion	26.7367	0.48211	0.27835	End of depletion	29.0000	2.00000	1.15470
fodder	27.3333	0.15275	0.08819	fodder	30.6667	1.52753	0.88192
White (f)	27.6667	0.40415	0.23333	White (f)	35.3333	2.08167	1.20185
White (u)	28.3333	0.37859	0.21858	White (u)	44.3333	4.50925	2.60342
Brown (f)	27.5667	0.98658	0.56960	Brown (f)	38.0000	1.00000	0.57735
Brown (u)	29.6000	1.65227	0.95394	Brown (u)	46.6667	2.08167	1.20185
Bran (f)	29.2333	0.35119	0.20276	Bran (f)	42.0000	1.00000	0.57735
Bran (u)	30.1333	0.58595	0.33830	Bran (u)	49.6667	2.08167	1.20185

Table (13): MEAN, Std. Deviation, Std Error values for TIBC and TRF.

TIBC	Mean	Std. Deviation	Std. Deviation	TRF	Mean	Std. Deviation	Std. Error
End of depletion	330.0000	4.35890	2.51661	End of depletion	6.5667	0.20817	0.12019
fodder	330.6667	1.52753	0.88192	fodder	6.2667	0.32146	0.18559
White (f)	340.0000	8.54400	4.93288	White (f)	6.8333	0.25166	0.14530
White (u)	348.6667	17.55942	10.13794	White (u)	7.7000	0.78102	0.45092
Brown (f)	343.6667	4.16333	2.40370	Brown (f)	7.4333	0.25166	0.14530
Brown (u)	355.3333	5.13160	2.96273	Brown (u)	8.0000	0.36056	0.20817
Bran (f)	340.0000	11.13553	6.42910	Bran (f)	7.9000	0.30000	0.17321
Bran (u)	348.6667	10.50397	6.06447	Bran (u)	7.8667	045092	0.26034

Table (14): MEAN, Std. Deviation, Std Error values for Ferritin.

Ferritin	Mean	Std. Deviation	Std. Deviation
End of depletion	30.0167	0.66033	0.38124
fodder	30.7733	0.47057	0.27168
White (f)	23.0667	0.15275	0.08819
White (u)	26.0867	3.47365	2.00551
Brown (f)	26.0267	0.88500	0.51096
Brown (u)	27.6333	0.47258	0.27285
Bran (f)	22.2000	0.36056	0.20817
Bran (u)	30.3733	1.05973	0.61184

Conclusion:

We note from the study that hematological and biochemical tests for the fortified diet groups was higher than unfortified ones, this increase can be seen with the transition from white to brown and bran bread because of the higher content of bran rich in minerals including iron, although the content of anti-nutrients such as phytic acid has also increased this can be explained by the fermentation process which can be considered one of the most important global measures in reducing the phytic acid content and one of the most essential steps in making Arabic bread, which increases the bioavailability of minerals including iron.

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