

Nutritional and Sensory Properties of Flat Bread Made from Fenugreek Seeds and Rice Bran and Its Effect on Hypercholesterolemic Rats

Manal M. E. M. Shehata* & Hanan El-sayed**

*Food Sci. Dept. (Rural Home Economics - Food Science and Nutrition), Fac. Agric., Zagazig Univ., Egypt

**Food Sci. Dept. (Rural Home Economics), Fac. Agric., Zagazig Univ., Egypt

E-mail: manal.m.e.shehata@gmail.com



مجلة البحوث في مجالات التربية النوعية

معرف البحث الرقمي DOI: 10.21608/jedu.2021.85365.1410

المجلد الثامن العدد 39 . مارس 2022

التقييم الدولي

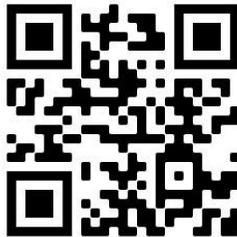
P-ISSN: 1687-3424

E- ISSN: 2735-3346

موقع المجلة عبر بنك المعرفة المصري <https://jedu.journals.ekb.eg/>

موقع المجلة <http://jrfse.minia.edu.eg/Hom>

العنوان: كلية التربية النوعية . جامعة المنيا . جمهورية مصر العربية



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Abstract

The resurgence of food-related chronic diseases has become a public health problem. The main target of the present research was to investigate the utilize of germinated fenugreek seeds (GFS) and stabilized rice bran (SRB) as a source of nutrients and bioactive compounds to prepare functional bread (FB) for lowering blood lipids in hypercholesterolemic rats. FB was formulated by wheat flour (WF), GFS and SRB by the ratio of 100:0:0 (B1), 90:10:0 (B2), 80:10:10 (B3), 75:10:15 (B4) and 70:10:20 (B5), respectively. FB was examined for chemical, sensory and antioxidant characteristics. Effect of FB on growth, biochemical parameters and histological examination of the liver of hypercholesterolemic rats were also evaluated. SRB was an excellent source of fiber (14.00%) and fat (16.92%), while GFS were rich in protein (32.80%) and fiber (10.80%). GFS and SRB had significant levels of total phenolic (TP), total flavonoids (TF) and antioxidant activity (AA). FB was sensory accepted up to 10%GFS+15%SRB (B4). Protein and AA values of (B4) increased by 24.0 and 63.0%, respectively, moreover (TP), (TF) and fiber values were almost 11-fold, 33-fold and 6-fold, respectively, more than control. Feeding hypercholesterolemic rats bread containing 10%GFS(G4), 10%GFS+10%SRB (G5) and

10%GFS+15%SRB (G6) significantly improved ($P\leq 0.05$) lipid profile, liver & kidney functions. The best findings were recorded in (G6), where the biochemical & histological changes were restored close to the normal status. This study recommends that intake of food products containing GFS and SRB may be beneficial for patients who suffer from hypercholesterolemia due to its nutritional and therapeutic properties.

Key words: Hypercholesterolemia, germinated fenugreek seeds, stabilized rice bran, sensory evaluation, bread

Introduction

Diet and nutrition are closely related to the etiology of hypercholesterolemia. Definition of hypercholesterolemia is a lipid disorder described by elevated cholesterol and low-density lipoprotein serum levels, also usually know as dyslipidemia, which may be followed by a reduction in high-density lipoprotein, a rise in triglycerides, or qualitative lipid anomalies (**Verbeek et al., 2018**). Hypercholesterolemia is one of the main risk factors for causing cardiovascular diseases (CVDs). These diseases (CVDs) are a dangerous problem that usually occurs in developing countries (**Woudberg et al., 2016**). According to WHO, the first reason for death worldwide is CVDs. Each year, 17.9 million persons die from CVDs, an estimated 31 percent of all deaths worldwide. Moreover, in countries with low and middle incomes, more than 75 percent of deaths occur by CVDs. Unhealthy diets, lack of sports activity and tobacco use are the most important risk factors to consider for CVDs (**WHO, 2020**). WHO reports that 40 percent of total deaths in Egypt are caused by CVDs. These diseases (CVDs) are a main public health concern with substantial economic and social consequences in terms of health care needs, productivity loss and premature death (**WHO, 2018**).

Rice (*Oryza sativa*) bran (RB) is considered a beneficial by-product of milled rice, and it can be utilized for the nutrition of

humans and animals. RB can be utilized in bakery products as nutritional improvement, meat substitute, functional and pharmaceutical products (**Sharif et al., 2014**). Consequently, value addition to by-products improves the product for consumers in addition generates extra income for industries (**Iriundo-dehond et al., 2018**). RB includes almost 10% of the weight of coarse rice but 60% of the nutrients (**Hu et al., 1996**). RB contains high amounts of protein (11–17%), fat (12–22%), fiber (6–14%), ash (8–17%) and vitamins such as vitamin E, B1 & B3, moreover substantial content of minerals including Fe, Ca, Mg and K (**Sharif et al., 2014**). RB is an excellent source of bioactive phytochemicals as tocopherols, γ -oryzanol and tocotrienols, which have health useful characteristics and antioxidant activity (**Moongngarm et al., 2012**). RB can prevent several diseases such as cancer, obesity and diabetes, as it has many bioactive components (**Bodie et al., 2019**).

Fenugreek (*Trigonella foenum-graecum*) is a member of the *Fabaceae* family, which is dicotyledonous (**Parchin et al., 2021**). It is named “Halba” in Egypt. Fenugreek seeds (FS) are an excellent source of protein (25–35%), fatty acids (5.0–7.5%), which are predominantly linoleic, linolenic and oleic. FS have a substantial amount of vitamins A, C, B1, B2, B3 and nicotinic acid, moreover minerals like calcium and iron (**Naidu et al., 2011; Singh et al., 2020**). FS have medicinal properties like hypocholesterolemic, antibacterial, anticancer, hepatoprotective effect, lactation-aiding and antidiabetic. FS have been utilized as a food stabilizer, food emulsifier, food adhesive and gum, and also to produce different kinds of bakery products moreover, extruded products (**Wani and Kumar, 2018**).

Bread is considered a popular baked product throughout the world (**Kourkouta et al., 2017**). Hence, bread can be targeted as an effective delivery means for bioactive components and essential nutrients found in plant-based food by-products (**Boll et al., 2016**). There is an elevated in consumers acceptance and

demand for bread that contains health benefits in addition its usual nutritional value and this bread is known as functional bread (**Birch and Bonwick, 2019**).

Previous researchers are used RB and FS separately in fortifying food products (**Chaubey et al., 2018; Bultum et al., 2020**). Combining cheaper by-products such as RB with main food components and FS which have nutritional and therapeutic benefits, are useful for developing food products for low-income consumers in Egypt. Moreover, diet plays an important role in reducing hypercholesterolemia. Therefore, this paper aimed to study the nutritional and sensory properties of flat bread made from fenugreek seeds and rice bran and its effect on hypercholesterolemic rats.

Materials and Methods

Materials

Fresh rice bran was collected from Sharkia Mills Company, Sharkia Governorate, Egypt. Fenugreek seeds, wheat flour (72%), dry yeast and salt were purchased from the local market in Sharkia Governorate, Egypt. Purchasing of minerals, vitamins, casein and cellulose were from El-Gomhoriya Company for Trading Drugs, Chemicals and Medical Instruments, Sharkia Governorate, Egypt. Folin-Ciocalteu reagent, Gallic acid and 2,2-diphenyl- 1- picrylhydrazyl were procured from Sigma Chemical Co. (St. Louis, MO, USA). Kits for blood analysis were procured from Alkan-Medical Division Biocon, Germany.

Methods

Stabilization of RB by microwave

The moisture level of fresh RB was adjusted to 21% by adding deionized water before treatment. RB (100g) was packed in a microwave-safe polyethylene bag (thickness 0.5 cm). The microwave oven (LG MH8082X, Korea) was preheated at 800W for 3 min. RB was heated at 100% power for three min, and the temperature of SRB after heating was $108 \pm 2^\circ\text{C}$. The sample was cooled at room temperature (25°C), packed in air-tight

polyethylene bags and stored at 4-5°C (**Ramezanzadeh et al., 2000**).

Germination of FS

FS were cleaned and soaked in water for 12 h at 37°C. The soaked seeds were allowed to germinate in a muslin cloth tied loosely for 48 h in an incubator at 37°C with frequent watering. GFS were washed in distilled water, then dried at 55–60°C. The dried sample was ground to fine powder, then packed in zip-lock bags and stored in an air sealed container till use (**Lalit and Kochhar, 2017**).

Preparation of flat bread

Shamy flat bread preparation was done as described by **Yaseen and Shouk, (2011)** in five types (B1 to B5) with WF, GFS and SRB in the ratio of 100:0:0 (B1), 90:10:0 (B2), 80:10:10 (B3), 75:10:15 (B4) and 70:10:20 (B5), respectively. The ingredients were 100g flour, 0.5g active dry yeast, 1.5g salt & water as required. Flour and other ingredients were mixed, then the dough formula was left to ferment at 30°C for 30 min. The dough was divided into loaves and was left to re-ferment for 30 min at 30°C. Loaves were baked in an electric oven at 250°C for 3 min. Then loaves were cooled and packed in polyethylene bags till analysis.

Chemical analysis and energy value

Moisture, protein, ash and fiber were determined in tested samples according to **AOAC, (2005)**. Total carbohydrates were calculated by difference. Calculation of the energy values were as described by **Chaney, (2006)** as follows: Energy value (kcal/100g) = 4 (Protein + carbohydrates) + 9 (Fat).

DPPH radical scavenging activity assay (RSA)

RSA of GFS, SRB and bread was done by using 2,2 diphenyl-1-picrylhydrazyl according to **Brand-Williams et al. (1995)**.

Measurement of total phenolic (TP)

The amount of TP was determined as described by Singleton *et al.*, (1999). TP were expressed as milligrams of gallic acid equivalents (GAE) per g of sample.

Measurement of total flavonoids (TF)

TF were examined according to Zhishen *et al.*, (1999) method, and expressed as milligrams of quercetin equivalents (QE) per g of sample.

Sensory evaluation

A number of 20 trained panelists from the staff members of the Food Sci. Dept., Fac. Agric., Zagazig Univ., Egypt, was listed for sensory assessment of prepared bread. Prepared bread was given to the panelists in randomly with coded plates. Panelists were asked to assess each type of bread for crust color, odor, taste, texture, appearance & overall acceptability using a 9-points hedonic scale, ranging from 9 as like extremely to 1 as dislike extremely according to Meilgaard *et al.*, (2007).

Animals

Thirty-six male albino rats (Wistar strain) weighing about 120-130g were procured from the Faculty of Veterinary Medicine, Zagazig University, Sharkia Governorate, Egypt. Rats were lived individually in stainless steel cages under standard conditions of temperature, humidity with a 12-h light per 12-h dark cycle. Animals had access to the designated diet and water *ad libitum*. Rats were maintained for 7 days as adaptation period and fed on a standard diet (Reeves *et al.*, 1993).

Experimental design

Rats were separated randomly into six groups (named as G1, G2, G3, G4, G5 and G6), comprising 6 rats in each group. Normal control group (G1) was fed only the standard diet (-ve), while the other groups were fed the hypercholesterolemic high fat diet (HCHFD) which was prepared by adding 16% fat, 2% cholesterol and 0.2% cholic acid to the standard diet according to Harnafi *et al.*, (2009) for six weeks, substituting an equivalent

quantity of starch. Group (G2), rats were left as hypercholesterolemic control group and fed on HCHFD (+ve). Other groups (G3, G4, G5 and G6) were fed on HCHFD and 40% of control bread, 10% GFS bread, 10% GFS+10% SRB bread and 10% GFS+15% SRB bread, respectively (40% of the starch in the diet was replaced with bread) during the experiment period (6 weeks). Feed intake was estimated daily. The variation in body weight of experimental groups were recorded weekly throughout the test period.

Blood sampling

After the period of the experiment (6 weeks) and under diethyl ether anesthesia, blood samples were collected via cardiac puncture from animals after being fasted overnight. The serum was separated by centrifugation at 3000 rpm for 20 min, which was kept at -20 °C until the determination of blood parameters. Livers were removed from the rats and washed with normal saline. Afterward, blotted on filter paper and weighed. Livers of experimental rats were kept to be examined microscopically.

Biochemical analysis

Serum glucose levels were examined by **(Trinder, 1969)** method. Triglycerides (TG), Cholesterol (TC) and High-density lipoprotein (HDL-C) were estimated according to **Fossati and Prencipe, (1982), Richmond, (1973) and Burstein et al., (1970)**, respectively. Calculation of LDL-C, VLDL-C and atherogenic index (AI) was as described by **(Friedewald et al., 1972)**. Serum aspartate amino transferase (AST) & alanine amino transferase (ALT) were estimated by **(Reitman and Frankle, 1957)** method. Alkaline phosphatase (ALP) was determined as described by **Klein and Kaufman, (1967)**. Total protein and albumin levels were estimated by **Dumas, (1975)** and **Dumas et al., (1971)** methods, respectively. Urea & creatinine were measured by **(Patton and Crouch, 1977)** and **(Larson, 1972)** methods, respectively.

Histological examination

The liver intended for histological examination by light microscopy was removed and immediately fixed in 10% formalin solution. The histopathological was examined by **Suvarna *et al.*, (2013)** method.

Statistical analysis

The data were statistically analyzed by SPSS program, version 25. Statistical significance was carried out by using a one-way analysis of variance (ANOVA) and post-hoc Duncan test. Results were reported as mean \pm standard deviation. P-values were established as significant when ($p \leq 0.05$) (**Bailey, 1995**).

Results and Discussion

Chemical characteristics of WF, SRB and GFS

Chemical characteristics of WF and SRB, as well as GFS, are clarified in Table (1). Results displayed that fat (16.92%), ash (12.00%) and fiber (14.00%) were significantly higher ($p \leq 0.05$) in SRB than WF and GFS. **Bultum *et al.*, (2020)** stated that SRB had 16.85% (fat), 11.76% (ash), 17.05% (fiber), 14.68% (protein), 32.11% (carbohydrate) and 7.54% (moisture). The highest protein value was found in GFS (32.80%), and similar finding was obtained by **Badr, (2021)**. The highest amount of carbohydrate was estimated in WF (74.42%), followed by GFS (36.50%) and SRB (33.68%). WF contained significantly lower values ($p \leq 0.05$) of protein (11.50%), fat (1.06%), ash (0.51%) and fiber (0.61%) comparing with SRB and GFS. **Bultum *et al.*, (2020)** estimated that WF contained 11.21% protein, 1.67% fat, 1.84% ash, 0.61% fiber and 72.77% carbohydrate.

Table (1): Chemical characteristics of WF, SRB and GFS (on a fresh weight basis)

Component %	Raw Material		
	WF	SRB	GFS
Moisture	11.90±0.36 ^a	7.90±0.20 ^c	8.90±0.30 ^b
Protein	11.50±0.34 ^c	15.50±0.42 ^b	32.80±0.50 ^a
Fat	1.06 ±0.04 ^c	16.92±0.41 ^a	5.60±0.16 ^b
Fiber	0.61±0.02 ^c	14.00±0.35 ^a	10.80±0.30 ^b
Ash	0.51±0.02 ^c	12.00±0.32 ^a	5.40±0.20 ^b
Carbohydrate	74.42 ±0.90 ^a	33.68±0.60 ^c	36.50±0.65 ^b

Values with different letters (a-c) in the same row indicate a significant difference at ($p \leq 0.05$).

WF: Wheat flour; SRB: Stabilized rice bran; GFS: Germinated fenugreek seeds.

Table (2) demonstrates that TP and TF levels in SRB were 47.20 mg GAE/g and 20.00 mg QE/g, respectively, and these findings are close to the data get by **Hasim et al., (2017)**. Meanwhile, the antioxidant activity was 71.00% in SRB. Whereas, GFS had 72.22 mg GAE/g TP and 22.30 mg QE/g TF. **Pandey and Awasthi, (2015)** found that GFS contained 80.8 mg GAE/g TP. **Dixit et al., (2005)** reported that GFS contained 19.14 mg QE/g TF. Moreover, GFS contained 87.20% antioxidant activity. **Saleh et al., (2019)** stated that FS had 87.68% antioxidant activity.

Table (2): Phenolics, flavonoids & DPPH radical scavenging activity of SRB and GFS

Raw Material	DPPH %	TP mg GAE/g	TF mg QE/g
SRB	71.00±0.80	47.20±0.60	20.00±0.43
GFS	87.20±0.90	72.22±0.82	22.30±0.45

All values are means of 3 determinations ± SD.

SRB: Stabilized rice bran; GFS: Germinated fenugreek seeds.

Chemical analysis of different kinds of bread

The chemical analysis of bread prepared from GFS and SRB is clarified in Table (3) (on a fresh weight basis). Protein (13.36%), fiber (1.64%), ash (0.98%) and moisture (31.51%) contents in 10% GFS bread significantly increased ($p \leq 0.05$), while carbohydrate & energy values significantly decreased ($p \leq 0.05$) comparing with control bread. These findings probably are result to GFS contain higher amounts of protein, fiber & ash than WF. Similar findings were published by **Chaubey et al., (2018)**. Fat contents of bread did not differ significantly ($p \leq 0.05$) between

10% GFS and control. All component contents except carbohydrate and energy levels significantly increased ($p \leq 0.05$) as the supplementation level of SRB increased in bread compared with 10% GFS and control bread. Similar data were stated by **Bultum et al., (2020)**. The highest protein level (14.12%) was recorded in bread prepared from 10% GFS + 20% SRB, while the lowest protein level (11.27%) was determined in control. Also, the highest fiber, ash and fat levels were estimated in bread containing 10% GFS + 20% SRB. These results might be because of SRB has higher values of protein, fiber, fat and ash comparing with WF. Protein values of bread (B4 and B5) increased by 24.0 and 25.3%, respectively, comparing to (B1). Ash, fat and fiber contents in (B4) were approximately 5-fold, 3-fold and 6-fold, respectively, more than control bread. Whereas in (B5) were approximately 6-fold, 4-fold and 7-fold, respectively, higher than (B1). Energy value of bread in treatments B4 and B5 was 8.0 and 9.4% lower than control, respectively. The highest moisture value (34.72%) was noticed in (B5), while the lowest content (30.10%) was estimated in (B1). These findings perhaps are result to the elevate in water retention ability of fibers in GFS and SRB comparing with WF. RSA, TP and TF values significantly increased ($p \leq 0.05$) in bread prepared from 10% GFS or bread containing both 10% GFS and various values of SRB comparing with control (Table 3). These results perhaps are due to the elevate of RSA, TP and TF contents in GFS and SRB comparing to WF. The same findings were stated by **Chaubey et al., (2018)** and **Irakli et al., (2015)** for GFS and SRB, respectively. The highest RSA, TP and TF levels were showed in bread prepared from 10% GFS + 20% SRB, while the lowest amounts were estimated in control. TP and TF values in (B4) were almost 11-times and 33-times, respectively, more than (B1). Whereas in (B5) were approximately 12-times and 38-times, respectively, higher than (B1). RSA level of bread (B4 and B5) increased by 63.0 and 73.4%, respectively, comparing with (B1).

Table (3): Chemical analysis of bread containing GFS and SRB

Component	Flat bread sample				
	B1	B2	B3	B4	B5
Moisture%	30.10±0.60 ^c	31.51±0.63 ^d	33.22±0.61 ^c	33.91±0.70 ^b	34.72±0.65 ^a
Protein %	11.27±0.18 ^d	13.36±0.23 ^c	13.73±0.31 ^b	13.96±0.41 ^{ab}	14.12±0.38 ^a
Fat%	1.04±0.02 ^d	1.49±0.02 ^d	3.04±0.05 ^c	3.87±0.11 ^b	4.55±0.15 ^a
Fiber%	0.60±0.01 ^c	1.64±0.03 ^d	2.94±0.04 ^c	3.65±0.10 ^b	4.26±0.11 ^a
Ash%	0.50±0.01 ^c	0.98±0.02 ^d	2.11±0.03 ^c	2.70±0.03 ^b	3.20±0.07 ^a
Carbohydrate%	56.49±0.85 ^a	51.02±0.73 ^b	44.96±0.80 ^c	41.91±0.77 ^d	39.15±0.65 ^c
EV Kcal/100g	280.40±2.12 ^a	270.93±2.20 ^b	262.12±2.00 ^c	258.31±1.55 ^d	254.03±1.45 ^e
DPPH %	21.60±0.20 ^e	27.96±0.22 ^d	32.80±0.32 ^c	35.20±0.28 ^b	37.45±0.50 ^a
TP mg GAE/g	1.37±0.06 ^e	8.34±0.14 ^d	12.83±0.25 ^c	15.10±0.30 ^b	17.20±0.34 ^a
TF mg QE/g	0.16±0.01 ^e	2.34±0.03 ^d	4.26±0.05 ^c	5.26±0.10 ^b	6.10±0.12 ^a

Values with different letters (a-e) in the same row indicate a significant difference at ($p \leq 0.05$). B1: 100% WF (control); B2: 90% WF + 10% GFS; B3: 80% WF + 10% GFS + 10% SRB; B4: 75% WF + 10% GFS + 15% SRB; B5: 70% WF + 10% GFS + 20% SRB.

Sensory evaluation of different kinds of bread

The sensory evaluation of bread prepared from GFS and SRB are explained in Table (4). The data indicated that no any significant changes ($p \leq 0.05$) were noticed among control bread (B1) and (B2, B3 and B4) in crust color, odor, taste, texture, appearance & overall acceptability scores. While, all sensory properties significantly decreased ($p \leq 0.05$) in 10% GFS + 20% SRB (B5) bread comparing with control (B1). **Chaubey et al., (2018)** stated that 10% fortified toast bread with GFS were organoleptically acceptable. **Olusengun et al., (2017)** found that breads containing 10 and 15% levels of defatted RB were acceptable.

Table (4): Sensory evaluation of bread containing GFS and SRB

Sample	Crust color	Odor	Taste	Texture	Appearance	Overall acceptability
B1	8.50±0.36 ^a	8.13±0.33 ^a	8.30±0.32 ^a	8.50±0.33 ^a	8.00±0.31 ^a	8.40±0.36 ^a
B2	8.42±0.32 ^a	8.05±0.36 ^a	8.19±0.34 ^a	8.39±0.32 ^a	7.91±0.30 ^a	8.31±0.35 ^a
B3	8.30±0.21 ^a	7.92±0.25 ^a	8.10±0.26 ^a	8.23±0.27 ^a	7.80±0.27 ^a	8.20±0.24 ^a
B4	8.22±0.22 ^a	7.85±0.23 ^a	8.00±0.23 ^a	8.10±0.24 ^a	7.70±0.22 ^a	8.17±0.20 ^a
B5	7.80±0.20 ^b	7.40±0.21 ^b	7.45±0.21 ^b	7.40±0.22 ^b	7.30±0.20 ^b	7.20±0.18 ^b

Values with different letters (a-b) in the same column indicate a significant difference at ($p \leq 0.05$). B1: 100% WF (control); B2: 90% WF + 10% GFS; B3: 80% WF + 10% GFS + 10% SRB; B4: 75% WF + 10% GFS + 15% SRB; B5: 70% WF + 10% GFS + 20% SRB.

Biological evaluation

After a period of 6 weeks, the HCHFD fed group (G2) showed a statistically higher ($p \leq 0.05$) body weight gain (BWG), feed intake (FI) and feed efficiency ratio (FER) than the healthy

group (G1) fed with a standard diet (Table 5), and these data are in concurrence with the data published by **El-Anany and Ali, (2018)**. The incorporation of bread containing 10% GFS, 10% GFS + 10% SRB and 10% GFS + 15% SRB into the HCHFD caused significant reductions ($p \leq 0.05$) in the BWG, FI and FER of rats. The BWG of rats consumed the HCHFD containing 10% GFS (G4), 10% GFS + 10% SRB (G5) and 10% GFS + 15% SRB (G6) bread was 46, 51 and 55%, respectively, lower than that of rats consumed the HCHFD only (G2). These decreases in BWG might be due to the presence of the high level of fiber in GFS and SRB than WF (Table 1). **Konopelniuk, (2017)** demonstrated that treatment with FS significantly suppressed the elevate in the BWG of rats fed a high-calorie diet. Relative liver weight values revealed a significant elevate ($p \leq 0.05$) in (+ve) control group comparing with (-ve) control group. Rats that received diets containing 10% GFS (G4), 10% GFS + 10% SRB (G5) and 10% GFS + 15% SRB (G6) bread, showed a significant decrease ($p \leq 0.05$) in relative liver weight in comparison with (+ve) control group. The best finding was estimated in the 10% GFS + 15% SRB bread group (G6) comparing with (G2). **Bahnasy et al., (2020)** proved that rats fed on a diet that had FS clarified a significant decrease ($P < 0.05$) in relative liver weight comparing with (+ve) group in hepatic intoxicated rats.

Table (5): Effect of feeding hypercholesterolemic rats with bread containing GFS and SRB on BWG, FI, FER and relative liver weight

Group	Body weight gain (BWG) (g)	Feed intake (FI) (g/rat/42days)	Feed efficiency ratio (FER)	Relative liver weight (g)
G1	110.00±1.00 ^c	672.00±2.30 ^c	0.164±0.004 ^c	2.60 ±0.10 ^d
G2	200.00±1.20 ^a	798.00±2.50 ^a	0.251±0.006 ^a	4.82±0.22 ^a
G3	195.00±1.11 ^b	793.80±2.40 ^b	0.246±0.005 ^b	4.72±0.20 ^a
G4	108.00±1.00 ^d	672.00±2.35 ^c	0.161±0.003 ^d	3.90±0.15 ^b
G5	98.00±0.86 ^e	622.00±2.20 ^d	0.158±0.002 ^e	3.25±0.13 ^c
G6	90.00±0.72 ^f	588.00±2.00 ^e	0.153±0.002 ^f	2.55±0.11 ^d

Values with different letters (a-f) in the same column indicate a significant difference at ($p \leq 0.05$). G1: Healthy rats; G2: Hypercholesterolemic rats; G3: Hypercholesterolemic rats consumed control bread; G4: Hypercholesterolemic rats consumed 10% GFS bread; G5: Hypercholesterolemic rats consumed 10% GFS + 10% SRB bread; G6: Hypercholesterolemic rats consumed 10% GFS + 15% SRB bread.

The serum lipid profile of rats consumed bread containing GFS and SRB is presented in Table (6). Hypercholesterolemic rats (G2) revealed that significantly elevated ($p \leq 0.05$) in TG, TC, LDL-C and VLDL-C levels, whereas significantly decreased ($p \leq 0.05$) was noticed in HDL-C value compared with healthy rats (G1). Results did not indicate significant changes ($p \leq 0.05$) in the hypercholesterolemic rats consumed control bread (G3) comparing with (G2). Feeding hypercholesterolemic rats bread containing 10% GFS (G4), 10% GFS + 10% SRB (G5) and 10% GFS + 15% SRB (G6) significantly improved ($p \leq 0.05$) all lipid parameters by decreasing TG, TC, LDL-C and VLDL-C values, and increasing HDL-C level as comparing with (G2). The best findings were recorded in (G6), where TG, TC, LDL-C and VLDL-C values reduced by 60, 51.5, 64.8 and 60%, respectively, and HDL-C level increased by 42.6% comparing with (G2). All lipid profile values of (G6) were reached close to the normal range. The fiber, gum and saponin components of FS are most likely responsible for its hypocholesterolemic effect. Also, these findings probably as a result of RB contains bioactive ingredients, like fatty acids, protein and γ -oryzanol which lead to improving the lipid profile. **Bruce-Keller et al., (2020)** stated that feeding mice a high-fat diet with 2% FS led to reducing its blood lipids. **Zhang et al., (2020)** proved that rice bran phenolic extracts significantly improved the lipid profile of mice fed a high-fat diet.

Table (6): Effect of feeding hypercholesterolemic rats with bread containing GFS and SRB on serum lipid profile (mg/dl)

Group	TG	TC	HDL -C	LDL-C	VLDL-C
G1	87.30±0.90 ^d	140.00±1.10 ^d	49.00±0.60 ^a	73.54±0.80 ^f	17.46±0.22 ^d
G2	220.00±1.12 ^a	290.00±1.50 ^a	34.00±0.20 ^d	212.00±1.10 ^a	44.00±0.30 ^a
G3	219.50±1.11 ^a	289.40±1.32 ^a	34.10±0.24 ^d	211.40±1.12 ^a	43.90±0.32 ^a
G4	158.40±1.00 ^b	214.60±1.24 ^b	41.14±0.50 ^c	141.78±1.00 ^b	31.68±0.26 ^b
G5	112.00±0.91 ^c	166.00±1.33 ^c	45.80±0.63 ^b	97.80±0.81 ^c	22.40±0.20 ^c
G6	88.00±0.85 ^d	140.65±1.23 ^d	48.50±0.60 ^a	74.55±0.76 ^d	17.60±0.21 ^d

Values with different letters (a-f) in the same column indicate a significant difference at ($p \leq 0.05$). G1: Healthy rats; G2: Hypercholesterolemic rats; G3: Hypercholesterolemic rats consumed control bread; G4: Hypercholesterolemic rats consumed 10% GFS bread; G5: Hypercholesterolemic rats consumed 10% GFS + 10% SRB bread; G6: Hypercholesterolemic rats consumed 10% GFS + 15% SRB bread.

Calculation of atherogenic index (AI) and LDL-C/HDL-C ratio proved that the best result was in (G6) where 10% GFS + 15% SRB bread significantly suppressed ($p \leq 0.05$) the elevated values which returned close to the normal range. The reduction was 75% in the AI and LDL-C/HDL-C ratio (Table 7).

Table (7): Effect of feeding hypercholesterolemic rats with bread containing GFS and SRB on atherogenic index (AI) and LDL-C/HDL-C ratio

Group	AI	Change %	LDL-C/HDL-C ratio	Change %
G1	1.86±0.02 ^c	-	1.50±0.01 ^c	-
G2	7.53±0.11 ^a	+ 305	6.24±0.11 ^a	+ 316
G3	7.49 ±0.10 ^a	- 0.53	6.20±0.12 ^a	- 0.64
G4	4.22±0.04 ^b	- 44	3.45±0.05 ^b	- 45
G5	2.62±0.03 ^c	- 65	2.14±0.03 ^c	- 66
G6	1.90±0.02 ^c	- 75	1.54±0.02 ^c	- 75

Values with different letters (a-c) in the same column indicate a significant difference at ($p \leq 0.05$). G1: Healthy rats; G2: Hypercholesterolemic rats; G3: Hypercholesterolemic rats consumed control bread; G4: Hypercholesterolemic rats consumed 10% GFS bread; G5: Hypercholesterolemic rats consumed 10% GFS + 10% SRB bread; G6: Hypercholesterolemic rats consumed 10% GFS + 15% SRB bread.

Levels of the liver profile of animals in all groups are clarified in Table (8). AST, ALT and ALP activities significantly elevated ($p \leq 0.05$) in rats consumed HCHFD when comparing to the healthy group, and these results are in concurrence with the data obtained by **Zhang et al., (2020)**. Hypercholesterolemic rats treated with control bread (G3) did not appear any significant changes ($p \leq 0.05$) in all liver function parameters comparing to (G2). A significant reduction ($p \leq 0.05$) in AST, ALT and ALP activities was noticed in hypercholesterolemic rats consumed 10% GFS (G4), 10% GFS + 10% SRB (G5) and 10% GFS + 15% SRB (G6) bread comparing with (G2). Results revealed that (G6) recorded the best findings for liver biomarkers where AST, ALT and ALP levels decreased by 39, 41.2 and 46%, respectively in comparison to (G2), and values were reached close to the normal range. **Konopelniuk, (2017)** found that treatment with FS powder for 21 days significantly improved liver functions of rats fed a high-calorie diet. **Zhang et al., (2020)** stated that rice bran

phenolic extracts significantly improved liver biomarkers of mice fed a high-fat diet.

Serum total protein & albumin significantly reduced ($p \leq 0.05$) in hypercholesterolemic rats comparing to control ones (Table 8). In hypercholesterolemic rats consumed 10% GFS + 15% SRB bread (G6), serum total protein & albumin levels were restored close to the normal values. These findings perhaps are the result of antioxidant properties in both GFS and SRB which improve organ functions. **Bahnasy et al., (2020)** mentioned that rats who received diets containing FS (5%) revealed a significant elevate ($p \leq 0.05$) in total protein and albumin comparing to (+ve) group in hepatic intoxicated rats.

Table (8): Effect of feeding hypercholesterolemic rats with bread containing GFS and SRB on liver profile

Group	AST (U/L)	ALT (U/L)	ALP (U/L)	Protein (g/dl)	Albumin (g/dl)
G1	35.70±0.50 ^d	28.50±0.43 ^e	69.10±0.72 ^c	7.20±0.15 ^a	4.20±0.09 ^a
G2	60.00±0.72 ^a	50.00±0.54 ^a	130.00±1.12 ^a	4.90±0.08 ^c	2.90±0.04 ^c
G3	59.30±0.65 ^a	49.50±0.50 ^a	129.40±1.10 ^a	5.20±0.09 ^c	2.95±0.05 ^c
G4	48.00±0.51 ^b	39.50±0.40 ^b	98.80±0.90 ^b	6.13±0.10 ^b	3.50±0.06 ^{ab}
G5	40.00±0.50 ^c	32.90±0.35 ^c	80.00±0.86 ^c	6.55±0.20 ^{ab}	3.70±0.06 ^{ab}
G6	36.60±0.40 ^d	29.40±0.31 ^d	70.20±0.81 ^d	6.80±0.22 ^{ab}	3.82±0.08 ^a

Values with different letters (a-e) in the same column indicate a significant difference at ($p \leq 0.05$). G1: Healthy rats; G2: Hypercholesterolemic rats; G3: Hypercholesterolemic rats consumed control bread; G4: Hypercholesterolemic rats consumed 10% GFS bread; G5: Hypercholesterolemic rats consumed 10% GFS + 10% SRB bread; G6: Hypercholesterolemic rats consumed 10% GFS + 15% SRB bread.

Urea & creatinine values significantly increased ($p \leq 0.05$) in (G2) comparing with healthy rats (G1) (Table 9). Hypercholesterolemic rats that consumed control bread (G3) did not display any significant changes ($p \leq 0.05$) in all kidney function parameters that were measured in the serum, when compared with (G2). While, hypercholesterolemic rats consumed bread containing 10% GFS (G4), 10% GFS + 10% SRB (G5) and 10% GFS + 15% SRB (G6) recorded a significant reduction ($p \leq 0.05$) in urea & creatinine values compared with (G2). Urea & creatinine values in (G6) which were treated with 10% GFS + 15% SRB bread were restored close to the normal values. These findings

probably are the result of the antioxidant effect of GFS and SRB. **Darwish et al., (2020)** mentioned that Co-administration of dried and GFS along with gentamicin significantly prevented nephrotoxicity. **Siqueira et al., (2021)** proved that RB prevented renal disease by modulating risk factors in rats who consumed a high sugar-fat diet.

Serum glucose content was significantly elevated ($p \leq 0.05$) in animals fed on HCHFD (G2) in comparison to the normal group (G1) (Table 9). Six weeks after treating hypercholesterolemic rats with 10% GFS (G4), 10% GFS + 10% SRB (G5) and 10% GFS + 15% SRB (G6) bread, glucose values significantly reduced ($p \leq 0.05$) in treated groups comparing with (G2). These findings perhaps are the result of GFS and SRB contain high amounts of phenolic and flavonoid compounds than WF. **Bafadam et al., (2021)** noticed that glucose levels significantly decreased ($P < 0.001$) in animals with diabetes treated with fenugreek seed extract than the diabetic group. **Kubota et al., (2020)** estimated that rice bran protein improves diabetes, diabetic nephropathy and fatty liver in rats.

Table (9): Effect of feeding hypercholesterolemic rats with bread containing GFS and SRB on kidney profile and glucose (mg/dl)

Group	Urea	Creatinine	Glucose
G1	43.20±0.50 ^d	0.60±0.01 ^c	86.70±0.88 ^d
G2	60.00±0.80 ^a	1.60±0.05 ^a	230.00±1.20 ^a
G3	59.50±0.70 ^a	1.58±0.04 ^a	229.40±1.10 ^a
G4	49.20±0.50 ^b	1.12±0.03 ^b	144.90±1.00 ^b
G5	45.40±0.40 ^c	1.00±0.03 ^{bc}	106.00±0.83 ^c
G6	43.80±0.40 ^d	0.96±0.01 ^{bc}	87.40±0.73 ^d

Values with different letters (a-d) in the same column indicate a significant difference at ($p \leq 0.05$). G1: Healthy rats; G2: Hypercholesterolemic rats; G3: Hypercholesterolemic rats consumed control bread; G4: Hypercholesterolemic rats consumed 10% GFS bread; G5: Hypercholesterolemic rats consumed 10% GFS + 10% SRB bread; G6: Hypercholesterolemic rats consumed 10% GFS + 15% SRB bread.

Histological examination of liver

Histological examination of the healthy rat's liver was showed normal hepatic parenchyma with the preserved lobular pattern, portal triad structures, vascular tree, Kupffer cells and stromal component (Photo. 1 and Table 10). While, hypercholesterolemic rat's liver was displayed widely distributed areas of steatosis which represented by enlarged, sharp and clear vacuoles with peripherally located nuclei beside minute perivascular round cells infiltration and congested hepatic blood vessels were also detected (Photo. 2 and Table 10). The liver section of hypercholesterolemic rats consumed control bread revealed widely distributed areas of steatosis in the form of large or small sharp, clear vacuoles with peripherally located nuclei. Moreover, congestion of hepatic blood vessels and sinusoids were also seen (Photo. 3 and Table 10). Liver section of hypercholesterolemic rats consumed 10% GFS bread showed moderately distributed areas of degenerative changes mostly steatosis and hydropic degeneration besides congested hepatic blood vessels (Photo. 4 and Table 10). Photo. 5 and Table 10 clarified that mildly distributed areas of steatosis with hydropic degenerated cells in liver section of hypercholesterolemic rats fed on 10% GFS + 10% SRB bread. Photo. 6 and Table 10 displayed that apparently normal hepatic parenchyma with healthy hepatocyte and prominent Kupffer cells in liver section of hypercholesterolemic rats consumed 10% GFS + 15% SRB bread. These results probably are because of GFS and SRB contain a high amount of phenolic and flavonoid compounds comparing to WF. **Kandhare *et al.*, (2017)** reported that liver tissue of rats treated with glycosides based standardized fenugreek seed extract (20 and 40 mg/kg) clarified a reduction in bleomycin-induced hepatic injury. **Zhang *et al.*, (2020)** indicated that rice bran phenolic extracts could alleviate liver injury of mice fed a high-fat diet.

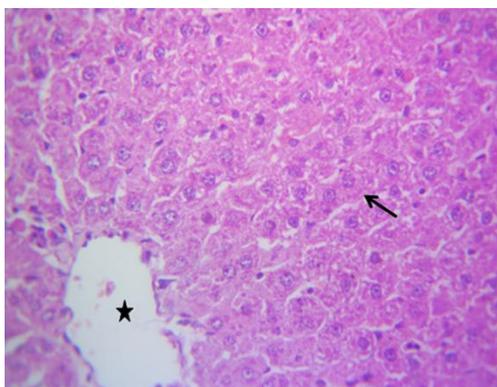


Photo. 1 (G1): Photomicrograph of liver showing normal hepatic parenchyma with the preserved lobular pattern (arrow) and vascular tree (star) (H&E X400).

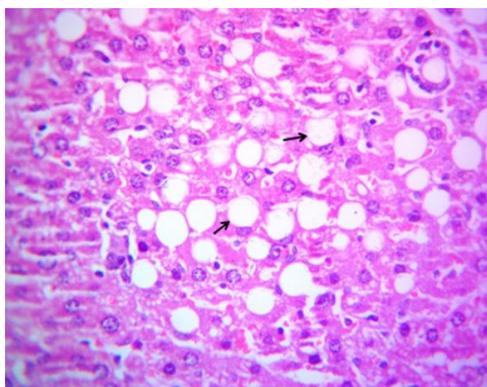


Photo. 2 (G2): Photomicrograph of liver showing widely distributed areas of steatosis (arrows) (H&E X400).

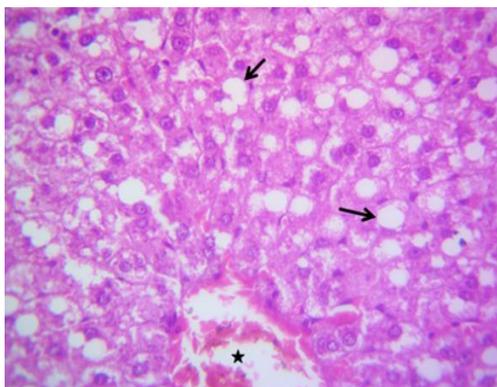


Photo. 3 (G3): Photomicrograph of liver showing widely distributed areas of steatosis (arrows) and congestion of hepatic blood vessels (star) (H&E X400).

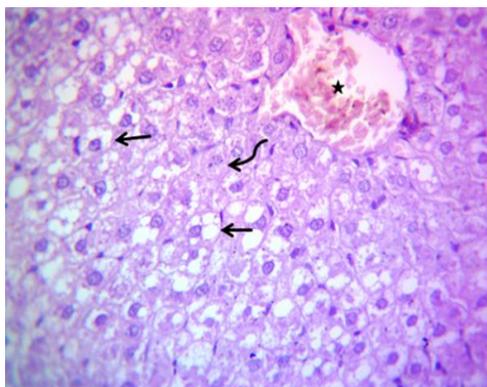


Photo. 4 (G4): Photomicrograph of liver showing moderately distributed areas of steatosis (arrows) and hydropic degeneration (curved arrow) with congested hepatic blood vessels (star) (H&E X400).

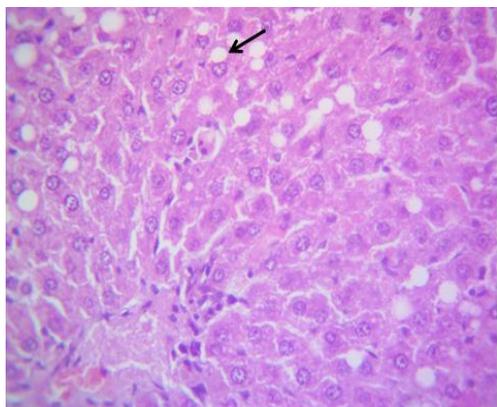


Photo. 5 (G5): Photomicrograph of liver showing mild distributed areas of steatosis (arrow) (H&E X400).

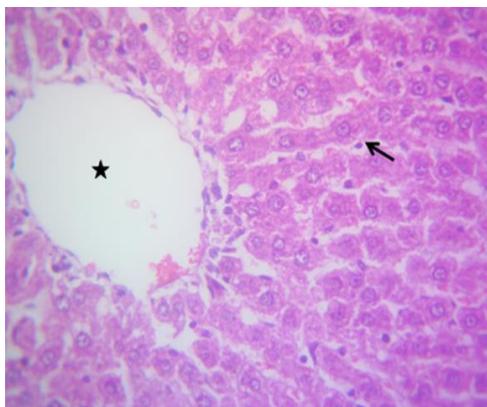


Photo. 6 (G6): Photomicrograph of liver showing normal hepatic parenchyma with healthy hepatocyte (arrow), prominent Kupffer cells and central vein (star) (H&E X400).

Results proved that the best finding of histopathological examination was noticed in liver section of hypercholesterolemic rats fed on 10% GFS + 15% SRB bread as comparing with hypercholesterolemic rats (Photo. 6 and Table 10). This finding is in concurrence with the data of serum lipid values in the current study. This data is also consistent with previous results for kidney and liver functions in this study.

Table (10): Effect of feeding with bread containing GFS and SRB on cholesterol-induced alterations in liver histology of rats

Hepatic changes	G1	G2	G3	G4	G5	G6
Fatty change	-	+++	+++	++	+	-
Congestion of hepatic Blood vessels and sinusoids	-	++	++	+	-	-
Inflammatory cells	-	+	+	-	-	-
Hydropic degeneration	-	++	++	+	-	-

-: No changes; +: Mild (25-39% changes); ++: Moderate (40-65% changes); +++: Severe (more than 65% changes). **G1:** Healthy rats; **G2:** Hypercholesterolemic rats; **G3:** Hypercholesterolemic rats consumed control bread; **G4:** Hypercholesterolemic rats consumed 10% GFS bread; **G5:** Hypercholesterolemic rats consumed 10% GFS + 10% SRB bread; **G6:** Hypercholesterolemic rats consumed 10% GFS + 15% SRB bread.

Conclusion

Fortification of bread with 10% GFS and 15% SRB was organoleptically acceptable and had enhanced protein, fiber, ash, antioxidant activity, total phenolic and flavonoids. Treating of hypercholesterolemic rats by 10% GFS and 15% SRB bread restored the biochemical and histological changes close to the normal range. The present work is considered one of the best strategies for preventing and treating hypercholesterolemia through a food-based strategy. Where FS and RB are locally available at cheap prices. Therefore, this type of functional bread improves people's nutritional status, and also benefits those who suffer from hypercholesterolemia.

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الخصائص الغذائية والحسية للخبز المسطح المحضر من بذور الحلبة ونخالة الأرز وتأثيره على الفئران المصابة بارتفاع نسبة الكوليسترول في الدم

منال محمد السيد محمد شحاتة* & حنان السيد**

*قسم علوم الأغذية شعبة الاقتصاد المنزلي الريفي (التغذية وعلوم الاطعمة) - كلية الزراعة -
جامعة الزقازيق - مصر

**قسم علوم الأغذية شعبة الاقتصاد المنزلي الريفي- كلية الزراعة - جامعة الزقازيق - مصر

أصبح زيادة ظهور الأمراض المزمنة المتعلقة بالغذاء مشكلة صحية عامة. يهدف البحث الحالي إلى دراسة استخدام بذور الحلبة المنبته (GFS) ونخالة الأرز المثبتة (SRB) كمصدر للعناصر الغذائية والمركبات النشطة بيولوجياً لإعداد الخبز الوظيفي (FB) لخفض نسب دهون الدم في الفئران المصابة بارتفاع نسبة الكوليسترول. تم تحضير FB من دقيق القمح (WF) و GFS و SRB بنسب (B1) 0:0:100 و (B2) 0:10:90 و (B3) 10:10:80 و (B4) 15:10:75 و (B5) 20:10:70 على التوالي. تم تقدير الخصائص الكيميائية والحسية ومضادات الأكسدة للخبز الوظيفي. كما تم تقييم تأثير الخبز الوظيفي على النمو والقياسات البيوكيميائية والفحص النسيجي للكبد للفئران المصابة بارتفاع نسبة الكوليسترول في الدم. وتشير النتائج إلى أن SRB مصدراً ممتازاً للألياف (14.00%) والدهون (16.92%)، بينما كانت GFS غنية بالبروتين (32.80%) والألياف (10.80%). وأظهرت النتائج أن GFS و SRB يحتويان على كميات معنوية من المركبات الفينولية (TP) والفلافونويدات (TF) ومضادات الأكسدة (AA). تم قبول الخبز الوظيفي حسيًا حتى 10% GFS+15% SRB (B4). وزادت قيم البروتين و AA في B4 بنسبة 24.0% و 63.0% على التوالي، علاوة على ذلك، كانت قيم (TP) و (TF) والألياف تقريباً 11 مرة و 33 مرة و 6 مرات زيادة عن الكنترول على التوالي. وأثبتت النتائج أن هناك تحسناً معنوياً ($P \leq 0.05$) عند تغذية الفئران المصابة بارتفاع نسبة الكوليسترول في الدم على الخبز الذي يحتوي على 10% GFS (G4) و 10% GFS+10% SRB

(G5) و (G6) SRB%15+GFS%10 في نسب دهون الدم ووظائف الكبد والكلية. تم تسجيل أفضل النتائج في (G6)، حيث عادت التغيرات البيوكيميائية والنسجية إلى قرب المستويات الطبيعية. وتوصي هذه الدراسة بأن تناول المنتجات الغذائية المحضرة من GFS و SRB قد يكون مفيداً للمرضى الذين يعانون من ارتفاع نسبة الكوليسترول في الدم بسبب خصائصها الغذائية والعلاجية.

الكلمات المفتاحية: ارتفاع كوليسترول الدم - بذور الحبة المنبثة - نخالة الارز
المنبثة - التقييم الحسى - الخبز