

## Nutritional, Sensory and Biological Study of Biscuits Fortified With Red Beet Roots

Amnah, M. A. Alsuhaibani

Nutrition and Food Sciences Dept, Home Economic Collage, Princess Nora Bint Abdul Rahman -University,  
Riyadh, Saudi Arabia  
[amalsuhaibani@pnu.edu.sa](mailto:amalsuhaibani@pnu.edu.sa)

**Abstract:** The use of red beet roots powder and extract in the preparation of biscuits and the effect of consumption of these biscuits on injured liver in experimental rats were studied. The results revealed that beet powder or beet extract incorporation to biscuits increased protein and ash contents as well as fiber, moisture contents and caloric value. Biscuit fortified with beet extract showed higher sensory values in comparing with biscuit with beet powder. Biological study was carried on forty male rats which administered 0.5 ml/rat /by back subcutaneous of CCl<sub>4</sub> in paraffin oil for two days from the start of the experimental period for inducing rats liver injury. Rats were classified into control (+ve) group, control biscuit group, beet powder biscuit group and beet extract biscuit group. The experimental period was 45 days. Biscuit with beet powder and biscuit with beet extract groups showed a significant increase in body weight gain and FER and liver antioxidant enzymes but showed a significant decrease in liver function enzymes in serum and liver cholesterol and total lipids compared with both control (+ve) and control biscuit rat groups. This study investigated that addition of red beet root to biscuits increase nutritional values and acceptability, and also showed improvement of liver function enzymes and antioxidant in injured liver rats.

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**Key wards:** Biscuit- red beet roots-liver injury – rats.

### 1. Introduction

Biscuits are a mixture of flour and water but contain fat, sugar and other ingredients mixed together into dough which is rested for a period and then passed between rollers to make a sheet (**Okaka 1997**). Biscuits are the most popular bakery items consumed nearly by all levels of society because of low cost among other processed foods, good nutritional quality and availability in different varieties with longer shelflife. Most of bakery products are used as a source for incorporation of different nutritionally rich plants (**Hooda and Jood 2005**).

Red beet (*Beta vulgaris* L.) is cultivated throughout the world for its roots, which are used as a food and as a source of natural dye. Beets are small herbaceous plants with broad dark-green leaves. Its underground taproot matures in 50-60 days of sowing and weighs about 100 to 150 g. The unique crimson-red color of red beet is due to betalain pigments, such as *asbetanin* and *betacyanin*. Yellow varieties are rich in  $\beta$ -*xanthin* pigment. Beet pigments, betalains, have been examined as natural colorants in food products such as processed meat, ice cream, baked goods, candies, and yogurt. Beetroot is a rich source of potent nutrients including magnesium, sodium, potassium and vitamin C., and betaine. Results from several *in vitro* studies have demonstrated that betalains from beetroots possess powerful antiradical and antioxidant activity (**Kujala et al. 2002**).

The objectives of this research work are to identify the proximate composition and the sensory evaluation of biscuits fortified with beetroots powder or extract and also to determine the treatment effect on liver injury in rats.

### 2. Materials and Methods

#### A- Materials:

Red beet roots, wheat flour, margarine, egg, sugar, vanilline, salt and baking powder were purchased from local market in Riyadh, Saudi Arabia. Carbon tetrachloride (CCl<sub>4</sub>) purchased from El Gomhorya Co. in the form of 40% liquid dispensed in 1 L plastic bottles which used to induce rat hepatitis at dose 0.5 ml/rat /by back subcutaneous injection according to **Lee et al. (2005)** and **Abd El-Ghany and Nanees (2010)**. BioMerieux Kits were purchased from Alkan Co. for Chemicals and Biodiagnostics. Forty male albino rats of Sprague Dawley strain were provided from experimental animals' center in Medicine collage of King Saudi University in Riyadh. The average weight was 120 ± 10 g. The rat basal diet consisted of 140 g casein (83 % protein), 100 g sucrose, 50 g corn oil, 50 g cellulose, 35 g mineral mixture, 10 g vitamin mixture, 1.8 g L-cystine, 2.5 g choline bitartrate and the remainder (610.6 g) corn starch. Diets were formulated according to **Reeves et al. (1993)**.

**B- Methods:**

Red beet roots were washed with tap water, chopped into small pieces and then separately dried in an air-circulated oven at 40°C to complete dryness. The dried material was separately reduced into powder form as far as possible and added as 5% for preparation of biscuit fortified with beet powder (AOAC, 1997). 270 g of fresh red beet roots were chopped with both 300 ml ethanol by using a blender for 1 min at average speed. The mixture was macerated during 24h at the 4°C. After that, resulting extract was filtered using a 0.45 µm pore size cellulose acetate membrane filter. The extract was used as 2.5% in preparation of biscuit fortified with beet extract (Fritschee *et al.* 2002). Phenols, flavonoids and flavonols were determined in red beet roots according to Singleton and Rossi (1965) and Georgiev *et al.* (2010).

Control biscuit dough was prepared according to the formula presented in table (1). Powdered sugar, eggs and margarine were creamed in Braun Mixer with a flat beater for 2 minutes at 6 rpm. Water containing salt, baking powder and vanillin was

added to the cream and mixed for 5 minutes at 125 rpm to obtain a homogenous cream. Thereafter flour was added slowly to the above cream and was mixed for 2 minutes at 60 rpm to obtain biscuit dough. The dough was sheeted to a thickness of about 3 mm using and cut into round shape using a 45 mm diameter cutter and baked on an aluminum tray in an electric oven at 180°C for 6 minutes. The biscuit was cooled for 30 minutes, packed in polyethylene bags (Hanan and Rasha 2012). Fortified biscuits were prepared as control biscuit with adding 5% red beet roots powder or 2.5 % of red beet roots extract in substituted of flour. Moisture, protein, crude fibers, fat content and ash contents were determined according to the method described in the AOAC (1997). Total carbohydrates were calculated by difference. Energy value was calculated according to Livesy (1995). Sensory evaluation for the appearance, exterior color, interior color, softness, tender, degree of sweetness, odour, taste, and general acceptability were done by a numerical hedonic scale ranging from 1 to 10. Ten experienced judges participated in the test (Larmond 1977).

Table (1): Composition of control biscuit, biscuit with beet powder and biscuit with beet extract

Variables	Samples	Control biscuit	Biscuit with beet powder	Biscuit with beet extract
Wheat flour		41	36	38.5
Sugar		12	12	12
Salt		0.30	0.30	0.30
Margarine		29	29	29
Egg		14	14	14
Vanillin		1.5	1.5	1.5
Baking powder		2.2	2.2	2.2
Beet powder		-	5	-
Beet extract		-	-	-

All rats were fed on the basal diet for five day for adaptation then were subcutaneously administered a single dose of CCl<sub>4</sub> in paraffin oil for two days from the start of the experimental period for inducing rats liver injuries. Rats were classified into control (+ve) group which fed on the basal diet only, control biscuit group which fed on 10% control biscuit in basal diet, beet powder biscuit group which fed on the 10% beet powder biscuit in basal diet and beet extract biscuit group which fed on 10% beet extract biscuit in basal diet.

The food intake was calculated daily and the body weight gain was recorded daily. Food efficiency ratio was determined according to the method of Chapman *et al.* (1959). After 45 days, rats were fasted overnight then sacrificed to immediately collect blood in clean and dried Wiesserman tubes from the portal vein. The blood samples were

centrifuged at 3000 rpm for 15 minutes to obtain serum. Serum (ALT& AST) activity, alkaline phosphatase, gama glutamyl transferase (γGT) and bilirubin were estimated according to Reitman and Frankel (1957), Kind and King (1954), Henry (1974) and Jendrassik (1938), respectively. The liver of sacrificing rats was perfused with 50 to 100 of ice cold 0.9% NaCL solution. Liver cholesterol, total lipids, superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase were determined according to Abell *et al.* (1952), Folch *et al.* (1957), Beuchamp and Fridovich, (1971), Beuther *et al.* (1987) and Cohen *et al.* (1970), respectively.

Collected data were statistically analyzed using one way analysis of variance (ANOVA). Student "t" test was used for significance according to Artimage and Berry (1987).

### 3. Results and Discussion

Table (2) showed that the values of polyphenols, flavenoids and flavenols in red beet roots were 1.41, 0.88 and 1.24, respectively. These results indicate that red beet roots have good antioxidant properties. Such data are in good agreement with those previously reported by Kapadia *et al.* 2011 who proved that extract of beetroot exhibited chemopreventive and antioxidant. The effect was evident as a significant reduction in tumor incidence, multiplicity, and delay in tumor latency period. Váli *et al.* (2007) reported that table beet contains important bioactive agents (betaine and polyphenols), which have a wide range of physiologic effects. Pal *et al.* 2010 and Georgiev *et al.* 2010 reported the presence of flavonoids, carbohydrate, betain, neobetain and anthocyanin pigments in *Beta vulgaris* root of phytochemical studies.

The mean value of gross chemical composition and caloric value of control biscuits and biscuits fortified with beet powder or beet extract were given in table (3). The data revealed that beet powder or beet extract incorporation to biscuits increased protein and ash contents as well as fiber, moisture contents and caloric value. However, such supplementation decreased carbohydrate content, fat and energy. The difference than control biscuits attributed to chemical composition of red beet root. The chemical composition of red beet roots showed that the values of carbohydrate, protein, fiber, fat, sugar and moisture are 9.56, 1.61, 2.8, 0.17, 6.76 and 87.58 in raw. while the values in cooked beet root are 9.96, 1.68, 2.0, 0.18, 7.96 and 87.06 g/100gram. Besides the colour pigments the juice or extract consists of sugars, salts and/or proteins naturally occurring in red beets (USDA 2013)

The scores of the flair sensory evaluation of the biscuits produced from control biscuit, biscuit with beet powder and biscuit with beet extract were shown in table (4). In general, a control biscuit was the most accepted by the judges. The scores of various sensory were low in fortified biscuits with beet powder or extract compared to control biscuit where the score values of appearance, exterior color, interior color, softness, tenderness, degree of sweetness, odor, taste and general acceptability were lower in comparing with values of control biscuit. On the other side, biscuit fortified with beet extract showed higher values of appearance, exterior color, softness, tenderness, odor, taste and general acceptability in comparing with biscuit with beet powder. The obtained results were in good accordance with Pedreno and Escribano 2001 who recorded that beetroot juice contains colorants that can be divided into two categories of betalains: the red betacyanins and the yellow betaxathines. Betalain is important for

cardiovascular health. Betanin, which makes up 95% of the total betacyanins, is more stable in the beetroot extract than in pure chemical form. Mridula *et al.* 2009 recorded increased hardness and breaking strength with increased levels of beetroot powder while redness of biscuits increased but lightness decreased with increased levels of beetroot powder in biscuits.

The present results in table (5) showed no significant difference in food intake and weight gain between control (+ve) and control biscuit groups. Biscuit with beet powder and biscuit with beet extract groups showed a significant increase in body weight gain and FER compared to control (+ve) group at  $p < 0.001$  and also compared to control biscuit group. These results may be due to nutritional values of red beet roots because of an excellent source of folates, carotenoids, flavonoid anti-oxidants, and vitamin A and B-complex vitamins such as niacin, pantothenic acid, pyridoxine and minerals (Mohammed *et al.* 2011). *Beta vulgaris* var. *rubra* (table beet root) contains several bioactive agents as betain, betanin, vulgaxanthine, polyphenols, folic acid and different metal elements as Al, B, Ba, Ca, Cu, Fe, K, Mg, Mn, Na, Zn, which act on the various physiological routes (Blázovics *et al.* 2007).

Control (+ve) and control biscuit rat groups showed non significant difference in the examined serum enzymes ALT, AST and ALP ( $P > 0.05$ ). On the other side, biscuit with beet powder and biscuit with beet extract showed a significant decrease in serum ALT, AST, ALP and  $\gamma$  GT at  $P < 0.001$  compared with both control (+ve) and control biscuit rat groups as shown in table (6). The obtained results were in accordance with results of Kapadia *et al.* (2003) who recorded that red beetroot (*Beta vulgaris* L.) is used as a popular folk remedy for liver and kidney diseases, for stimulation of the immune and hematopoietic systems, and as a special diet in the treatment of cancer. Pal *et al.* 2010 and Eman 2011 reported that the treatment with ethanolic extract of *Beta vulgaris* caused significant reduction of CCl<sub>4</sub> induced elevated serum levels of enzyme activities indicating that, the extract could preserve the normal functional status of the liver. Decrease in the levels of serum AST, ALT and ALP after treatment with *Beta vulgaris* indicated the effectiveness of the extract against CCl<sub>4</sub> induced hepatotoxicity. That may be due to presence of its chemical contents.

Biscuit with beet powder and biscuit with beet extract rat groups showed a significant decrease in liver cholesterol and total lipids compared to both control (+ve) and control biscuit rat groups as shown in table (7). This lipid lowering potential of beet root may be due to flavanoids and/or saponins which were found to be the main constituents of beet root and

these findings are in accordance with the earlier studies demonstrating the effect of flavonoids on cholesterol metabolism. Saponins from some medicinal plants reduced the triglycerides and cholesterol levels in rats and also reduced heart diseases. Also, flavonoids are considered as active principles in many medicinal plants and natural products with positive effect on human health (**Wettasinghe et al.2002 and Mohammed et al. 2011**). The hepatoprotective activity of beetroot ethanolic extract against CCl<sub>4</sub>-induced liver injury in rats assessed on the basis of routine serum markers of liver function and the concentration of cholesterol and triglycerides (**Agarwal et al. 2006**).

Control (+ve) and control biscuit rat groups showed a significant increase in liver catalase ( $P<0.01$ ). On the other side, biscuit with beet powder and biscuit with beet extract group showed a significant increase in liver SOD, GPX and catalase at  $P<0.001$  compared with both control (+ve) and control biscuit rat groups as shown in table (8). The present findings showed that, Betalains of beet root are water-soluble plant pigments that are widely used as food colorants, and have a wide range of desirable biological activities, including antioxidant, anti-inflammatory, hepatoprotective, anti-cancer properties (**Georgiev et al., 2010**).

Table (2): The content of polyphenols, flavenoids and flavenols ( $\mu\text{g/g}$ ) of red beet roots

Variables \ Samples	Polyphenols	Flavenoids	Flavenols
Red beet roots	1.41	0.88	1.24

Table (3): Gross chemical composition of control biscuit, biscuit with beet powder and biscuit with beet extract

Variables \ Samples	Control biscuit	Biscuit with beet powder	Biscuit with beet extract
Carbohydrate	70.81	69.01	69.89
Protein	6.20	6.33	6.26
Fat	16.5	16.35	16.31
Moisture	4.11	4.32	4.13
Ash	1.10	1.31	1.45
Fiber	1.28	2.68	1.96
Energy	456.54	448.51	451.39

Table (4): Sensory evaluation of control biscuit, biscuit with beet powder and biscuit with beet extract

Variables \ Samples	Control biscuit	Biscuit with beet powder	Biscuit with beet extract
Appearance	9.11	8.14	8.33
Exterior color	9.01	7.11	7.33
Interior color	8.77	8.55	8.33
Softness	8.55	6.99	7.14
Tenderness	8.71	7.11	7.71
Degree of sweetness	9.14	8.21	7.96
Odour	9.31	6.41	7.55
Taste	9.51	7.55	8.11
General acceptability	9.44	7.50	8.33

Table (5): Mean values  $\pm$  SD of body weight gain, food intake and FER of the experimental rat groups

Variables \ Groups	Control (+ve)	Control biscuit	Biscuit with beet powder	Biscuit with beet extract
Body weight gain(g)	25.14 $\pm$ 2.14 <sup>b</sup>	30.41 $\pm$ 5.41 <sup>b</sup>	67.41 $\pm$ 7.71 <sup>a***</sup>	75.71 $\pm$ 9.01 <sup>a***</sup>
Food intake (g/w)	15.33 $\pm$ 1.25 <sup>a</sup>	15.61 $\pm$ 1.31 <sup>a</sup>	16.21 $\pm$ 1.21 <sup>a</sup>	17.31 $\pm$ 1.31 <sup>a</sup>
FER	0.036 $\pm$ 0.001 <sup>d</sup>	0.043 $\pm$ 0.002 <sup>c*</sup>	0.092 $\pm$ 0.005 <sup>ab***</sup>	0.097 $\pm$ 0.001 <sup>a***</sup>

Significant with control (-ve) group \*  $P<0.05$  \*\*  $P<0.01$  \*\*\*  $P<0.001$

Mean values in each raw having different superscript (a, b, c, d) are significant

Table (6) The Mean values  $\pm$  SD of serum ALT, AST, ALP and  $\gamma$  GT of the experimental rat groups

Groups Variables	Control (+ve)	Control biscuit	Biscuit with beet powder	Biscuit with beet extract
ALT( $\mu$ /ml)	103.77 $\pm$ 9.61 <sup>a</sup>	95.41 $\pm$ 8.81 <sup>ab</sup>	78.61 $\pm$ 7.61 <sup>c***</sup>	65.31 $\pm$ 7.80 <sup>cd***</sup>
AST( $\mu$ /ml)	145.76 $\pm$ 12.61 <sup>a</sup>	130.55 $\pm$ 13.33 <sup>ab</sup>	81.16 $\pm$ 8.20 <sup>c***</sup>	75.41 $\pm$ 5.21 <sup>cd***</sup>
ALP( $\mu$ /ml)	139.61 $\pm$ 13.31 <sup>a</sup>	120.45 $\pm$ 14.14 <sup>ab</sup>	92.67 $\pm$ 10.21 <sup>c***</sup>	88.14 $\pm$ 9.40 <sup>cd***</sup>
$\gamma$ GT( $\mu$ /ml)	13.60 $\pm$ 1.75 <sup>a</sup>	11.31 $\pm$ 1.51 <sup>b*</sup>	7.88 $\pm$ 1.33 <sup>c***</sup>	7.31 $\pm$ 1.41 <sup>c***</sup>

Significant with control group \* P<0.05 \*\* P<0.01 \*\*\* P<0.001

Mean values in each raw having different superscript (a, b, c, d) are significant

Table (7): Mean values  $\pm$  SD of liver cholesterol and total lipids of the experimental rat groups

Groups Variables	Control (+ve)	Control biscuit	Biscuit with beet powder	Biscuit with beet extract
Cholesterol	8.11 $\pm$ 1.22 <sup>a</sup>	8.41 $\pm$ 1.16 <sup>a</sup>	6.31 $\pm$ 0.77 <sup>b*</sup>	6.11 $\pm$ 0.65 <sup>b*</sup>
Total lipids	49.61 $\pm$ 7.33 <sup>a</sup>	46.21 $\pm$ 6.96 <sup>ab</sup>	41.71 $\pm$ 6.44 <sup>b**</sup>	37.14 $\pm$ 6.21 <sup>bc**</sup>

Significant with control group \* P<0.05 \*\* P<0.01 \*\*\* P<0.001

Mean values in each raw having different superscript (a, b, c, d) are significant

Table (8): Mean values  $\pm$  SD of liver SOD, GPX and catalase of the experimental rat groups.

Groups Variables	Control (+ve)	Control biscuit	Biscuit with beet powder	Biscuit with beet extract
SOD ( $\mu$ /mg)	32.15 $\pm$ 3.17 <sup>c</sup>	41.71 $\pm$ 5.11 <sup>c</sup>	60.21 $\pm$ 7.33 <sup>a***</sup>	60.11 $\pm$ 7.20 <sup>a***</sup>
GPX ( $\mu$ /mg)	29.77 $\pm$ 2.91 <sup>b</sup>	35.11 $\pm$ 3.96 <sup>b</sup>	58.45 $\pm$ 6.11 <sup>a***</sup>	57.71 $\pm$ 6.12 <sup>a***</sup>
Catalase ( $\mu$ /l)	25.11 $\pm$ 3.24 <sup>d</sup>	38.41 $\pm$ 4.71 <sup>c**</sup>	49.11 $\pm$ 5.22 <sup>ab***</sup>	55.61 $\pm$ 5.41 <sup>a***</sup>

Significant with control (-ve) group \* P<0.05 \*\* P<0.01 \*\*\* P<0.001

Mean values in each raw having different superscript (a, b, c, d) are significant

## Conclusion

Biscuits fortified with red beet root powder either powder or extract of acceptable quality were nearly similar to control biscuit. From the study, it was observed that the addition of red beet roots to biscuits blends had better proved to be nutritious functional and healthful foods and also improves liver activity function.

So it is advice to add beet root powder and extract to bakery product and consume it as routine diet to hepatic disease patients.

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