Utilization of Coffee Husks to Prepare Functional Products

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Abstract: Coffee husks are a by-product resulting from dehulling the coffee cherries during dry processing. Our study aimed to investigate the effect of coffee husks on hyperlipidemic rats and utilization it to prepare the balady bread loaves as a function product. For this purpose, 25 albino rats were divided to 5 subgroups and fed on a high fat diet with different levels of coffee husks. After two months, the lipid profile showed a reduction in total cholesterol, LDL-C, VLDL-C, and an increase in HDL-C which recorded 117.41, 24.46, and 47.46 mg/dl respectively, at 15% coffee husks replacement. Balady bread was prepared by wheat flour (82% extraction rate) with 5, 10, 15, and 20% coffee husks level. The bread was evaluated by 15 panelists for sensory evaluation. All samples were acceptable and good except 20 % coffee husks. These results were confirmed by analyzing the texture of the bread in the lab. In addition, the paper valued the coffee husks as antioxidant source as showed by different antioxidant activity assays (DPPH, ABTS, and FRAB). The DPPH, ABTS, and FRAP were 207.254, 2.054, and 0.832 g Trolox/g sample, respectively. Generally, the coffee husks have a potential effect in improving lipid profile of hyperlipidemic rats and a good source for making a new functional food product.

***Keywords*:** Coffee husks, Antioxidants, bread, phenolic acids

INTRODUCTION

Coffee is considered one of the most favorite beverages in the world. In 2020, the rate of global coffee consumption reached about 165 million bags contained à 60 kg of coffee, and Brazil is the largest producer in the world (ICO, 2020), this amount was approximately equal to 19.7 billion packages of coffee which were bought all over the world. Ethiopia is the main home of the coffee tree, and it is cultivated in over 80 countries worldwide (Murthy and Naidu, 2012a). The coffee tree is a perennial tree that takes 3-4 years to start blooming, and it produces the maximum yield after 10-15 years (Berlitz *et al*., 2009). After harvesting, coffee cherries can be subjected to dry or wet processing methods to obtain green coffee beans which constitute only 50–55% of the dry matter of the ripe cherry. Coffee husks are the fibrous mucilaginous by-product resulting from dehulling the coffee cherries during dry processing (Bekalo and Reinhardt, 2010; Castaldo *et al*., 2018). The burning or disposal of coffee husks without any safe treatment causes environmental problems, especially in the producing countries (Rebollo-Hernanz *et al*., 2021). For many years, coffee husks using to prepare a traditional beverage "Cascara beverage" that consume in Yemen and Ethiopia (Heeger *et al*., 2017). But recently, many studies have been carried out to use coffee husks in other fields such as silage, aerobic composting, animal feed, vermiculture, production of biogas, vinegar, biopesticides, enzymes, single-cell protein, probiotics, food industries, and pharmaceutical industries because it contains high levels of caffeine, bioactive compounds with antioxidant activity, and dietary fiber (Mennini, 2013; Rebollo-Hernanz *et al*., 2021). The coffee husks contains three important compounds that affect the body (Hoseini *et al*., 2021): Caffeine increases weight loss by encouraging heat production and lipid peroxidation in the body, diterpenes exert anti-inflammatory effects (Bidel and Tuomilehto 2013; Meng *et al*., 2013), and chlorogenic acid impact glucose metabolism by preventing hepatic glucose-6-phosphatase activity and inhibition of glucose absorption in the small intestine by preventing glucose-6-phosphate translocase 1 as well (Naveed *et al*., 2018). For many years, many studies carried out to investigate the relationship between obesity and caffeine. Obesity is a global problem in adults and children worldwide. The World Health Organization (WHO) indicates that overweight and obesity cause more people die than underweight (Pan *et al*., 2016). In 2016, more than 1.9 billion adults, 18 years and older, were overweight. Of these over 650 million were obese (WHO, 2020). Obesity is related to the risks of many diseases such as hypertension, heart diseases, type 2 diabetes, some types of cancer, and non-alcoholic fatty liver disease (Lee *et al*., 2017). The objective of this study was using coffee husks in improving lipid profile of hyperlipidemic rats and manufacturing a good functional product fortified by different levels of coffee husks.

MATERIALS AND METHODS

Sample collection

The Coffee husks was obtained from the dry coffee roaster (coffee roaster STA - Italy) in Shebin El-Kom, Menofia Governorate, Egypt. The coffee husks were cleaned and carefully sieved to remove foreign materials. After that, the coffee husks were ground by an electric mill (Retsch mill, 5657 HAAN, Germany) and passed through 80 mesh sieves (British standard screen). The fine powder was kept in glass containers and stored at -20ºC until used.

Chemical composition

Total ash, total protein, total fiber, and total lipids content of the dry coffee husks powder were determined as described by the AOAC (2000).

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Identification of phenolic compounds by HPLC

A known weight of the dried coffee husks was soaked in 25 ml sterilized water and still on a shaker for 24 h. The aqueous extract was filtered through Whatman 3 MM filter paper under vacuum, followed by centrifugation at 10000 rpm for 30 min at 80ºC. Phosphoric acid was used to acidify an aqueous extract to pH 2.5. Each sample was partitioned three times with an equal volume of diethyl ether. The layer of diethyl ether was evaporated to dryness under low pressure at 30ºC. The resulting residue was dissolved in 3 ml of spectral grade methanol and filtered through a 0.2 mm Sterilized filter membrane prior to HPLC analysis. Identification of individual phenolic compounds of the coffee husks samples was performed on a Hewlett-Packard HPLC (Model 1100), using C_{18} reversed-phase column (250 × 4.6 mm) with 5 mm particle size. A constant flow rate (1 ml/min) was used with two mobile phases. (A) 0.5% acetic acid in distilled water at pH 2.65, and solvent (B) 0.5% acetic acid in 99.5% acetonitrile. The gradient of elution was starting with mobile phase A and ending with mobile phase B during 35 min, using an UV detector set at wavelength 254 nm. The relative retention times were used to identify the phenolic compounds in each sample comparing with standard mixture of phenolic compounds. Peak area measurements were used to calculate the concentration of each phenolic compound, then converted to µg phenolic acid/g dry weight.

Antioxidant Capacity Assays

Sample preparation

Ethanol extract of coffee husks was prepared as follows: 0.5 g of ground coffee husks was added to 50 ml ethanol. The mixture was left on a shaker for 24 h, then centrifuged under cooling at 10000 rpm for 10 min, and the supernatant was filtered through Whatman No. 41 filter paper. The volume of filtered supernatant was adjusted to 50 ml again, kept at -20^{0}C, and for up to one week to use .

DPPH, ABTS, and FARP antioxidant activity assays

Three activity assays were carried out to measure the free radical scavenging capacity of coffee husks ethanolic extract using the DPPH assay (1,1-diphenyl-2-picryl hydrazyl) according to the method described by Akillioglu and Karakaya (2010), the ABTS•+ assay was carried out according to the method of Gouveia and Castilho (2011) and FRAP assay was carried out according to the method reported by Benzie and Strain (1996).

Preparation of balady bread.

Balady bread preparation was done on an automatic commercial baking line according to Eissa *et al*. (2007) as in the official baking house, North of Cairo city, Egypt.

In the control sample, balady bread was prepared from wheat flour (82% extraction). The baking recipe was as follows: 100 g flour, 0.5 g of active dry yeast, 1.5 g of sodium chloride, and 75–80 mL of water. All ingredients were mixed by hand for about 6 min to form the needed dough. The dough was left 1 h to have a good fermentation at 30°C and 85% relative humidity (RH). After that, the dough was divided into 125 g pieces and was arranged on a wooden board which was covered by a fine layer of bran. The pieces of dough were left again to ferment for about 45 min at the previous temperature and RH. The pieces of fermented dough were flattened to be about 20 cm in diameter. After the flatting process, the loaves were proof at 30 ^{0}C and 85 % RH for 15 min., and then baked at 400 - 500 ^{0}C for 1-2 min. the loaves were left at room temperature to cool for 2 h.

The experimental samples were executed in the same steps of control sample, but with different levels of coffee husks (5, 10,15, and 20 %) on account of wheat flour.

Loaf measurements

The following measurements of loaf quality were taken in triplicate according to Dawoud (1989). The standing height (cm) was measured in the center of the products. Volume was measured by rapeseed displacement after cooling for 1 hour at room temperature (~25^{0}C). The products were weighed after baking and specific volume was also calculated (Volume/weight).

Sensory evaluation

All samples were presented to fifteen panelists. The samples were coded with a three-digit number and were evaluated for their sensory attributes and the scoring scheme was as follow: taste (20), flavor (15), crumb distribution (15), color of crumb (15), color of crust (15) and general appearance (20) as described by Atia (1986). The average of total score was converted to a descriptive category as follows: 90-100: very good 80-90: good 70-79: satisfactory less than 70: questionable.

Textural properties analysis (TPA) of bread

Crumb texture was determined at Food Technology Research Institute, Agricultural Research Center Giza, Egypt, by a universal testing machine. (Cometech, B type, Taiwan) provided with software An Aluminum 25 mm diameter cylindrical probe was used in a "Texture Profile Analysis" (TPA). Double compression test to penetrate to 50% depth, at 1 mm/s speed test. Firmness (N/cm^2), gumminess (N/cm^2), chewiness (N/cm2), cohesiveness (ratio), springiness and resilience were calculated from the TPA graphic. Both, springiness and resilience give information about the after-stress recovery capacity. But, while the former refers to retarded recovery, the latter concerns instantaneous recovery (immediately after the first compression, while the probe goes up) Texture determination carried out removing the crust, in (40* 40 * 30 minimized samples (Bourne, 2003).

Biological experiment

Animals:

Twenty-five adult male albino rats of Sprague Dawley strain (150:170 g) were purchased from the Giza Memorial Institute for Ophthalmic Research, Animal House, Ministry of Health, Giza, Egypt. The animals were adapted by keeping them in cylindrical wire cages. The feeding of animals was carried out by

special food cups (mangers) to avoid scattering of food. Also, water was provided to the rats by glass tube projection through the wire cage. Food and water were provided and checked daily. The animals were housed under controlled normal laboratory conditions in a temperature of 20-23^{0}C, with a 12:12h light: Dark. After acclimatization

The rats were randomly divided into 5 equal groups according to the weight, the difference in weight between groups do not exceed ± 10 g, and ± 5 g between rats in each group. The first group (G1) was a negative group which received the normal diet ad libitum (rat chow), the second group (G2) Group was a positive group which received the high fat diet (HFD). Other groups (G3, G4 and g5) were received the high fat diet and different ratios of coffee husks according to the following table:

Table (1): Composition of the experimental diets (g/kg) fed to rats.

Ingredients	G 1 Negative group	G2 Positive group	G3	G4	G5
	*ND	HFD	High Fat Diet (HFD) + Coffee husks		
Coffee husks	-	-	50	100	150
Protein (Casein)	200	200	200	200	200
Corn starch	150	111	91	71	51
Sucrose	500	370	340	310	280
Cellulose	50	50	50	50	50
Fat (Corn oil)	50	30	30	30	30
Beef tallow	-	170	170	170	170
Mineral mixture	35	42	42	42	42
Vitamin mixture	10	12	12	12	12
Choline biartrate	2	2	2	2	2
Cholesterol	-	10	10	10	10
Tert-Butylhydroquinone	0.01	0.04	0.04	0.04	0.04
DL-Methionine	3	3	3	3	3
Total	1000	1000	1000	1000	1000
Fat, % calorie	11.11	38.10	38.10	38.10	38.10

*Composition of the normal and experimental diet (g/1000 diet) was according to the formula of Kim *et al*. (2009) with some modifications.

Normal and high fat diet ingredients were purchased from El-Gomhoria Company, Cairo, Egypt. Both of them were kept in refrigerator at 4°C until used. Obesity was induced in two months (El-Anany and Ali, 2018).

Sample collection

At the end of the two months, animals were fasted overnight and sacrificed under diethyl ether anesthesia. Blood samples were collected in a clean dry centrifuge tube from the hepatic portal vein. Tubes were separated to obtain the serum by centrifugation at 5000 rpm for 10 min at room temperature and then kept in a plastic vial until analysis. Serum was used for estimation of lipid profile including triglycerides, total cholesterol, High-density lipoprotein cholesterol (HDL-cholesterol), and total lipids-by enzymatic colorimetric methods using kits (Buccolo *et al*., 1973; Meiattini *et al*., 1978; Grove, 1979; Kaplan *et al*., 1984), respectively. Calculation of very low-density lipoprotein cholesterol (VLDL-C) and low density lipoprotein cholesterol (LDL-cholesterol) in mg/dl was done by using the following equation: VLDL-C = triglycerides/5, and LDL-cholesterol = Total cholesterol – [HDL + VLDL)] (Lee and Nieman, 1996). The atherogenic index (AI) was calculated by using the following equation: Log [triglycerides (mg/dL)/HDL-C (mg/dl)] (Dobiásová and Frohlich, 2001).

This experiment was carried out in accordance with the guidelines of the Ethical Committee of Giza Memorial Institute for Ophthalmic Research, Animal House, Ministry of Health, Giza. Egypt.

Statistical analysis

Results were presented as mean (M) ± standard deviation (SD). To assess significant difference among experimental animal groups or samples of sensory evaluation. The One-Way ANOVA analysis of samples was performed using Statistical Package for Social Sciences (SPSS version 20.0 IBM, Armonk, NY, USA), followed by Duncan's multiple range test, with a significance level set at P≤ 0.01.

RESULTS AND DISCUSSIONS

Chemical composition

Data in Table (2) displayed the proximate chemical composition of the coffee huskss powder. The coffee husks had 10.12 g/100 g crude protein, 2.56 g/100g total fat, 18.22 g/100g crude fiber, 2.7 g/100 g ash, and 66.40 g/100g carbohydrates. These results were lower than that recorded by Navya and Pushpa (2013) for protein, Murthy and Naidu (2012a) for total

fiber but were higher than that recoded by Pandey *et al*. (2000) and Franca and Oliveira (2009) for total lipids and total carbohydrates. In general, the chemical composition of coffee husks presents a wide range of values compared with other studies because of the differentiation in varieties, geographical location, cultivation conditions, resources in the production region, and development stage on which the coffee fruits were harvested (Mullen *et al*., 2013; Bonilla-Hermosa *et al*., 2014).

Table (2): Chemical composition of coffee husks (g/100 g dry weight)

Parameters	M ± SD
Crude protein (g)	10.12 ± 0.82
Fat (g)	2.56 ± 0.051
Crude fiber (g)	18.22 ± 1.22
Ash (g)	2.70 ± 0.003
Carbohydrates[1] (g)	66.40 ± 3.45

Each value in the table is the average of three replicates and followed by ± standard deviation.
[1]Carbohydrates were calculated by difference

Antioxidant Capacity Assays

From the data displayed in Table (3), it could be noticed that DPPH test is a frequent assay used to evaluate the radical-scavenging potential of a sample including coffee husks. Usually, the high percentage or concentration of DPPH reflects the high levels of antioxidant potential in sample, that able to scavenge the free radicals in human body. The value of DPPH radical-scavenging activity of the coffee husks was recorded 53.47% (207.25 mg Trolox/g sample). Overall, the DPPH scavenging of the coffee husks was higher than that previously reported for husks derived from the dry and wet methods coffee processing (Ribeiro *et al*., 2019), but a lower 65-69% value was detected than that recorded by Murthy and Naidu (2012b) who studied the coffee-by-products such as coffee pulp, coffee husks, and silver skin.

Data from the previous Table showed the value of ABTS, antioxidant capacity assay which recorded 2.054 g Trolox/g sample. The cation radical of ABTS ($ABTS^{+}$) results from the loss of electron yields bluish-green colored substance to form 2,2-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt. In the presence of hydrogen donation atom from test article or standard Trolox, the charges are suppressed, and the solution becomes uncolored or clear. Hence The ABTS whose cationic radical form is generated by treating it with oxygenating reagents such as potassium permanganate or manganese dioxide (Miller and Rice-evans 1997). This assay is unique because it can be applied at different pH conditions and estimated in both organic and aqueous extracts.

Coffee husks are need to more studies about its role as an antioxidant. Andrade *et al*. (2011) studied the antioxidant activity of coffee husks extracts and found that the low-pressure method and extraction with ethanol were the best method to obtain the high free radical scavenging activity extract evaluated with ABTS radical (161±3 mmol TEAC/kg). This value could be associated to the different coffee varieties and to different conditions of technological process. Our results about antioxidant activity measured with ABTS assay are high value to those reported in literature, which shows that the coffee by-products have an appreciable free radical scavenging activity measured with ABTS assay. Silver skin has the highest antioxidant activity (21.2 mmol Trolox/kg) followed by spent waste (20.4 mmol Trolox/kg), cherry husks (18.4 mmol Trolox/kg), and coffee pulp (15.3 mmol Trolox/kg) among the coffee byproducts (Murthy & Naidu, 2012b).

FRAP method is another assay used to estimate the power of substance as an antioxidant. The value of FRAP antioxidant capacity assay recorded 0.832 g Trolox/g sample Table (3). The principle of this assay depended on the reduction of ferric (Fe^{3+}) form of substance to ferrous (Fe^{2+}) form, this assay was carried out in acidic conditions (pH 3.6) to maintain the solubility of iron in ferric and ferrous form (Hagerman *et al*., 1998). The FRAP method is simple, fast, and efficient method which finds is applications in antioxidant estimation from body fluids, food, and plant extracts.

The result of FARP was high recorded by silva *et al*., (202١) who evaluated the effect of different methods (water bath and ultrasonic) and solvents (different ratios of water, and ethanol) for obtaining bioactive compounds from coffee husks and found that the wide variation between FRAP values extractions (23.38 – 2639.4 µmole Trolex /g for water bath method, and 27.62 – 3136.4 µmole Trolex /g for ultrasonic method) due to the experiment time method and kind of solvent. According to Barros *et al*. (2019) few phenolic compounds, such as quercetin cannot reduce Fe^{3+} within 30 min. Another factor is the reduction of Fe3+/Fe2+ occurs much fast in using orthophosphoric acid for extraction comparing with other solvents.

Table (3): Determinations of antioxidant capacity assays (DPPH, ABTS, and FRAB) of the coffee husks powder extract

Parameters	M ± SD
DPPH (%)	53.472 ± 0.306
DPPH (mg Trolox/g sample)	207.254 ± 0.722
ABTS (g Trolox/g sample)	2.054 ± 0.074
FRAB (g Trolox/g sample)	0.832 ± 0.050

Each value in the Table is the average of three replicates and followed by ± standard deviation.

HPLC Phenolic Compounds Profile

From Table (4) chlorogenic acid, gallic acid, and caffeine are the main phenolic acids of coffee husks (780, 19.78 and 18.20 µg/g, respectively). The esterification of caffeic acid with quinic acid produces chlorogenic acid, which is a soluble polyphenol (Gauthier *et al.*, 2016). In the intestine, chlorogenic acid is hydrolyzed into caffeic acid which has a stronger antioxidant activity than chlorogenic acid, it is possible that caffeic acid plays an important role in the protective effect of chlorogenic acid against ischemia-reperfusion injury (Sato *et al*., 2011).

Table (4): HPLC phenolic compounds profile of the coffee husks (mg/g dry weight)

Compound	Coffee husks
Gallic acid	19.78
Protocatechuic acid	92.23
4-hydroxybenzoic acid	6.45
Vanillic acid	2.45
Syringic acid	0.29
Salicylic acid	2.84
Chlorogenic acid	780
Caffeic acid	15.87
Caffeine	18.20
p-coumaric acid	1.89
Ferulic acid	2.2
Catechin	1.55
Epicatechin	7.94
Coumarin	1.243

Coffee husks contained a moderate amount of caffeic acid, protocatechuic acid, and Epicatechin, the less amounts of phenolic compounds were observed in syringic acid, catechin and coumarin which was recorded 0.29, 1.55, and 1.243 respectively. These results were agreed with Rebollo-Hernanz *et al*. (2021).

Polyphenols and flavonoids are considered the main components of non-enzymatic antioxidant defense system, which have the high capacity to catch free radicals and protect body against increasing of ROS (Ferguson, 2001). Therefore, coffee husks are a good example for non-enzymatic antioxidant due to the presence of higher amounts of phenolic compounds.

Sensory evaluation of balady bread

Table (5) presents the sensory properties score and statistical analysis for balady bread which was prepared by 82% flour and different levels of coffee husks powders. From this Table, it could be observed that balady bread had no significant ($p > 0.01$) effects on all sensory characteristics at level 5 % coffee husks replacement compared with control balady bread characteristics except appearance, color of crust, and color of crumb but at 20% level replacement, significant ($p < 0.01$) differences were observed for all characteristics compared with balady bread control. Statically, the best balady bread treatment contained 10% coffee husks powder while the balady bread containing 20% coffee husks was displayed the worst characteristics among all treatments prepared with coffee husks powder.

According to the grade, the replacement of coffee husks powder at 5 to 15% was acceptable, though 20% was unacceptable.

Table (5): Sensory evaluation of balady bread fortified with different levels of coffee husks

Parameters	Control	Coffee husks levels			
		5 %	10 %	15 %	20 %
Appearance (20)	$19.40^{a} \pm 1.350$	$18.0^{ab} \pm 1.633$	$18.0^{ab} \pm 1.886$	$17.20^{b} \pm 1.687$	$12.00^{c} \pm 1.886$
Color of crust (15)	$14.40^{a} \pm 1.049$	$13.80^{ab} \pm 0.949$	$13.80^{ab} \pm 1.378$	$12.75^{ab} \pm 1.061$	$10.05^{c} \pm 2.127$
Color of crumb (15)	$14.10^{a} \pm 1.049$	$13.50^{ab} \pm 1.225$	$13.35^{ab} \pm 1.107$	$12.45^{ab} \pm 1.589$	$9.75^{c} \pm 1.768$
Crumb distribution (15)	$14.10^{a} \pm 0.775$	$13.95^{a} \pm 1.235$	$13.75^{a} \pm 1.549$	$13.20^{a} \pm 1.317$	$9.15^{b} \pm 1.492$
Flavor (15)	$14.25^{a} \pm 1.049$	$14.10^{a} \pm 1.061$	$13.5^{a} \pm 1.414$	$12.3^{b} \pm 1.183$	$9.15^{c} \pm 1.492$
Taste (20)	$18.80^{a} \pm 1.033$	$18.60^{a} \pm 1.647$	$17.60^{ab} \pm 2.06$	$16.40^{b} \pm 1.690$	$12.20^{c} \pm 1.989$
Overall scores (100)	$95.05^{a} \pm 4.234$	$91.95^{a} \pm 5.91$	$90.00^{ab} \pm 6.57$	$84.30^{b} \pm 5.02$	$62.30^{c} \pm 7.616$
Grade	Very good	Very good	Very good	Good	Questionable

Each value in the Table is the average of 15 panelists and followed by ± standard deviation.
Means in the same raw bearing different superscript letters are different significantly ($P \leq 0.01$)

Texture profile analysis of balady bread and loaf measurements

Data from Tables (6 & 7) showed that the firmness of bread samples became less hardness with increasing levels of coffee husks powder from 0 to 20%. In addition, the replacement of wheat flour by different levels of coffee husks showed the same effect on firmness except for 20% coffee husks replacement. In this study, the weight of samples was not significant ($p>0.01$) different among bread samples. Thus, the increase in firmness was mainly related to the volume of bread samples. These results are compatible with Wanga *et al*. (2002) and Yamsaengsung *et al*., (2010), who stated that the increased bread volume is directly related to the decreased hardness values.

Cohesiveness is the ability of the material food to resist the deformation before rupturing during biting the food product. TPA results showed insignificant ($p>0.01$) differences in the bread cohesiveness except at 20% coffee husks replacement, and it was decreased with increasing the level of coffee husks powder.

Gumminess and chewiness are closely related. Gumminess is the energy needed to break up a semi solid food until it is ready for ingestion, and it can be calculated by multiplication of hardness and cohesiveness. While as, Chewiness is determined by gumminess multiplied by springiness and represents the amount of energy needed to disintegrate a food for swallowing. The chewiness and gumminess values had a similar trend of hardness. These results agree with those obtained by Ibrahim (2011) who reported that both gumminess and chewiness are parameters dependent on hardness.

Springiness is defined as the ability of a sample to return to the original size through the two compressions as follow the end of the first one and the start of the second one. The results showed significant ($p<0.01$) differences among all bread treatments, but the best one was showed in bread with 5% coffee husks replacement which recorded 0.809.

Resilience is the ratio of recoverable energy as the first compression is relieved. The resilience values had a similar trend of cohesiveness.

Table (6): Texture profile analysis parameters of balady bread fortified with different levels of coffee husks

Parameters	Control	Coffee husks levels			
		5 %	10 %	15 %	20 %
Firmness	23.39 ^{a} ± 1.560	8.34 ^{b} ± 0.550	8.040 ^{b} ± 0.410	7.890 ^{b} ± 0.360	5.540 ^{c} ± 0.120
Cohesiveness	0.850 ^{a} ± 0.030	0.813 ^{a} ± 0.015	0.780 ^{a} ± 0.020	0.760 ^{a} ± 0.020	0.660 ^{b} ± 0.060
Gumminess	19.882 ^{a} ± 1.230	6.780 ^{b} ± 0.590	6.271 ^{b} ± 0.360	5.996 ^{b} ± 0.120	3.656 ^{c} ± 0.420
Springiness	0.943 ^{a} ± 0.045	0.890 ^{ab} ± 0.030	0.820 ^{bc} ± 0.030	0.773 ^{cd} ± 0.015	0.730 ^{d} ± 0.020
Chewiness	18.740 ^{a} ± 1.860	6.035 ^{b} ± 0.450	5.142 ^{b} ± 0.530	4.635 ^{b} ± 0.140	2.669 ^{c} ± 0.260
Resilience	0.790 ^{a} ± 0.030	0.780 ^{a} ± 0.020	0.780 ^{a} ± 0.020	0.760 ^{a} ± 0.020	0.500 ^{b} ± 0.080

Each value in the Table is the average of three replicates and followed by ± standard deviation.
Means in the same raw bearing different superscript letters are different significantly ($P \leq 0.01$)

Table (7): Loaf measurements of balady bread fortified with different levels of coffee husks powders.

Parameters	**Control**	**Coffee husks levels**			
		5 %	10 %	15 %	20 %
Weight (g)	104 ^{a} ± 2.300	103.6 ^{a} ± 1.960	103.1 ^{a} ± 1.890	102.2 ^{a} ±1.770	101 ^{a} ± 1.650
Volume (cc)	201 ^{a} ±1.320	182 ^{d} ± 1.330	186 ^{c} ± 1.260	190 ^{b} ± 1.530	193 ^{b} ± 1.020
Specific volume (cc/g)	1.932 ^{a} ± 0.030	1.756 ^{b} ± 0.025	1.804 ^{b} ± 0.019	1.859 ^{b} ± 0.019	1.910 ^{c} ± 0.014
Height (cm)	5.4 ^{a} ± 0.220	5.1 ^{ab} ± 0.410	4.9 ^{ab} ± 0.610	4.7 ^{ab} ± 0.120	4.3 ^{b} ± 0.320
Loaf diameter (cm)	15.3 ^{a} ± 0.990	14.5 ^{a} ± 1.120	13.5 ^{a} ± 1.060	13 ^{a} ± 1.200	12.6 ^{a} ± 1.510

Each value in the Table is the average of three replicates and followed by ± standard deviation.
Means in the same raw bearing different superscript letters are different significantly ($P \leq 0.01$)

Serum lipid profile of rats administered experimental diets

The effect of coffee husks powder substitutions for 8 weeks on serum total lipids, triglycerides, total cholesterol (T-C), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), and very low density lipoprotein cholesterol (VLDL-C) concentrations in rats fed on HDF is shown in Table (8). All lipid profile parameters were significantly increased ($p \leq 0.01$) in rats, which were fed on the HDF compared with the control (-) rats group fed on basal diet except, high density lipoprotein cholesterol (HDL-C) parameter. The coffee husks powder level at 15 % showed a significant improvement in all lipids profile parameters followed by 10 %, and 5 %, except total lipids which have the same effect at 10 or 15 % coffee husks replacement. The high density lipoprotein cholesterol (HDL- C) concentration of rats fed on HFD was sharply decreased by 41.18% as compared with the control group.

In general, the administration of various levels of coffee husks to rats fed on HFD significantly improved all lipid parameters ($P \leq 0.01$) by decreasing serum total lipids, total cholesterol, triglycerides, low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C), and increasing serum high density lipoprotein cholesterol (HDL-C).

The comparison between the rats fed on the HFD and rats fed on the HFD containing 15% coffee husks showed that values of the total lipids, triglycerides, total cholesterol, low density lipoprotein cholesterol (LDL-C), and very low density lipoprotein cholesterol (VLDL-C) were enhanced by 9.57, 29.14, 35.41, 36.63, and 29.14%, respectively. It is clear that the higher level of coffee husks at 15% has improved the lipid parameters more than the level at 5%.

The incorporation of different levels of coffee husks into the HFD caused a significant reduction ($P < 0.01$) in the Atherogenic index value. The value of Atherogenic index of rats administered with HFD at 5, 10, and 15 % coffee husks recorded 0.606, 0.510, and 0.411, respectively. The Atherogenic index from the previous values was lower than the Atherogenic index value of rats fed on HFD.

The obtained results indicated the coffee husks served an important role in reducing serum lipids (Table 8). The coffee husks powder is a complex mixture contains several bioactive compounds including caffeine, polyphenols, and diterpenes that affect serum lipids (Hoseini *et al*., 2021). After two months of coffee husks powder intake, caffeine from the husks elevated the breakdown of fat cells and fat oxidation *in vivo* through lipolysis in fat cells and the release of catecholamines, thereby inhibiting weight gain and body fat accumulation in animals (Zheng *et al.*, 2004; Lee *et al.*, 2017). Caffeine has been reported to facilitate the release of norepinephrine from sympathetic nervous system resulting in accelerating the consumption of energy and loss of body fats (Westerterp-Plantenga *et al.*, 2006; and Acheson *et al*., 2004). Same results were observed by Shimoda *et al.* (2006) who studied the effect of green coffee bean extract on fat accumulation and body weight gain in mice and found that caffeine, chlorogenic acid and other polyphenolic compounds act as a synergist agent to suppress body weight gain and visceral fat accumulation in mice. The explanation of chlorogenic mechanism depends on inhibiting the hepatic Peroxisome Proliferators'-Activated Receptor γ (PPARγ), which promotes the fatty acids uptake into liver cells (Naveed, *et al*., 2018).

Table (8): Effect of different coffee husks powder levels on serum total lipids profile of hyperlipidemic rats.

Parameters	G1 Control (-)	G2 Control (+)	Coffee husks levels		
			G3 (5 %)	G4 (10 %)	G5 (15 %)
Total lipids (md/dl)	$277^{a} \pm 3.29$	$355^{d} \pm 6.36$	$340^{c} \pm 3.65$	$332^{bc} \pm 5.20$	$321^{b} \pm 2.92$
Triglycerides (md/dl)	$89.51^{a} \pm 2.45$	$172.64^{e} \pm 2.13$	$153.22^{d} \pm 2.68$	$131.18^{c} \pm 3.35$	$122.33^{b} \pm 2.78$
Total cholesterol (md/dl)	$136.23^{a} \pm 2.88$	$293.15^{e} \pm 2.32$	$244.89^{d} \pm 3.45$	$207.45^{c} \pm 3.36$	$189.33^{b} \pm 3.76$
HDL-cholesterol (md/dl)	$55.36^{a} \pm 0.85$	$32.56^{e} \pm 0.93$	$37.89^{d} \pm 0.72$	$40.54^{c} \pm 0.88$	$47.46^{b} \pm 0.29$
LDL-cholesterol (md/dl)	$62.97^{a} \pm 3.45$	$226.07^{e} \pm 1.89$	$176.36^{d} \pm 2.88$	$140.68^{c} \pm 3.45$	$117.41^{b} \pm 3.33$
VLDL- cholesterol (md/dl)	$17.90^{a} \pm 0.49$	$34.52^{e} \pm 0.42$	$30.64^{d} \pm 0.53$	$26.23^{c} \pm 0.67$	$24.46^{b} \pm 0.55$
Atherogenic index	$0.206^{a} \pm 0.005$	$0.724^{e} \pm 0.007$	$0.606^{d} \pm 0.001$	$0.510^{c} \pm 0.002$	$0.411^{b} \pm 0.007$

Each value in the Table is the average of 5 rats from each group and followed by ± standard deviation.
Means in the same raw bearing different superscript letters are different significantly ($P \leq 0.01$)

CONCLUSION

This study aimed to the utilization of coffee husks as a by-product in the preparing of balady bread loaves. The different levels of Coffee husks had significant effects on the textural, physical, and sensory attributes of balady bread loaves. Panelists decided the best levels added to the bread 5 to 15 % of coffee husks replacement. On the other side, coffee husks have a therapeutic role in reduction lipid profile (total lipids, triglycerides, total cholesterol, low density lipoprotein cholesterol, and very low density lipoprotein cholesterol) and raising high density lipoprotein cholesterol. These balady bread loaves are an excellent option for diabetic and hyperlipidemic patients because they are rich in fiber, total phenols, and antioxidants. Coffee husks are still need more research about the effect of coffee husks as antinutritional factors and how to use them in other different functional products such as cakes, beverages, pizza, and bakery products.

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الاستفادة من قشور القهوة لتحضير منتجات وظيفية

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تعد قشور القهوة منتجا ثانويا ينتج من نزع حبوب البن أثناء عملية التصنيع الجاف. استهدفت هذه الدراسة إلى معرفة تأثير قشور القهوة على الفئران المصابة بارتفاع دهون الدم والاستفادة منه في إعداد خبز بلدي كمنتج وظيفي. من اجل هذا الغرض، ٢٥ فأر من نوع البينو تم تقسيمهم إلى مجموعات صغيرة وتم تغذيتها على غذاء مرتفع الدهن مع مستويات مختلفة من قشور القهوة، بعد شهرين لوحظ انخفاض كلا من الكوليسترول الكلي، البروتين الدهني منخفض الكثافة، البروتين الدهني منخفض الكثافة جدا، وارتفاع البروتين الدهني العالي الكثافة وقد سجلوا قيما ١١٧.٤١ ، ٢٤.٤٦ ، ٤٧.٤٦ ميللجرام/ ديسيلتر على التوالي، عند مستوى ١٥٪ من قشور القهوة. تم تجهيز الخبز البلدي من دقيق بلدي استخلاص ٨٢٪ مع مستويات مختلفة من قشور القهوة (٥ ، ١٠ ، ١٥، ٢٠٪). تم تقييم الخواص الحسية للخبز بواسطة ١٥ محكم، وكانت كل المعاملات مقبولة وجيدة فيما عدا مستوى ٢٠٪. تم تأكيد هذه النتائج عن طريق تحليل قوام الخبز معمليا. بالإضافة إلى ذلك تم تقييم قشور القهوة كمضاد للأكسدة بثلاث اختبارات ووجد إن DPPH, ABTS, and FRAP سجلوا قيما ٠.٨٣٢، ٢.٠٥٤، ٢٠٧.٢٥٤ جرام تروليكس/جرام عينة على التوالي. بوجه عام اظهر قشور القهوة انه لها تأثير فعال في خفض صورة الدهون في الفئران المصابة بارتفاع دهون الدم، وكونه مصدرا جيدا لعمل منتج غذائي وظيفي جديد.

الكلمات الدالة: قشور القهوة، مضادات الأكسدة، الخبز، الأحماض الفينولية