Egyptian Journal of Chemistry
http://ejchem.journals.ekb.eg/

Microwave/ultrasonic treatments of *Jatropha curcas* shell: effect on phenolic compounds, antioxidant activities, and its incorporation into cookies

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Abstract

The manufacture of phenolic compounds as food additives requires maximizing their extraction from plant material. The present investigation evaluated the combination of microwave and ultrasonic for phenolic compound extraction as a "green extraction" with high antioxidant activities, with regard to the effect of different concentrations of additives in Jatropha curcas shell (1, 3, 5, and 7%) on the chemical, physical, and sensory properties of the cookies. Results displayed that the combination of ultrasonic and microwave extraction was the most efficient method for increasing total phenolic compounds as well as releasing phenolic acids in a synergistic manner and providing the strongest antioxidant activities when compared to separate treatments using either ultrasonic or microwave using the DPPH and FRAP methods. Results indicated that, with steadily increasing amounts of Jatropha seed shell flour, cookies' thickness and spread ratio were enhanced. On the other side, the increased amount of fiber steadily decreased as the percentage of flour made from jatropha shells increased. Therefore, 3.0% jatropha shell flour may be suggested for the creation of excellent cookies with acceptable sensory qualities, as well as extending its shelf life, which holds promise for novel functional cookies for industrial use as powerful antioxidant and nutritional supplement..

Keywords: Jatropha curcas shell, ultrasonic, microwave, green extraction, antioxidant activities, functional cookies.

1. Introduction

As a biofuel plant, Jatropha curcas has been found to possess a number of desirable traits, including seeds with a high oil content (27–40%), rapid growth, ease of cultivation, tolerance to drought, ability to grow on poor soil and waste land, low nutrient and maintenance requirements, and no negative effects on current food crops, insects, or pest resistance. According to [1], the seeds of the Jatropha curcas plant have an inner kernel with a high oil content that may be used to make biodiesel and shell that makes up around 35–40% of the seed [2]. Also, Jatropha curcas contains several bioactive substances such as tannins, phenolic compounds, flavonoids, phenolic acids and plant extract, have demonstrated significant antioxidant activities [3]. According to [4], the Jatropha curcas shell is abundant in phenolic compounds with strong antioxidant activity; as a result, it might be regarded to be a useful source of natural antioxidants. Phenolic compounds may be present in the seed shell's potential chemical composition. Additionally, [5] evaluated the total phenolic content, antioxidant activity, and potential as a source of natural antioxidants from J. curcas seed shells. Additionally, the bioactivity of phenolic compounds as antioxidants and anticancer agents has drawn a lot of interest to diets high in these compounds. In order to gain the health advantages of phenolic compounds, new strategies for adding them to our diet are being inspired by the rising need for phenolics in our diet [6]. The manufacture of phytochemicals as additions to nutritional supplement and food component formulations requires the extraction of bioactive compounds from plant material, which is a crucial step. The use of ultrasonic and microwave technology for extraction is regarded as a "green" idea since it might increase yield, shorten processing time, and consume less energy and solvent. The introduction of low-light, functional, natural, and organic products is one of the market's

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Receive Date: 13 March 2023, Revise Date: 06 May 2023, Accept Date: 14 May 2023
DOI: 10.21608/EJCHEM.2023.199872.7724

most popular trends as consumers want healthier bakery items [7]. All age groups around the world now regard cookies as one of the most popular and well-liked snacks. This is as a result of the fact that cookies are "ready-to-eat" foods and a strong source of energy. The cookies could be a valuable source of immediate nourishment due to their inexpensive cost of production and stable shelf life (caused by their low water activity) [8]. People are becoming more and more conscious of their health and the numerous issues that it might cause. Thus, a trend toward consuming nutritious as well as healthful food is developing along with changing lives and mindsets of people. To the best of our knowledge, no bakery-based food products, including cookies, have been created using the seed shell of *J. curcas*. To provide insight into the added value of this by-product as a source of antioxidants to evaluate the phenolic compound profile and antioxidant activity of *J. curcas* shell of extracted with ultrasonic and/or microwave extraction methods to improve biscuit quality by including different scale levels of *Jatropha curcas*, the current research is conducted.

2. Materials and Methods

2.1. Materials

Jatropha specie (Jatropha curcas L.) was carried out during 2019 of Jatropha curcas trees (6 years old) grown in South Sinai Government which irrigated with saline fresh water. All used chemicals and reagents were purchased from Sigma, Aldrich & Fluka Chemical Co. (St. Ouis, Mo, 63103 USA). All other chemicals and reagents used were of analytical grade.

2.2. Ultrasonic and/or microwave treatments of Jatropha seed shell

Jatropha shell was made by removing the seed's outer, black coating and milling it to a 0.45 mm severance.

A) Ultrasonic: Jatropha shell powder (10 g) was extracted with 100 ml of 70% ethanol using ultrasonic (200 W, 59 kHz, Shanghai Kudos Sonication Machine Company Ltd., China) for 60 min at room temperature. Extract was then filtered through filter paper Whatman no. 4, and evaporated [9].

B) Microwave: Jatropha shell powder (10 g) was treated with microwave Samsung, Model MF245 (Korea), power was 900 W for 10 min [10]. It was extracted using 100 ml of 70% ethanol, filtered through Whatman no. 4, then evaporated.

C) Microwave and ultrasonic: Jatropha shell powder (10 g) was treated with microwave for 10 min, extracted with 100 ml of 70% ethanol in an ultrasonic for 60 min. The mixture was filtered, evaporated [9].

2.3. Determination of total phenolic compounds

Shells extracts were performed according to [11] using ethanol 70%. The Folin– Ciocalteu assay, adapted from [12] was used for the determination of total phenolics present in samples. The absorbance was read at 685 nm against the blank standard. Gallic acid was used to estimate standard curve and the results were expressed in mg of gallic acid per g.

2.4. HPLC analysis of phenolic compounds

It was carried out using an Agilent 1260 series, C18 column (4.6 mm x 250 mm i.d., 5 μm). The multi-wavelength detector was monitored at 280 nm. The injection volume was 10 μl. The column temperature was maintained at 35 °C [13].

2.5. Antioxidant Activities

DPPH radical scavenging activity

Extracts effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical at 517nm was evaluated according to[11]. where Ac and As are the control and sample absorbance respectively.

Ferric reducing power (FRAP) assay

The FRAP assay [14] is based on the ability of phenolics to reduce Fe3+ to Fe 2+.

2.6. Preparation of cookies

Cookies were prepared using [15] as ingredients were: 100 g flour, 50 g sugar, 40 g shortening, 0.75 g sodium bicarbonate, 0.5 g vanilla and 30 g water weighed accurately. Jatropha seed shell powder: 0.0, 1.0, 3.0, 5.0 and 7.0 %.

2.7. Physical characteristics of cookies

Diameter (D), thickness (T), spread ratio (SR) and spread factor (SF) of six biscuits were measured as described in the [17] method. The spread factor (SF) was calculated as:

SF = D X CF X10 T

CF is a correction factor (1.0).

2.8. Chemical analysis formulated cookies samples

2.8.1. Proximate analysis

Proximate analysis of Jatropha seed shells and cookies samples were determined according to the method of [16].

2.8.2. Lipid oxidation (TBARS)

The Thiobarbituric acid reactive substances TBARS were determined according to [18]. A standard curve was prepared using 1, 1, 3, 3-tetraethoxy-propane (TEP).

2.9. Sensory evaluation of the cookies

Evaluation of the quality and acceptability of the cookies with Jatropha seed shells flour (0, 1.0, 3.0, 5.0 and 7.0 %) was performed using panelists of ten well-trained judges on a 9-point hedonic scale (1-dislike extremely to 9=like extremely) at the Food Technology Department, National Research Centre, Egypt., the sensory evaluations were carried out for color, flavor, taste, texture, odor, and overall-acceptability according to [19].

2.10. Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) with P < 0.05 statistically significant [20].

3. Results and Discussion

3.1. Effect of microwave and ultrasonic treatments on jatropha shell total phenolic compounds

The total phenolic content of the Jatropha seed shell is shown in Table 1. It is obvious that every treatment led to increase the concentration of phenols in a substantial (P <0.05) way. It can be shown that the ultrasonic method extracts total phenols more effectively than the microwave method. The amount of total phenolic compounds increased when ultrasonic and microwave processing were combined, and the greatest value of phenolic compounds was at 12.94 mg gallic acid per gram under these conditions as opposed to 9.57 mg gallic acid per gram under control (Table 1). The combination of microwave and ultrasonic extraction seems to be the most effective way of extraction.

3.2. Characterization of phenolic compounds by HPLC

Table 1 displays the effects of the therapies. 13 phenolic compounds were detected by HPLC, as shown in Figure 1. Gallic acid predominated in the phenolic 2082.59 µg/g sample from Shell, followed by vanillin (733.33 µg /g), dihydroxyisothiocyanate (568.8 µg/g), coffeic acid (434 g/g), and propyl gallate (424.5 µg /g). When the various extraction methods were contrasted, greater phenolic acid concentrations were seen in gallic acid, coffeic acid, vanillin, querectin, propyl gallate, and dihydroxyisoflavone (Table 1). Ultrasonic extraction was determined to be the most efficient extraction method that maximised the phenolic acid concentration. However, the extract from the microwave treatment was more abundant in gallic acid, cinamic acid, syringic acid, cinamic acid, and naringenin. However, both ultrasound and microwave treatments demonstrated the ability to dissolve ester and ether bonds and liberates the phenolic acids in the majority of instances [21]. It seemed that microwave or ultrasonic processing reduced several phenolic acids, including catechin, syringic acid, and coumaric acid. Combining ultrasonic and microwave extraction released phenolic acids in a synergistic manner. That might be the result of increased ester or ether bond breakdown. Combining more than one treatment, such as microwave and ultrasonic, may be helpful for phenolic acid extraction in order to increase phenolic acid concentration.

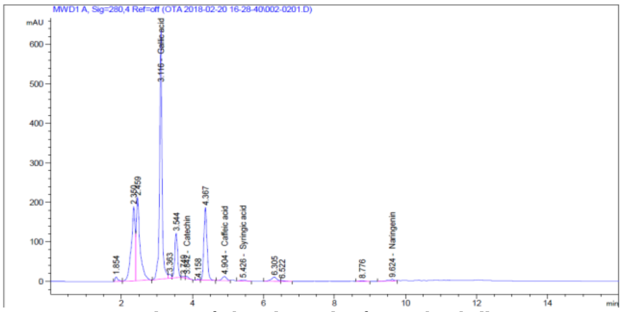

Fig. 1: HPLC analysis of phenolic acids of Jatropha shell.

Table 1
Phenolic acids (µg/g) of Jatropha shell by HPLC

Conc. (µg/g)	Control	Ultrasonic	Microwave	Ultrasonic/Microwave
Gallic Acid	2082.5	2346.0	2439.1	2663.4
Catechin	95.0	90.3	79.7	125.8
Coffeic Acid	443.0	583.4	470.3	609.0
Syringic Acid	41.5	26.0	115.2	0.0
Rutin	72.0	96.5	0.0	87.2
Coumaric Acid	23.0	21.2	19.2	17.1
Vanillin	737.3	1031.2	952.7	1054.8
Ferulic Acid	0.0	0.0	0.0	0.0
Naringenin	0.0	0.0	16.9	0.0
Querectin	124.0	437.0	417.8	569.0
Cinnamic Acid	70.8	145.8	161.8	183.2
Propyl Gallate	424.5	1353.9	608.6	1368.8
Dihydroxyisoflavone	568.8	923.1	640.6	947.6
FRAP µg Trolox eq/g sample	651.56d±1.06	906.03b±4.82	788.67c±3.53	997.53a±6.01
DPPH antioxidant activity as mg Vit. C/g sample	40.24d±0.68	56.96b±0.44	51.45c±0.10	58.31a±0.23
Total Phenol mg/g	45.73d±0.31	95.73b±0.45	84.9c±0.44	121.35a±0.39

*Values FRAP, DPPH, and total phenol are means of triplicate determinations ± standard deviation (SD).
*Means within rows with different letters are significantly different (P < 0.05).

3.3. Antioxidant activities of jatropha shells affected by microwave and ultrasonic treatments

The combination of ultrasonic and microwave extraction treatment offered the significant (P <0.05) strongest antioxidant activity power when compared to separate treatments of either ultrasonic or microwave, according to tests of their activities using two distinct methods: DPPH and FRAP. In addition to the aforementioned, the extract obtained using ultrasound had better (P <0.05) antioxidant activity than that obtained using microwave treatment (56.96 51.45 mg ascorbic acid/g DM), which was indicative of its ability to scavenge DPPH radicals. The microwave extract and ultrasound had the highest antioxidant activity (906.03-788.67 g Trolox eq/g sample) of the FRAP radical in comparison to the control (Table 1). The antioxidant activity of the phenolic compound was shown to be enhanced by ultrasonic and microwaves by [22]. Brand-Williams [23] reported that the phenolic compounds antioxidant activities are influenced by their structural form where; para and ortho hydroxyl group positions are more active than meta position.

3.4. Chemical composition of Jatropha curcas shells flour and the cookies prepared from its different levels

According to data, the shells of *Jatropha curcas* seeds are mostly made of fiber (84.6%) and contain relatively little total carbs, protein, and fat. Table 2 shows the approximate composition of cookies made with various amounts of *Jatropha curcas* shells flour. Cookies made with more *Jatropha curcas* shell flour had lower moisture content and a substantial (P < 0.05) increase in crude fibre and ash content. These findings concur with those made public by [24]. Compared to control sample A (100% wheat flour), cookies with low moisture content would have longer storage times because microbial activity would be reduced [25]. Probably as a result of the minerals (calcium, magnesium, potassium, and others) present in the *Jatropha curcas* shell, the ash content increased from 0.54% to 2.31% [26]. According to observations made by [27] an increase in the ash content might make the product a suitable source of minerals. The cookies' fat content dropped from 21.07% to 17.76, a significant reduction (P < 0.05). The decreased fat level in *Jatropha curcas* shells flour was claimed to be the cause of this. Consumers who are concerned about their health may appreciate the cookies' comparatively low-fat level.

Table 2
Chemical composition (% on dry weight) of cookies formulated with different levels of Jatropha seed shell flour

Treatments	Moisture	Fat	Ash	Protein	Crude fiber	Total phenols mg/g
Jatropha shell flour	6.32±1.02	1.15 ±0.02	6.16 ^{a}±1.02	4.41±1.02	84.65 ^{a}±3.0	45.73^{a}±0.31
Control	3.26 ^{a}±0.02	21.07 ^{a} ± 3.0	0.54 ^{e} ±0.03	9.8±0.66	1.87 ^{e} ±0.02	1.17^{cd}±0.01
1.0 %	2.94^{b}±0.02	20.99^{b} ± 3.2	1.33 ^{d} ±0.02	9.36±0.9	2.53^{d} ±0.02	1.37^{c}±0.01
3.0 %	2.83^{c}±0.02	19.94 ^{c} ± 2.0	1.85 ^{c} ±0.02	9.02±0.51	3.14 ^{c} ±0.02	1.76^{bc}±0.01
5.0 %	2.35^{d}±0.02	18.51 ^{d} ±3.0	1.98 ^{b} ±0.02	8.83±0.63	4.15 ^{c} ±0.02	2.0^{b}±0.03
7.0 %	2.26^{e}±0.02	17.76 ^{e} ±2.0	2.31 ^{a} ±0.02	8.33±0.87	5.42 ^{a} ±0.02	2.75^{b}±0.02

All values are means of triplicate determinations ± standard deviation (SD).
Means within columns with different letters are significantly different (P < 0.05).

3.5. The total phenolic content of formulated cookies

In addition to their function as naturally occurring antioxidants in cookies and as an accessible source of functional food, the total phenolics of Jatropha shell cookies were evaluated in order to assess their impact on the suppression of lipid oxidation. Table 2 displays the findings of phenolic compouns of prepared cookies as gallic acid equivalents (GAE). The total phenol content of all treatments increased significantly (p < 0.05), which indicates that the inclusion of Jatropha shell increased the total phenol content of cookies. In comparison to the control (1.17 mg/g), the total phenolic content of cookies varied from 1.37 mg/g with 1% Jatropha shell additions to 1.75 mg/g with 7% Jatropha shell additives. This suggested that the integration of Jatropha shell may have been the source of several phenolic chemicals.

3.6. Effect of jatropha total phenols on lipid oxidation (TBA value) of cookies

The TBA value is considered to be a reliable indication of lipid oxidation since it produces malonaldehyde (MDA). The TBA of cookies with various jatropha shell additions is shown in results in Fig. 1. The lowest TBA value was found in samples of cookies containing 7% jatropha shell additives (0.63 mg malonaldhyde/kg sample), and the highest values were found in samples of cookies containing 1% jatropha shell additives (0.91 mg malonaldhyde/kg sample) in comparison to control samples (0.94 mg malonaldhyde/kg sample), as shown in Fig.2. The sudden decrease in TBA levels at zero time amply demonstrates the phenolics found in jatropha shells' capacity to prevent lipid oxidation by scavenging free radicals right away after addition. During storage of cookies for three month, TBA values were increased dramatically for all samples. TBA values were decreased (P < 0.05) when jatropha shell levels increased, in comparison to the control, indicating that the jatropha shell added to cookies had antioxidant activities and emphasizing its ability to inhibit lipid peroxidation during storage to extend its shelf life. These findings indicated that these antioxidants slowed lipid oxidation both during storage and just after making of cookies. Jatropha products include bioactive substances (phenolics), which have antioxidant qualities, according to [28].

3.7. Lipid oxidation and phenolic compounds correlation

A strong negative correlation between the total phenol concentration and TBA (P <0.05) was found. The correlation, which was -0.94, explained that total phenolic compounds also play a role in reducing the formation of thiobarbituric acid reactant substance (TBA), as well as delaying the oxidation of lipids in cookie samples. This is because these compounds interact with molecules to reduce their levels, which in turn reduces the formation of TBA.

Table 3
Physical characteristics of cookies prepared by different levels of Jatropha curcas seed shells flour

Treatments	Diameter (cm)	Thickness (cm)	Spread ratio	Spread factor
Control	7.3a±0.03	0.33 e ±0.02	22.30 a ±3.14	223.05 a ±3.44
1.0 %	7.1b±0.02	0.36 d ±0.04	17.75 b ±0.22	177.50 b ±2.24
3.0 %	6.93c±0.57	0.45 c ±0.02	15.62c ±2.06	156.17 c ±2.64
5.0 %	6.87d±0.02	0.50 b ±0.02	13.73cd ±0.11	137.33 cd ±3.03
7.0 %	6.75e±0.02	0.55a ±0.02	12.78d ±1.26	123.78d ±2.69

[a]All values are means of triplicate determinations ± standard deviation (SD).
[b]Means within columns with different letters are significantly different (P<0.05).

3.8. Physical characteristics of cookies prepared by different levels of Jatropha curcas seed shells flour

Table 3 provides information on the width, thickness, spread ratio, and spread factor of cookies made with various percentages of *Jatropha curcas* shell flour blends and cookies made entirely with wheat flour. The findings show that the cookies' diameters ranged from 6.75 to 7.30 cm. is steadily reduced as the percentage of flour made from jatropha shells grows. The spreading properties of the fat and gluten in composite flour may be to blame for this [29]. The thickness of the cookies changed substantially (P <0.05) from 0.33 to 0.55 cm on the opposite side. The amount of flour made from jatropha shells steadily increased the thickness of the cookies. Increased fiber content prevented cookies from spreading as quickly, decreasing their width and increasing their thickness as a result [30].

The spread ratio of the cookies is a crucial factor in determining how well they rise. The spread ratio of the cookies made with *Jatropha curcas* shell flour additions differed substantially (P <0.05) from the treatment (12.78 to 22.30%), according to Table 3's findings. As the quantity of Jatropha seed shell flour was increased, the spread ratio of the cookies significantly dropped. The findings showed that when the amount of additives in Jatropha seedshell flour grew from 223,055 to 123,78, respectively, the spread factor steadily reduced. An increase in the amount of Jatropha seed shell flour, which is known to have better water-bending ability, may have reduced the spread ratio by increasing the amount of dietary fiber present [31]. Previous research linked high spread ratios with high fat levels Better rising properties will be exhibited by cookies with a low spread ratio than by those with a large spread ratio [32].

3.9. Sensory evaluation of cookies prepared by different levels of Jatropha curcas seed shells flour

The sensory evaluation of cookie samples made with various amounts of Jatropha seed shell flour is summarized in Table 4's results. According to the data shown in Table 4, significant variations (P <0.05) between samples for the sensory qualities of color, flavor, taste, texture, odor, and overall acceptability were found. In sensory evaluation, the product's color is crucial. According to the statistics, the ratings for flavor and taste of cookies varied from 7.23 to 8.55 and 7.09 to 8.85, respectively, while the scores for cookie color ranged from 6.64 to 8.82. The overall acceptance ranged from 7.23 to 8.68, the texture score was 7.14 to 8.86, and the smell score was 7.45 to 8.68. In comparison to the control sample and other treatments, the treatment with 3.0% Jatropha seedshell flour received superior ratings for color (8.55), flavor (8.05), taste (8.86), texture (8.59), odor (8.0), and overall acceptability (8.68). The amounts of Jatropha shell flour increased while general acceptability declined. Among other treatments (Figure 3), however, 3.0% was preferable and largely acceptable (8.32). Therefore, 3.0% additives might be suggested for production as cookies with acceptable sensory qualities.

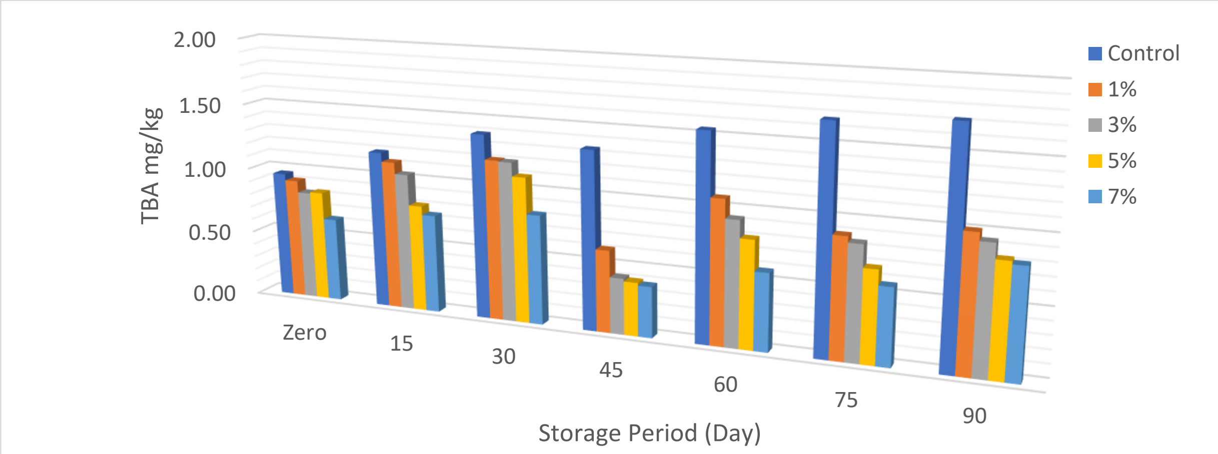

Fig. 2. TBA values (mg/kg) of wheat flour cookies formulated with different concentrations of Jatropha seed shell

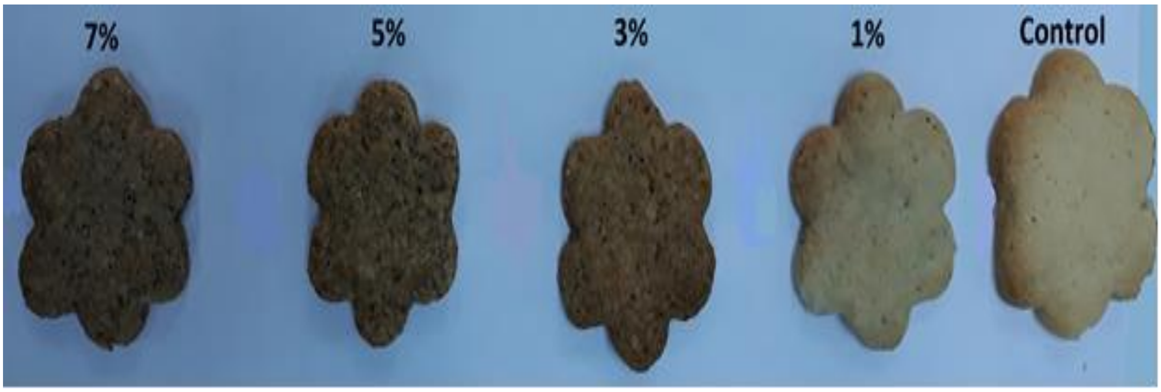

Fig. 3. Cookies prepared by different levels of Jatropha curcas seed shells flour

Table 4
. Sensory evaluation of wheat flour cookies formulated with different levels of Jatropha seed shell flour

Treatments	Color	Taste	Odour	Flavour	Texture	Overall-acceptability
Control	8.82 a ±0.72	8.85 a ±0.91	8.68 a ±1.01	8.55 a ±1.04	8.86 a ±0.70	8.84 a ±0.49
1.0 %	7.64 c ±0.71	8.18 c±0.87	7.82 ab ±0.98	7.73 c ±1.02	8.23 ab ±0.93	8.32 bc ±0.64
3.0 %	8.55b ±1.13	8.68 b ±0.35	8.0 ab ±1.07	8.05 b ±1.06	8.59a ±0.80	8.68 b ±0.64
5.0 %	7.0 cd ±0.63	7.64 cd ±1.18	7.55b ±0.93	7.55 d ±1.04	7.68bc ±1.10	7.95 c ±0.91
7.0 %	6.64d ±0.95	7.09 d ±0.83	7.45 b ±0.93	7.23e ±1.13	7.14c ±1.14	7.23 d ±0.93

[a]Each value is expressed as mean ± standard deviation (SD) (n =10).
[b]Means within columns with different letters are significantly different (P<0.05).

4. Conclusion

In order to gain the health advantages of phenolic compounds, new strategies for adding them to our diet are being inspired by the rising need for phenolics in our diet. Combining more than one treatment, such as microwave and ultrasonic, may be helpful for phenolic acid extraction in order to increase phenolic acid concentration. It should be emphasized that using ultrasonic plus microwaves for extraction is thought of as a "green" idea since it might increase the output of phenolic compounds, cut down on processing time, and consume less energy. The aforementioned findings demonstrated that jatropha shell is a potent antioxidant as well as enhance food's nutritional content, extends shelf life, and offers promise as a material for novel functional food cookies items for industrial use.

5. Conflicts of interest

There are no conflicts to declare.

6. Formatting of funding sources

This work was supported by the National Research Centre, Giza, Egypt (Grant No: 11090407/2016–2019 National Research Centre) as a part of an ongoing project.

7. Acknowledgments

The work team is really thankful to the National Research Centre that has funded the project (grantee No: 12010407 / 2019-2021) to allow for the potentiality to complete this research as a part of the project.

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