P-ISSN 1991-8941
E-ISSN 2706-6703
Vol. 19, No. 2 (2025)

Journal of University of Anbar for Pure Science
(JUAPS)

journal homepage: juaps.uoanbar.edu.iq

Open Access

Original Paper

Effect of heat treatment on phenolic content and antioxidant activity of peanut *(Arachis hypogaea* L.) extracts

Eman Gamal Mohamed[1], Souzan Saad Latif[1], Hani M. A. Mohamed[1], Hanaa M. Hassan[2*]

[1]Food Science Department, Faculty of Agriculture, Minia University, El-Minia 61519, Egypt
[2]Department of Agricultural Chemistry, Faculty of Agriculture, Minia University, El-Minia 61519

ARTICLE INFO

Received: 09/05/2025
Accepted: 02/07/2025
Available online: 07/11/2025
December Issue
10.37652/juaps.2025.160010.1377

Cite @ JUAPS

Corresponding author
Hanaa M. Hassan
hanaa_hassan@mu.edu.eg

ABSTRACT

Peanuts are rich in various bioactive compounds, including total phenolics and total flavonoids. The aim of this study is to evaluate the effects of heating on total phenolic content, total flavonoid content, and antioxidant activity in peanut *(Arachis hypogaea* L). Seven peanut treatments were prepared, including heated peanuts at 95 °C for 30, 35, 40, and 45 min, and three unheated forms (unheated whole peanut, inner shell peanut, and outer shell peanut). Changes in antioxidant activity in heated peanuts were assessed by determining total phenolics, total flavonoids, and antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reduction activity potential (FRAP), and 2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) assays. These values were compared with those of unheated peanuts. The results showed that peanut extract heated at 95 °C for 30 min, and the inner shell of peanuts, contained the highest concentrations of phenolic and flavonoid components (22.33, 24.50 mg GAE/g and 5.23, 6.61 mg QE/g, respectively). The highest DPPH, ABTS, and FRAP scavenging activities were also observed in the peanut extract heated at 95 °C for 30 min and in the inner shell peanut extract.

Keywords: *ABTS, Arachis hypogaea L., DPPH, FRAP, Peanut extract*

1 INTRODUCTION

The peanut *(Arachis hypogaea)* belongs to the bean-vegetable family Fabaceae and is regarded as a pea. Despite being a legume, because of its high oil content it is usually classified as an oilseed. Protein, fat, and fiber abound in peanuts [1].

Peanut is an important crop that is grown worldwide. Although it is used commercially to produce oil, peanut by-products also contain a number of other beneficial components, including proteins, fibers, polyphenols, antioxidants, vitamins, and minerals, which can be used in a variety of processed foods. It contains all 20 amino acids, with a notably high concentration of essential amino acids such as arginine, and it is also a rich source of coenzyme Q10. These bioactive compounds are thought to extend life expectancy and are recognized for their ability to prevent illness. Pretreatment and boiling are two processing techniques that have been shown to increase the concentration of bioactive compounds [2]. Their effectiveness has been shown in several investigations, and some are being manufactured commercially by artificial synthesis. However, because there is a lower chance of toxicity or adverse effects, consuming naturally occurring phytochemicals is thought to be safer [3]. These bioactive compounds are also thought to increase longevity and are recognized for their ability to prevent illness. Roasting and boiling are two processing methods that have been shown to improve the concentration of these beneficial compounds [4].

The aim of this study is to evaluate the chemical composition and the quantitative levels of phytochemical compounds, including total phenolic compounds, total flavonoids, and antioxidant properties, in peanut extract.

2 MATERIALS AND METHODS

Peanuts (Giza 6 variety) were obtained from Assiut, Abnub Abu Sheel village, and all chemicals used in this study were purchased from SIGMA-ALDRICH (22 Abo Zar El-Ghafary St. from El-Tayaran St, Nasr City - Cairo).

2.1 Evaluation of total protein

2.1.1 Sample preparation and protein extraction

With a few minor adjustments, the salt-alkaline extraction was carried out according to [5]. Briefly, a homogenizer was used to mix 30 milliliters of 0.1 M sodium hydroxide (NaOH) in 3.5% sodium chloride (NaCl) with 0.5 grams of raw material. The homogenates were incubated for 90 minutes at 60 °C prior to centrifugation at 4000 rpm for 30 minutes at 4 °C. The resulted supernatants were frozen and stored at -20 °C.

2.1.2 Determination of crude lipid (conventional method)

Two grams of the dried powder were placed in a porous thimble of a Soxhlet extractor that had a cotton plug in its mouth. The thimble was then placed in an extraction chamber suspended above a pre-weighed flask containing a methanol-chloroform solvent mixture. To extract crude lipid, the entire assembly was adjusted, and the flask was heated using a heating mantle for eight to ten hours. After extraction was completed, the solvent was removed under low pressure to obtain the crude lipid, and the thimble was removed from the Soxhlet device. The lipid-containing flask was then cooled in a desiccator, weighed, and heated at 100 ºC for 30 minutes to eliminate any remaining solvent. The proportion of crude lipid content was determined by calculating the amount of crude lipid [6].

2.1.3 Protein quantification by bradford method

The Bradford test was carried out using the procedure outlined in [7]. It is a quick and accurate spectroscopic analytical technique for determining the amount of protein in a solution. The method is a colorimetric protein assay based on the absorbance shift of Coomassie brilliant blue G-250 dye. Coomassie brilliant blue G-250 exists in three forms: cationic (red), neutral (green), and anionic (blue). In acidic conditions, the dye shifts from its red form to its blue form and binds to the protein being measured. If no protein is present to bind, the solution remains brown. The dye and the carboxyl and amino groups of the protein form a strong, non-covalent complex through van der Waals forces and electrostatic interactions.

2.1.4 Carbohydrates quantification by phenol- sulfuric acid method (colorimetric method)

To create working standards for glucose, standard concentrations of 0.1, 0.2, 0.4, 0.6, and 0.8 mg of glucose were prepared and dissolved in 1 milliliter of distilled water. 1 mL of 5% phenol and 5 mL of 96% sulfuric acid were then added portion-wise (one at a time) to each boiling tube containing each concentration. The final volume was adjusted to 1 mL. The mixture was shaken vigorously to ensure that the phenol and sulfuric acid were fully combined with the working standard. To allow the reaction to finish, all tubes were submerged in a water bath set at 25-30 °C for 15 minutes after the 10-minute mark. One milliliter of distilled water was used as the blank. The color absorbance was measured at a wavelength of 490 nm using the bio system 310 plus spectrophotometer [8].

2.1.5 Determination of moisture content by thermogravimetric analysis

When a material is heated, its mass decreases due to water vaporization. This loss in mass is used to measure moisture content in thermogravimetric analysis. A precision balance continuously calculates and records the change in mass. The mass of the sample is measured before and after the drying process to determine the final moisture content on a percentage basis. According to [9], thermogravimetric analysis provides a comprehensive, precise, and rapid method for determining moisture content.

2.1.6 Ash content determination

A method was used to determine a sample's ash content [10], which measures the amount of noncombustible inorganic material it contains. Unlike the ash that remains after partial combustion, the residue obtained after complete combustion typically consists of oxides of the inorganic elements originally present in the sample.

$$\%\text{ASH} = \frac{\text{ashed wt.} - \text{crucible wt.}}{\text{crucible and sample wt.} - \text{crucible wt.}} \times 100 \quad (1)$$

2.1.7 Total crude fiber by gravimetrical method

Crude fiber was measured gravimetrically after chemical digestion and solubilization of other components [11]. After ignition, the weight of the remaining fiber was adjusted for the amount of ash.

2.2 Preparation of peanut

The experiment was carried out with two factors: roasting temperature and roasting time. The roasting temperature was 95 °C, and the roasting time consisted of four levels, including 30, 35, 40, and 45 minutes [12].

2.3 Preparation of peanut extract

Ten grams of roasted and unroasted peanuts were extracted independently using 100 mL of ethanol and left in a shaker at room temperature (22 ± 1 °C) overnight. Whatman No. 1 filter paper was then used for filtration. With a few slight adjustments, the residues were extracted again under the identical conditions as described by [13] .

2.4 Phytochemical characterization and antioxidant screening

2.4.1 Determine the total phenolic and flavonoid contents via colorimetry analysis

Using the Folin-Ciocalteu colorimetric method, the total phenolic content was measured and reported as gallic acid equivalents (mg gallic acid/g of extract) [14]. Using aluminum chloride colorimetry, the total flavonoid content was measured and expressed as quercetin equivalents (mg quercetin/g of extract) [15].

2.4.2 Measurement of antioxidant capacity using DPPH, ABTS and FRAP

The DPPH test is used to estimate antioxidant activity by determining the free radical scavenging capability and the mechanism by which antioxidants act to limit lipid oxidation. Because the analysis requires a very short amount of time, the procedure is commonly employed. The ability of the peanut extract to scavenge the stable free radical DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) was assessed using the methodology indicated in [16]. The IC_{50} value was determined by converting the concentrations to their logarithmic values and applying the nonlinear inhibitor regression equation (log inhibitor) vs. normalized response variable slope using GraphPad Prism 5® [17]. With a few minor adjustments, the method in [18] was used to assess the ability of the peanut extract to scavenge the free radical ABTS (2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid). The reducing power of the peanut extract was measured using the ferric reducing antioxidant power (FRAP) test as described by [19].

2.5 Statistical evaluation

The experimental data consisted of the averages and standard deviations of three measurements taken in parallel. ANOVA techniques were used for the analysis of variance. To compute statistics at $P < 0.05$, GraphPad Prism (GraphPad Software, San Diego, California, USA) was used [20].

3 RESULTS AND DISCUSSION

3.1 Chemical composition of peanut

The average values for the chemical composition of peanuts are given in Table 1. Peanut treatment is an important process in the food industry to ensure the quality, taste, and safety of the final product [21]. Proximate analysis is a vital part of this treatment, providing insights into the nutritional content and composition of peanut samples [22].

Our results indicated that the whole peanut contained 27% fiber, 19.12% total lipids, 25.4% protein, 3.51% ash, 1.31% moisture, and 23.66% carbohydrate. Peanut inner shell and peanut outer shell contained 38.7%, 50.5% fiber, 12.92, 2.51% total lipids, 15.7, 6% protein, 6.65, 4.54% ash, 3.40, 8.21% moisture, and 22.63, 28.24 carbohydrate, respectively. On the other hand, peanut treatment at 95 oC for 30, 35, 40, and 45 min contained 32.5, 37.3, 32.1, 38.1% fiber, 25.63, 26.91, 22.53, 24.22% total lipids, 16.31, 8.95, 17.75, 10.3% protein, 4.34, 3.64, 3.42, 3.06 ash, 2.92, 1.80, 3.72, 1.92% moisture, and 18.27, 21.38, 20.48, 22,4 carbohydrates, respectively. The proximate analysis conducted on the batch of peanuts revealed some observations about their nutritional and compositional profile [23]. According to [24], the chemical composition of peanut cultivars showed that the amount of moisture ranged from 5.53±0.20 to 5.93±0.02 percent, the ash ranged from 2.00±0.11 to 2.17±0.05%, the fats ranged from 49.80±3.54 to 50.90±0.93%, the proteins ranged from 23.83±1.71 to 26.43±1.15%, the carbohydrates ranged from 13.23±2.20 to 19.42±3.83%, and the fiber ranged from 4.95 to 8.53%.

Several key parameters were analyzed, including protein content, fat content, water content, ash content, and fiber content. This allowed us to collect a comprehensive dataset to draw meaningful conclusions about the quality and nutritional value of the peanuts in question [25]. By closely examining the results obtained from this analysis, we can better understand the advantages and limitations of using this particular batch of peanuts for various applications such as processing, cooking, and

Table 1 Proximate analysis of peanut treatment

Treatment	Moisture (%)	Ash content (%)	Crude fiber (%)	Total Lipids content (%)	Total protein content (%)	Total Carbohydrate (%)
Whole peanuts	1.31 ±0.1	3.51±0.14	27.0±1.7	19.12±0.64	25.4±1.5	23.66±0.81
Peanut Inner shell	3.40± 0.08	6.65±0.22	38.7±0.5	12.92±1.31	15.7±1.7	22.63± 0.36
Peanut Outer shell	8.21 ±0.2	4.54±0.24	50.5±1.5	2.51 ±1.2	6±1.03	28.24±0.91
Peanut Tm 95, 30min	2.92 ±0.1	4.34±0.41	32.5±1.1	25.63 ±1.5	16.34±0.58	18.27± 0.92
Peanut Tm 95, 35m in	1.80 ±0.1	3.64±0.20	37.3±1.32	26.91±1.04	8.95 ±0.65	21.38±0.61
Peanut Tm 95,40min	3.72 ±0.1	3.42±0.17	32.1±0.416	22.53± 1.5	17.75±0.8	20.48± 0.75
Peanut Tm95, 45min	1.92 ±0.2	3.06±0.24	38.1±1.40	24.22± 0.72	10.3±1.28	22.4± 0.55

Note. Each value is expressed as the mean± SD (n=3 Composition)

consumption [22].

3.2 Antioxidant activity of peanut

Total phenolic and flavonoid compounds were measured using gallic acid equivalents (mg GAE/g sample) and quercetin equivalents (mg QE/g sample), according to the present findings (Table 2). Peanut extract at 95 °C for 30 min and the inner shell of peanuts contained the highest concentrations of phenolic and flavonoid components (22.33, 24.50 mg GAE/g and 5.23, 6.61 mg QE/g), respectively. Flavonoids, carotenoids, and triterpenes have antioxidant properties that scavenge reactive oxygen species and protect biological components, including DNA, proteins, and lipids, from damage [26].

Antioxidants are substances that help to reduce the radical state of DPPH by donating an electron or hydrogen. Due to this reaction, DPPH turns yellow instead of purple [27]. The highest DPPH, ABTS, and FRAP scavenging activities were observed in the peanut extract at 95 °C for 30 min and in the inner shell peanut extract. Furthermore, it was noted that the extraction method may affect flavonoid content [28]. For example, the study found that when determining the overall flavonoid content of seeds and sprouts, ethanol was more effective than methanol. Additionally, in the DPPH and ABTS+ assays, a peanut variety developed in Korea demonstrated the highest levels of radical scavenging activity. Strong results in all assays were shown by germinated samples, demonstrating the advantages of germination in boosting phytochemical characteristics [29].

The inner shell, or skin, of peanuts is rich in dietary fiber and natural antioxidants with radical scavenging capacity. Consequently, the nutritional value of peanut butter is improved when the inner shell is included [30]. Peanut butter has a resveratrol content that is roughly three times higher than that of roasted peanuts with their skin and is comparable to that of grape juice [4].

4 CONCLUSION

Peanuts are a nutrient-dense food, rich in protein, healthy fats, and fibre. Changes in the antioxidant activity of heated peanuts were assessed by determining total phenolic content, total flavonoid content, and antioxidant activity using DPPH, FRAP, and ABTS assays, and these values were compared with those of unheated peanuts. The highest DPPH, ABTS, and FRAP scavenging activities were observed in the peanut extract heated at 95 °C for 30 min and in the inner shell peanut extract.

ACKNOWLEDGEMENT

N/A

FUNDING SOURCE

No funds received.

DATA AVAILABILITY

N/A

DECLARATIONS

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Consent to publish

All authors consent to the publication of this work.

Ethical approval

N/A

Table 2 Total phenolic compounds, total flavonoids, and antioxidant activity by (DPPH IC_{50} , ABTS and FRAP) of extracts

Constituent	Whole peanuts	Peanut 30 Min.	Peanut 35 Min.	Peanut 40Min.	Peanut 45 Min.	The outer shell of peanuts	The inner shell of peanuts
Total phenolics (mg gallic acid/g)[a]	12±1	22.33±0.57	20±2	9.67±1.15	10.50±0.5	23±0.0	24.50±0.5
Total flavonoids (mg quercetin /g)[b]	5.14±0.05	5.23±0.15	5.07±0.15	5.16±0.06	5.05±0.05	5.4±0.34	6.61±0.2
DPPH IC_{50} (μg/mL)*	61.98	21.02	50.73	58.86	51.12	38.09	19.40
ABTS (μM TE/mg)	58.59±3.92	127.77±8.46	64.22±0.71	91.67±6.73	83.65±2.15	649.31±42.45	3959.32±267.2
FRAP (μM TE/mg)	20.85±1.44	44.85±2.85	18.45±1.19	43.32±1.80	39.95±2.50	475.12±26.79	2886.4±110.68

Notes. a: mg GAE /g of dry leaves extract; b: mg QE/g of dry leaves extract. Each value is expressed as the mean.± SD.
The IC_{50} values correspond to the amount of extract required to scavenge 50% of the radicals present in the reaction mixture

REFERENCES

[1] Suchoszek-Lukaniuk K, Jaromin A, Korycińska M, Kozubek A. Nuts and seeds in health and disease prevention. Elsevier; 2011. 10.1016/B978-0-12-375688-6.10103-3

[2] Moharana A, Lenka B, Singh AP, Kumar N, Nagaraju B, Das SR. Peanut as a food source: A review. Journal of Pharmacognosy and Phytochemistry. 2020;9(6):225-32

[3] Kim J, Choi CH, Lee AY, Lee S. Antioxidant, Anticancer, and Neuroprotective Activities and Phytochemical Analysis of Germinated Shoots. Journal of Food Biochemistry. 2023;2023:1–9. 10.1155/2023/2074678

[4] Arya SS, Salve AR, Chauhan S. Peanuts as functional food: a review. Journal of Food Science and Technology. 2015;53(1):31–41. 10.1007/s13197-015-2007-9

[5] Mæhre H, Dalheim L, Edvinsen G, Elvevoll E, Jensen IJ. Protein Determination—Method Matters. Foods. 2018;7(1):5. 10.3390/foods7010005

[6] Khilari V, Sharma P. Determination of total lipids from five underutilized wild edible fruits in Ahmednagar district, Maharashtra (India). International Journal of Advanced Research in Biological Sciences. 2016;3(7):14-20

[7] Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry. 1976;72(1–2):248–254. 10.1016/0003-2697(76)90527-3

[8] Roberts R, Elias R. Determination of carbohydrate using phenol sulphuric acid method. Springer; 2011

[9] Matejtschuk P, Duru C, Malik K, Ezeajughi E, Gray E, Raut S, et al. Use of Thermogravimetric Analysis for Moisture Determination in Difficult Lyophilized Biological Samples. American Journal of Analytical Chemistry. 2016;07(03):260–265. 10.4236/ajac.2016.73023

[10] John St. (1939). JOACI,XXII, 630

[11] AOAC (1984) Official Methods of Analysis, Assoc. Off. Anal. Chem., Supplement, Secs. 7.066-7.069, pp. 160-161D. O. Holst, J. Assoc. Off. Anal. Chem. (Vol. 65, No. 2, 1982), pp. 265-269.

[12] Yanti SA, Soekardi C, Lubis MSY. Analysis of Parameter Roasting on Color and Peanuts Roasted Taste. Aceh International Journal of Science and Technology. 2018;7(2):138–143. 10.13170/aijst.7.2.8964

[13] Yu J, Ahmedna M, Goktepe I. Effects of processing methods and extraction solvents on concentration and antioxidant activity of peanut skin phenolics. Food Chemistry. 2005;90(1–2):199–206. 10.1016/j.foodchem.2004.03.048

[14] Ul-Haq I, Ullah N, Bibi G, Kanwal S, Ahmad MS, Mirza B. Antioxidant and cytotoxic activities and phytochemical analysis of Euphorbia wallichii root extract and its fractions. Iranian journal of pharmaceutical research: IJPR. 2012;11(1):241

[15] Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colometric methods. Journal of Food and Drug Analysis. 2020;10(3). 10.38212/2224-6614.2748

[16] Boly R, Lamkami T, Lompo M, Dubois J, Guissou I. DPPH free radical scavenging activity of two extracts from Agelanthus dodoneifolius (Loranthaceae) leaves. International Journal of Toxicological and Pharmacological Research. 2016;8(1):29-34

[17] Zhang H, Holden-Wiltse J, Wang J, Liang H. A Strategy to Model Nonmonotonic Dose-Response Curve and Estimate IC50. PLoS ONE. 2013;8(8):e69301. 10.1371/journal.pone.0069301

[18] Arnao MB, Cano A, Acosta M. The hydrophilic and lipophilic contribution to total antioxidant activity. Food Chemistry. 2001;73(2):239–244. 10.1016/s0308-8146(00)00324-1

[19] Benzie IFF, Strain JJ. The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "Antioxidant Power": The FRAP Assay. Analytical Biochemistry. 1996;239(1):70–76. 10.1006/abio.1996.0292

[20] Motulsky HJ. Analyzing data with graphpad prism. GraphPad Software. Inc., San Diego, CA. 1999.

[21] Wang F, Miao H, Zhang S, Hu X, Li C, Chu Y, et al. Identification of a major QTL underlying sugar content in peanut kernels based on the RIL mapping population. Frontiers in Plant Science. 2024;15. 10.3389/fpls.2024.1423586

[22] Daramola OS, Kharel P, Iboyi JE, Devkota P. Peanut response to 2,4-D plus glyphosate. Weed Technology. 2023;37(1):46–52. 10.1017/wet.2023.3

[23] Muñoz-Arrieta R, Esquivel-Alvarado D, Alfaro-Viquez E, Alvarez-Valverde V, Krueger CG, Reed JD. Nutritional and bioactive composition of Spanish, Valencia, and Virginia type peanut skins. Journal of Food Composition and Analysis. 2021;98:103816. 10.1016/j.jfca.2021.103816

[24] Shibli S, Siddique F, Raza S, Ahsan Z, Raza I. Chemical Composition and Sensory Analysis of Peanut Butter from Indigenous Peanut Cultivars of Pakistan. Pakistan Journal of Agricultural Research. 2019;32(1). 10.17582/journal.pjar/2019/32.1.159.169

[25] Ahsan T, Tian P, Gao J, Wang C, Liu C, Huang Y. Effects of microbial agent and microbial fertilizer input on soil microbial community structure and diversity in a peanut continuous cropping system. Journal of Advanced Research. 2024;64:1-13. 10.1016/j.jare.2023.11.028

[26] Antony RIC, Umarani V, Sankaranarayanan S, Bama P, Ramachandran J. Antioxidant, antibacterial and cytotoxicity studies from flavonoid rich fraction of Enicostemma axillare (LAM.) raynal leaves. African Journal of Pharmacy and Pharmacology. 2016;10(43):916–925. 10.5897/ajpp2016.4675

[27] Baliyan S, Mukherjee R, Priyadarshini A, Vibhuti A, Gupta A, Pandey RP, et al. Determination of Antioxidants by DPPH Radical Scavenging Activity and Quantitative Phytochemical Analysis of Ficus religiosa. Molecules. 2022;27(4):1326. 10.3390/molecules27041326

[28] Tran GH, Uy NP, Kang SH, Heo W, Lee ES, Roh SS, et al. HPLC/DAD Analysis and Antioxidant Activity of Adlay Sprouts and Seeds. Separations. 2024;11(1):32. 10.3390/separations11010032

[29] Yu HH, Park JS, Lee S. Enhanced Bioactive Compounds and Antioxidant Activity in Germinated Seeds of the New Peanut Variety. BioTech. 2025;14(1):12. 10.3390/biotech14010012

[30] Ma Y, Kerr WL, Swanson RB, Hargrove JL, Pegg RB. Peanut skins-fortified peanut butters: Effect of processing on the phenolics content, fibre content and antioxidant activity. Food Chemistry. 2014;145:883–891. 10.1016/j.foodchem.2013.08.125

How to cite this article

Mohamed EG, Latif SS, Mohamed HMA, Hassan HM. Effect of heat treatment on phenolic content and antioxidant activity of peanut *(Arachis hypogaea* L.) extracts. Journal of University of Anbar for Pure Science. 2025; 19(2):90-95. doi:10.37652/juaps.2025.160010.1377