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Evaluation of Pies Fortified with Aqueous Extract Rich in Phenols of Peanut

Alaa A. Gaafar * and Zeinab A. Salama

Plant Biochemistry Department, National Research Centre, Dokki, Giza, Egypt

Abstract

The present work aims to study the sensory and chemical evaluations of pies after adding the aqueous extract of peanut skin rich in phenols to increase general health as functional products. The pies were manufactured at a pilot-scale formulated by adding peanut skin extract at different concentrations (Zero, 250, and 500 µg/Kg pies flour) and baked and stored for one month at room temperature, protected from light and air. The antioxidant compounds include total phenolic (10.98 µg /g dry pies),and total flavonoids (9.81µg/g dry pies) at 500 µg/Kg. Functional pies (FPs) showed high oxidative stability during storage (30 days) periods (as assessed by antiradical scavenging activity of DPPH˙ and Fe2+-chelating activity compared with that in untreated pies products (control). Data of sensory evaluation revealed that FPs containing peanut skin extract were significantly acceptable as compared with control for main sensory characteristics (color, odor/ aroma, flavor, texture, global appreciation, and overall acceptability). Thus, it could be concluded that functional pies (FPs) had good sensory and nutritional profiles and can be developed as a food market.

Keywords: Phenolic; flavonoids; antioxidant; sensory evaluation; pies; functional foods.

1. INTRODUCTION

Functional food (FFs) is considered to be any food or food component that provides health benefits beyond basic nutrition. A great deal of interest has been paid by the consumers towards natural bioactive compounds as functional ingredients in the diets due to their various health-beneficial effects (AL-Azawi *et al*., 2017) [1]. However, the term functional foods (FFs) was considered to be a tool to promote health and well-being for humans and animals. Diplock *et al*., (1999) [2] define FFs as food compounds have positively affect one or more physiological functions (anti- carcinogenicity, anti-mutagenicity, ant oxidative and antiaging actions), that could lead to increasing the well-being and/or reducing the risk of suffering a disease by modulating physiological systems Vo and Kim (2013)[3]. Therefore, increasing health concerns, efforts have been made by food industries to develop new functional foods. The modern food industry produces cheap, healthy, and more convenient products, in response to increasing demand consumption. Peanut (*Arachis hypogaea* L.) is one of the major oilseed crops of the world. It is also an important source of food protein in many countries. Their oil is very easily digested, and for this reason, they are useful consumptives. Peanuts not only contain the so-called "good" fat (monounsaturated fat), but they are also high in a variety of helpful antioxidants, or chemicals that shield the damaging effects of free radicals. Peanuts are also a source of helpful biologically active components found in plant foods, such as phytochemicals. Some of the phytochemicals in peanuts include flavonoids and phenolic compounds. Recently, peanuts have gained much attention as functional (Pizzolitto *et al*., 2013 and Ibrahim *et al*., 2015)[4,5]. By-products of the peanut industry, which include peanut plant skins and peels have also been identified as rich sources of phytochemicals, suggesting that the bioactivity found in fruits and vegetables could be present, although currently, these plant parts have little economic value (Braga *et al*., 2016)[6]. Recent studies suggest that peanuts consumption might reduce the risk of heart diseases by lowering serum low-density lipoprotein (LDL)-cholesterol level and reduce

*Corresponding author e-mail: dr.gaafar2308@hotmail.com.; (Alaa A. Gaafar).
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the risk in the development of type II diabetes, Alzheimer's Disease, and Cancer prevention (Tamura *et al*., 2012)[7]. The health benefits of peanuts have been attributed to the presence of minerals and vitamins, fatty acids, fiber, and bioactive compounds (Pizzolitto *et al*., 2013)[8]. The aims of studying the sensory and chemical evaluations of pies after adding the aqueous extract of peanut skin rich in phenols to increase general health as functional products.

2. MATERIALS AND METHODS

2.1. Reagents

All reagents and chemicals used in the experiments were purchased from Sigma-Aldrich Chemicals. All solvents used were of analytical grade.

2.2. Material

Peanut skin was obtained from the local market at Giza, Egypt. Pies as functional foods were prepared at National Research Centre-Giza, Egypt.

Figure 1. Peanut skin

2.3. Extraction of peanut skin:

The dried peanut skin 10 g were soaked and extracted with 100 ml of distilled water (aqueous extract), and shaking at room temperature for 48h. . Each mixture was filtered through Whatman No. 1 filter paper and the extraction step was repeated three times. The filtrate was then concentrated to dryness at 40 °C in a rotary evaporator. The crude extracts were stored in a refrigerator until analysis.

2.4. Preparation of pies

The pies were prepared by adding (Flour, oil, egg, H_2O, salt, and yeast) and mixing aqueous extract of peanut skin at three concentrations (zero, 250, and 500 µg/Kg flour) and kneading and baking and stored for one month at room temperature.

2.5. Extraction of pies:

The dried functional pies (FPs) 50 g were soaked and extracted with 500 ml of ethanol, and shaking at room temperature for 48h. . Each mixture was filtered through Whatman No. 1 filter paper and the extraction step was repeated three times. The filtrate was then concentrated to dryness at 40 °C in a rotary evaporator. The crude extracts were stored in a refrigerator until analysis.

2.6. Chemical studies

2.6.1. Total phenolic content

The total phenolic (TP) content was determined in ethanolic extracts of Functional pies (FPs) at different concentrations (zero, 250, and 500 µg/Kg pies flour) by Folin Ciocalteu reagent assay (Singleton and Rossi, 1965)[9]. A suitable aliquot (1 ml) of each ethanolic extract was added to a 25 ml volumetric flask, containing 9 ml of distilled water. One milliliter of Folin Ciocalteu phenol reagent was added to the mixture and shaken. After 5 min, 10 ml of 7 % Na_2CO_3 solution was added to the mixture. The solution was diluted to 25 ml with distilled water and mixed. After incubation for 90 min at room temperature, the absorbance was determined at 750 nm with a spectrophotometer (Unicum UV 300) against the prepared reagent as blank. A total phenolic content in the sample was expressed as µg Gallic acid equivalents (GAE)/g pies dry weight. All samples were analyzed in triplicate.

2.6.2. Total flavonoid content

The aluminium chloride method was used for the determination of total flavonoid (TF) content in ethanolic extracts of functional pies (FPs) at different concentrations (zero, 250, and 500 µg/Kg pies flour) (Zhishen *et al*., 1999)[10]. One ml of each ethanolic extract was added to a 10 ml volumetric flask containing 4 ml of distilled water. To the flask, 0.3 ml 5 % $NaNO_2$ was added and after 5 min 0.3 ml 10 %, $AlCl_3$ was added. At the 6^{th} min, 2 ml of 1M NaOH were added and the total volume was made up to 10 ml with distilled water. The solution was mixed well and the absorbance was measured against the prepared reagent blank at 510 nm by using a spectrophotometer (Unicum UV 300). The total flavonoid in the sample was expressed as µg quercetin equivalents (QE)/ g pies dry weight. All samples were analyzed in triplicate.

2.7. Antioxidant activity of functional food products (FFP) during storage time

The antioxidant activity of FFP was measured by the scavenging ability of DPPH radical and Fe chelating methods. All measurement was replicated 3 times and averaged.

2.7.1. DPPH$^{\bullet}$ radical scavenging assay

The DPPH$^{\bullet}$ (0.1 mM) in methyl alcohol was prepared and 0.5 ml of this solution was added to 1 ml of different ethanolic extracts of three samples of pies (zero, 250, and 500) at different concentrations (25, 50, 75, 100 µg/ml). The mixture was shaken vigorously and was allowed to stand at room temperature in the dark for thirty minutes. Butyl hydroxytoluene (BHT, Sigma Aldrich, St. Louis, MO,

USA) was used as a positive control, whereas the negative control contained the entire reaction reagent minus the extract. Then the absorbance was measured at 515 nm against methanol (Chu *et al*., 2000)[11]. The capacity to scavenge the DPPH• radical was calculated using the following equation:

DPPH• scavenging effect (%) = $(A_c - A_s / A_c) \times 100$

Where A_c was the absorbance of the control reaction and A_s was the absorbance in the presence of the pies ethanolic extracts. The results were expressed as IC_{50} (the concentration (µg/ml) of the pies ethanolic extracts that scavenges 50 % of DPPH• radical).

2.7.2. Ferrous chelating activity

Chelating activities on ferrous ions were carried out colorimetrically (Hsu *et al*., 2003)[12]. One ml of different ethanolic extracts of three samples of pies (zero, 250, and 500) or EDTA solution as a positive control at different concentrations (25, 50, 75, 100 µg/ml) was spiked with 0.1 ml of 2 mM $FeCl_2$- $4H_2O$ and 0.2 ml of 5 mM ferrozine solution and 3.7 ml methanol were mixed in a test tube and reacted for 10 min at room temperature then the absorbance was measured at 562 nm. A mixture without a fraction was used as the control. A lower absorbance indicates a higher ferrous ion chelating capacity. The percentage of ferrous ion chelating ability was calculated using the following equation:

$$\text{Chelating activity (Inhibition \%)} = [(A_c - A_s) / A_c] \times 100$$

Where (A_c) was the absorbance of the control reaction and (A_s) the absorbance in the presence of the plant fractions. The results were expressed as IC_{50} (the concentration (µg/ml) of the pies ethanolic extracts that chelate 50 % of Fe^{2+} ions).

2.8. Sensory evaluation of pies

A twenty member of an un-trained panel comprising of staff and researchers from the Plant Biochemistry and Food Sciences and Nutrition Departments (National Research Centre) was asked to mark the scores of main sensory characteristics (color, odor/aroma, flavor, texture, and the global appreciation) of pies samples prepared with different amount of main peanut skin extracts (PSE) according to (Meilgaard *et al*., 1992)[13]. Participants were informed about the study and explained that their participation was entirely voluntary, that they could stop the interview at any point, and that the responses would be anonymous. Also, this study has been done by the National Research Centre Ethics Committee, Egypt. Evaluation of the pies was conducted for 24 hours after baking. The panelists have used the points hedonic scale method: 9 (excellent) to 1 (very poor).

2.9. Statistical analysis

The data collected were subjected to analysis of variance (ANOVA) using Costat Statistical Analysis software version 6.303, Cohort, 2004, all analyses were done in triplicate and the averages were presented with their standard deviation. The differences between means were evaluated using least significant differences (LSD) at P≤ 0.05 (Snedecor, and Cochran, 1989)[14].

3. RESULTS AND DISCUSSION

3.1. Chemical composition of functional pies (TP and TF)

Table 1: Phenolic and Flavonoids of functional pies extracts

Samples	TP µg G/g dry pies	TF µg Q /g dry pies
Control (zero)	6.34 ± 0.10	5.59 ± 0.16
250 µg/kg flour	8.20 ± 0.04	7.61 ± 0.16
500 µg/kg flour	10.98 ± 0.71	9.81 ± 0.08
LSD at 0.05	0.23	0.19

All values demonstrate as mean ± S.D. Mean with different letters are significantly different at p ≤0.05

The active ingredients (TP and TF) of the whole Pies supplemented with or without peanut skin extracts rich in polyphenol samples are as presented in Table (1). The total phenolic and flavonoids increased in the proportion of the peanut extracts levels. This observation is in support of the findings of Nepote *et al*., (2005)[15] as well as Gaafar *et al*., (2015, 2018)[16,17]. The increase in total phenolics and flavonoids may be due to the development of Millard reaction products and/or the liberation of phenolic compounds. Several studies reported that processing steps such as heat treatment can liberate the phenolic compounds from residual sources, yielding higher total phenolic content (Yu *et al*., 2005)[18]. They reported that one-gram dry peanut skin contained 0.090–0.125 g total phenolics, and the skin removal methods (such as direct peeling, blanching, and roasting) and extraction had significant effects on total extractable phenolic.

3.2. Antioxidant activity

3.2.1. DPPH• radical scavenging of functional pies extracts

Table 2: IC_{50} of functional pies extracts against DPPH• radical

Extracts	Scavenging activity %				IC_{50} µg/ml
	25 µg/ml	50 µg/ml	75 µg/ml	100 µg/ml	
Control (zero)	2.93 ^{a} ± 0.25	13.28 ^{a} ± 0.57	18.20 ^{a} ± 0.64	75.54 ^{b} ± 0.72	197.26^{d} ± 5.71
250 µg/kg flour	5.21^{b} ± 0.45	16.92^{b} ± 0.64	24.05^{b} ± 0.65	66.49 ^{a} ± 0.34	155.14^{c} ± 1.42
500 µg/kg flour	8.92^{c} ± 0.33	19.91^{c} ± 0.37	25.91^{c} ± 0.21	85.17 ^{c} ± 0.69	149.12^{b} ± 2.92
BHT	34.48 ^{d} ± 0.57	58.32 ^{d} ± 0.62	78.94 ^{d} ± 0.75	94.00 ^{d} ± 0.77	7.61^{a} ± 0.33
LSD at 0.05	0.84	1.37	0.91	1.34	1.77

All values demonstrate as mean ± S.D. Mean with different letters are significantly different at p ≤0.05

Previous studies demonstrated that peanuts have received much attention because peanuts not only contain the so-called "good" fat (monounsaturated fat), but they are also high in a variety of helpful antioxidants, or chemicals that shield the damaging effects of free radicals. Peanuts are also a source of helpful biologically active components found in plant foods, such as phytochemicals. Some of the phytochemicals in peanuts skin extract include flavonoids and phenolic compounds (Munir *et al*., 2018)[19] and (Gaafar *et al*., 2013, 2020)[20,21]. The effect of pie fortified or not fortified with peanut skin extracts on the DPPH· radical scavenging activity expressed as IC_{50} µg/ml, compared with BHT is shown in (Table 2). As reported by (Gaafar *et al*., 2015 and Yu *et al*., 2005)[16,18] the strong anti-oxidative properties of peanut skin extracts could be due to different antioxidant components present in the different parts of peanut. The differences in antioxidant activity were statistically significant and the values of IC_{50} were ranged from 149.12 to 197.26 µg /ml. The highest value for DPPH· scavenging activity was observed with the pie submitted with 500 µg/Kg. The effect of phenolic compounds on DPPH· is thought to be due to their hydrogen donating ability and for the presence of higher levels of total phenolics and flavonoids which play a key role as proton-donating ability and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants (Ghasemzaeh *et al*., 2010; Yingming +., 2004 & Gaafar *et al*., 2016)[22,23,24]

3.2.2. Fe^{2+}-chelating activity

The metal chelating capacity is expressed by the percentage of inhibition of ferrozine-Fe^{2+}complex formation by different extracts. In this essay both samples are interfered with the formation of ferrous and ferrozine complex, suggesting that they have chelating activity and can capture ferrous ions (Halliwell *et al*., 1995)[25]. As shown in (Table 3) the pie submitted with 500 µg /Kg flour exhibited the highest percentage of metal-chelating capacity 184.11 µg/ml compared to pie without peanut flour 200.68 µg/ml.

Table 3. Iron chelating activity of functional Pies extracts

Extracts	Chelating activity %				IC_{50} µg/ml
	25 µg/ml	50 µg/ml	75 µg/ml	100 µg/ml	
Control (zero)	2.94^{a} ± 0.24	5.98^{a} ± 0.40	13.94^{a} ± 0.42	17.52^{a} ± 0.56	228.20^{d} ± 7.84
250 µg/kg flour	5.13^{b} ± 0.73	6.46^{b} ± 0.33	15.81^{b} ± 0.33	21.74^{b} ± 0.49	200.68^{c} ± 6.55
500 µg/kg flour	5.66^{c} ± 0.40	9.03^{c} ± 0.56	18.70^{c} ± 0.24	24.20^{c} ± 0.16	184.11^{b} ± 0.83
EDTA	54.49^{d} ± 0.32	68.48^{d} ± 0.33	82.80^{d} ± 0.56	90.49^{d} ± 0.56	13.32^{a} ± 0.11
LSD at 0.05	0.86	1.28	0.92	1.12	1.28

All values demonstrate as mean ± S.D. Mean with different letters are significantly different at p ≤0.05

3.3. Sensory evaluations

Table 4. Sensory evaluation of functional Pies supplemented with peanut extract

Samples	Control (zero)	250 µg/Kg flour	500 µg/Kg flour
Taste	18^{a}	18^{b}	18^{a}
Aroma	17^{a}	18^{b}	19^{b}
Mouthfeel	7^{a}	9^{b}	$10^{b,c}$
Crumb texture	12^{a}	13^{b}	14^{c}
Crumb color	8^{a}	9^{b}	9^{b}
Crust color	8^{a}	9^{b}	$10^{b,c}$
Break and shred	7^{a}	7^{a}	9^{b}
Symmetry shape	3^{a}	3^{a}	5^{b}

Mean values in the same row which are not followed by the same letter are significantly different (p≤0.05).

Peanut skin aqueous extract rich with polyphenol was as an ingredient was supplemented some food products (Pies) to enhance the biofunctional and nutritional quality of their products and improve its stability against auto-oxidative. Different levels of peanut skin extract rich in polyphenol (zero, 250, and 500 µg/Kg flour) were substituted to obtain Pies, and Pies without polyphenol were used as control. Sensory evaluation of Pies is an important step to consider the possibility towards an industrial and commercial approach. The main sensory characteristics (Test-Aroma-Mouth feel- Crumb texture- Crumb Colour Crust color–Break and shared and Symmetry shape). The results showed that all sensory values had good scores for peanut skin extract at 500µg/Kg and recorded acceptable scores in terms of Aroma, Mouthfeel, crumb Texture, and Crumb color compared to control pie Table 4 and Figure 2. On the other hand, with the addition of 250 µg /Kg, the product was less acceptable to the panelists compared to control pies. Generally, sensory evaluation is an important step to consider the possibility of an industrial and commercial approach (Faustino *et al*., 2019 & Batu and Arslan, 2014)[26,27]. The results also confirm the possibility of using plant wastes of neglected vegetable industrial purposes, as rich sources of plant chemicals. Therefore, agro-food by-products are considered as a new source of functional food (Taha *et al*., 2012)[28]. It could be concluded that using natural polyphenol as an antioxidant compound from peanut skin extract as food additives will be healthier than artificial additives.

4. CONCLUSION

From these results, it can be concluded that polyphenol from peanuts skin extract has a good nutritional value besides its high content of antioxidants compounds such as flavonoids, phenolic acids which could be used as a natural antioxidant, antimicrobial, antitumor, antiviral, effects. Furthermore, this research suggested that the natural polyphenol from peanut skin extract could be used as food additives which will be healthier than artificial additives which play an important role in improving antioxidant intake in human health.

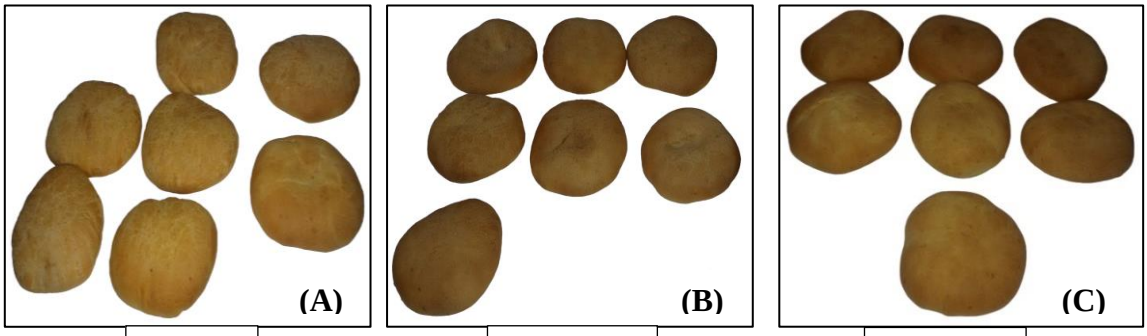

Figure 2: Sensory evaluation of functional Pie supplemented with peanut skin extract

5. CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

6. ACKNOWLEDGEMENT

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