Comparison between Antioxidant Activities of Phenolic Extracts from Different Parts of Peanut

Fakhriya S. Taha, Suzanne M. Wagdy and Fatma A. Singer

Fats and Oils Department, National Research Centre, Cairo, Egypt
suzan.wagdy@hotmail.com

Abstract: Peanut hull and skin are waste products of the food industry. Adding value to these products was the aim of this work. This goal was achieved by studying the phenolic content of the skin, hull and defatted flour of both roasted and unroasted peanuts. The antioxidant activity of the phenolic extracts was determined. The roasted peanut skin extract was then chosen and tested for its power of inhibition of flaxseed oil oxidation. The anticarcinogenic activity of the roasted peanut skin extract on different cell line carcinomas was examined. Both the extractable polyphenols (EPP) and the non extractable polyphenols (NEPP) were determined in the examined parts of the peanuts. Results revealed that NEPP was always higher than EPP and that highest phenolic content was found to be present in the skin. Roasted hull, unroasted hull, roasted skin, unroasted skin, roasted defatted flour, and unroasted defatted flour contained EPP 4.33, 3.38, 41.5, 56.2, 7.33 and 7.23 mg/g, respectively; and contained NEPP 7.4, 5.3, 102, 113.38, 10.3, and 13.4 mg/g, respectively; they also contain total polyphenol extract (TPE) 11.69, 8.73, 144.37, 169.19, 17.48, and 21.31 mg/g, respectively. Free radical scavenging activity (FRSA%) at 100µl conc. reached 87.0 % for roasted and unroasted peanut skin , ca. 79% for roasted and unroasted peanut hull, and least between 45.51-60.45% for unroasted and roasted defatted peanut flour, respectively. FRSA of BHT (0.1%) was 77.81%. Antioxidant activity (AOA) as measured by β-carotene/ linoleate method revealed AOA for roasted skin > unroasted skin > roasted hull > BHT > unroasted hull > unroasted defatted flour > roasted defatted flour, with values 89.13 > 86.65 > 80.33 > 76.33 > 75.27 > 39.34 > 30.37%, respectively. Roasted peanut skin extract (PSE) was chosen for further investigation. Percent reduction of oxidation in flaxseed oil when compared to control oil reached 27.10% and 22% for oil + PSE, and oil + BHT, respectively, as measured by p-anisidine value; and 41.99% and 38.88%, respectively, as measured by peroxide value. PSE exhibited potential as an anticarcinogenic agent, but needs further investigations.

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Key words: peanut hull, peanut skin, peanut flour, extractable polyphenol, non extractable polyphenol, antioxidant activity, anticarcinogenic activity.

1. Introduction

Peanut seed hulls and skins are considered as waste products of the food industry. Recently many seed hull extracts have been proven to exhibit antioxidant activities (Shahidi et al., 2006; Rao et al., 2010; Taha et al., 2011;Singer and Wagdy, 2011; Win et al., 2011). The antioxidant activities of these extracts are mainly due to presence of phenolic compounds. Phenolic compounds or polyphenols constitute one of the most numerous and widely distributed groups of substances in the plant kingdom and are an integral part of both human and animal diets (Panickar and Anderson, 2011). They are a structural class of organic chemicals characterized by the presence of one or more of phenol units (Quideau et al., 2011). The number and characteristics of these phenol structures underlie the unique physical, chemical and biological properties of particular members of the polyphenol class. Phenolic compounds can be divided into several groups including: phenolic acids, flavonoids, tannins, stilbenes and lignans (Han et al., 2007). The interest in phenolic compounds came from the discovery that they exhibit antioxidant properties (free radical scavenging and metal chelating activities) thus their possible beneficial implications in human health (Carrasco-Pozo et al., 2011). Flavonoids have applications as antibiotics, antiulcer, and anti-inflammatory agents (Oueslati et al., 2012), as well as in the treatment of diseases such as hypertension, vascular fragility, allergies, hypercholesterolemia (Bravo, 1998; Arts and Hollman, 2005). Epidemiological studies support the hypothesis that consumption of diets rich in fruits and vegetables decrease the risk of cardiovascular disease, diabetes, and cancer (Panickar and Anderson, 2011).

Peanut (Arachis hypogea L.) is an important oilseed crop. It is not only important for the production of oil, but also for direct consumption. They are consumed raw, roasted, pureed, or mixed with other foods or in different processed forms of which peanut butter is the most important. Recently, peanuts have gained much attention as functional food (Fransisco and Resurrection, 2008). Peanut

shells and skins are usually removed before processing or even when eaten as condiment. Shells and skins are sometimes burned or used in animal feed or as fertilizers. Peanut skin, shell, and kernel extracts were reported to exhibit different levels of antioxidant activity (Duh and Yen, 1997; Yu et al., 2005; Talcott et al., 2005 and Win et al., 2011). Roasting was reported to increase the antioxidant capacity of intact peanuts due to the formation of Maillard reaction products (Talcott et al., 2005). Fransisco and Resurrection (2009a) reported peanut skin to contain a complex series of procyanidin oligomers. While Win et al. (2011) studying peanut skins, hulls raw kernels and roasted kernel flour found that they contain p-hydroxy benzoic acid, chlorogenic acid (not detected in hull), p-coumaric acid (not detected in hull), Ferulic acid and epicatechin (present only in skin), resveratrol, quercetin (not detected in hull), luteolin (present only in hull), kaempferol (detected only in raw and roasted kernel flour).

Peanut is an international edible crop utilized in all countries. It would be an achievement if the huge amounts of hulls and skins resulting after its utilization could be upgraded to a valuable product by producing phenolic extracts from them. Peanuts are usually roasted before eating and before being added to many food recipes. The aim of the present research was to study the effect of roasting on the phenolic content and antioxidant activity of peanut hulls, skins, and defatted kernels (flour). Emphasis was made to extract the condensed tannins or nonhydrolysable polyphenols as they are usually an important but neglected part of the polyphenols. The antioxidant activity of the most effective peanut extract will be examined for their power to inhibit lipid oxidation.

2. Materials and Methods

Materials:

Peanut (*Arachis hypogea L.*) was brought from the local market. Peanuts were hulled and skinned manually, then ground using a pulverizor and sieved to pass through 60 mesh screen. The hulled skinned kernels were ground then defatted in a soxhlet apparatus using n-hexane. The defatted kernel (flour) was spread to dry, then reground in a Ball Mill and sieved to pass through 60 mesh screen. Peanuts with shells were roasted at 150°C in an air draft oven for 30 minutes.

Cell line Carcinomas:

Liver Carcinoma Cell Line (HEPG2), Colon Carcinoma Cell Line (HCT_{116}), Cervical Carcinoma Cell Line (HELA), Breast Carcinoma Cell Line (MCF7), were supplied and used in The National Cancer Institute, Biology Department, Cairo, Egypt.

Analytical methods:

Moisture, oil, protein, ash, crude fibre contents were determined in peanut hulls, skins and defatted flour according to A.O.A.C. (2005). Different crude phenolic extracts of the same samples were determined according to Hung et al. (2002).

Extractable polyphenols (EPP):

Powdered samples (500 mg) were extracted sequentially with 40 ml of methanol : water (50:50 v:v) and 40 ml of acetone: water (70:30 v:v) at room temperature for 60 min in each case, centrifuged at 2500 xg for 15 min. Combined extracts were made up to 100 ml with distilled water. EPP were determined by Folin Ciocalteu according to (Hung *et al.,* 2002) using Gallic acid as standard.

Condensed tannins or non hydrolysable polyphenols (NEPP):

The residue after centrifuge were treated with 40 ml conc. HCl in 1- butanol (50 ml/L) in a water bath at 100°C for 3 hrs, with occasional shaking, then centrifuged at 2000 xg, the supernatant was made up to 50 ml with the same solvent; and absorbance measured at 555 nm using tannin as standard.

The combined supernatants (EPP + NEPP) were designated total phenolic extract (TPE), and concentrated in a rotary evaporated at 50°C for the determination of the antioxidant activity, radical scavenging activity, and anticarcinogenic activity.

Antioxidant activity was determined by two methods: Free radical scavenging activity according to (Kuda*et al*., 2005). The second method used is the coupled oxidation of β- carotene/ linoleic acid method described by (Al-Shaikhan *et al.,* 1995).

Anticarcinogenic activity of the phenolic extract of roasted peanut skin was determined in the National Cancer Institute Cairo, Egypt (Biology Department) on several cell line carcinomas. This was determined by measurement of potential cytotoxicity of the phenolic extracts which was carried out by the Sulfo-Rhodamine-B stain (SRB) assay, according to the method of (Skehan *et al*., 1990).

Effect of phenolic extract on the inhibition of lipid oxidation

The oxidative stability of flaxseed oil with and without the addition of the phenolic extract resulting from roasted peanut skin was determined as follows: One hundred g flaxseed oil samples were stored in 200ml open beakers in a draught air oven at 60°C in the dark for 18 days. Combined phenolic extract (TPE) at 0.5% as well as 0.01% BHT were added to the oil samples. The oil samples were analysed at 2 days intervals to determine the progress in the formation of peroxide value (PV) and p-anisidine

value (p-AnV) of the oil. PV and p-AnV were determined according to (A.O.C.S, 1998).

Statistical analysis:

The results are presented as average ± standard deviation (SD). All results were evaluated statistically using analysis of variance according to McClave & Benson (1991).

Results and Discussion

Table 1 shows the chemical composition of roasted and unroasted peanut hull, skin and defatted flour. Roasting process decreased the crude fibre content in the hull and skin. On the other hand the protein content in the hull, skin and flour increased upon roasting. Oil and ash contents were hardly affected by roasting. Protein content of hull, skin, and flour were 3.9, 11.8, and 52.4%, respectively, upon roasting they reached 4.9, 13.0, and 56.0%, respectively. Statistical analysis indicated a significant difference at $p<0.05$ between roasted and unroasted peanut hull and skin for all the examined constituents. There was no significant difference in ash content of both roasted and unroasted hulls. On the other hand, roasting caused a significant difference between the moisture and protein contents of the roasted meal and the unroasted meal.

Kerr *et al.* (1986) reported that peanut hull contains 8.2% protein. While Hegazy *et al.* (1991) analysed peanut hull flour and found it to comprise 7.92 % moisture, 6.90% protein, 1.30% oil, 4.23% ash and 49.2% crude fibre. No chemical composition has been reported in the literature for peanut skin. Batal *et al.* (2005) studying the nutrient composition of 17 peanut meal samples, they reported that crude protein ranged between 40.1-50.9 with a mean of 45.6% and mean values of oil, fibre and ash were 2.5, 8.3 and 5.0%, respectively. While studying the physicochemical characterisation of heat (HPF) and cold (CPF) pressed peanut meal flours, Juliana and Zhengxing (2008) found that HPF contained 5.9% moisture, 49.8% protein, 0.9% fat, 8.6% ash and 8.0% crude fibre; and CPF contained 7.1% moisture, 52.1% protein, 1.6% fat, 7.6% ash and 9.7% fibre.

Table 1: Chemical Composition of Peanut Hull, Skin and Defatted flour.

Sample	Moisture %	Protein %	Oil %	Ash %	Fiber %	NFE %
Skin	8.01±0.1^{b}	11.8±0.20^{b}	7.1±0.45^{a}	2.1±0.11^{b}	55.0±0.17^{a}	15.9±0.29^{b}
Roasted skin	8.5±0.29^{a}	13.0±0.31^{a}	6.3±0.26^{b}	2.7±0.21^{a}	50.0±0.69^{b}	19.4±0.45^{a}
L.S.D.	0.0740	0.4532	0.2325	0.2222	0.4038	0.2772
Hull	8.5±0.040^{a}	3.90±0.12^{b}	0.13±0.02^{a}	3.10±0.22	60.0±0.40^{a}	23.47±0.28^{b}
Roasted hull	6.41±0.40^{b}	4.90±0.10^{a}	0.1±0.02^{b}	3.20±0.12	58.0±0.11^{b}	27.39±0.30^{a}
L.S.D.	0.0535	0.2267	0.0227	0.000	0.3322	0.5074
Defatted flour	7.3±0.33^{b}	52.40±0.53^{b}	0.50±0.02	5.10±0.22	8.3±0.18	26.40±0.05^{a}
Roasted Defatted flour	7.82±0.13^{a}	56.00±0.15^{a}	0.50±0.03	5.37±0.03	8.00±0.10	22.41±0.29^{b}
L.S.D.	0.0520	0.2240	0.000	0.000	0.000	0.5563

Different letters in each column (between roasted and unroasted) indicates significant differences at P<0.05, ± = Standard deviation, NFE= Nitrogen Free Extract

Phenolic compounds in different parts of roasted and unroasted peanuts

Phenolic compounds have attracted much interest recently because *in vitro* and *in vivo* studies suggest that they have a variety of beneficial biological properties which may play an important role in the maintenance of human health. Phenolic compounds exhibit a wide range of physiological properties, such as antioxidant, anti-allergenic, anti-artherogenic, anti-inflammatory, anti-microbial, anti-thrombotic, cardioprotective and vasodilatory effects (Benavente-Garcia *et al.,* 1997; Samman *et al.,* 1998; Puupponen-Pimia *et al.,* 2001; Manach, *et al.,* 2005).

According to the solubility criterion, polyphenols may be classified into extractable polyphenols (EPP) and nonextractable polyphenols (NEPP) (Saura-Calixto *et al.,* 2007). EPP are low and intermediate molecular mass phenolics that can be solubilized in organic or aqueous organic solvents. NEPP are high molecular mass compounds (proanthocyanidins or condensed tannins, and hydrolysable tannins) or polyphenols bound to other food matrix components such as dietary fibre and protein that can be found in the residues of aqueous organic extracts (Sayago-Ayerdi *et al.,*2007). Most studies on food polyphenols and dietary intake address exclusively EPP. In fact most studies ignore NEPP which remain in the residue, although these compounds possess high bioactivities. Arranz *et al.* (2009; 2010) in their study of EPP and NEPP in fruits reported that the amount of NEPP (112-126 mg/100g of fresh fruit) was much higher than the EPP (18.8-28mg/100g of fresh fruit). Most peanuts are eaten roasted. The determination of EPP and NEPP in the hull, skin and defatted meal of roasted and unroasted peanuts seemed important.

Table 2: Effect of roasting peanuts on EPP, NEPP and TPP extracts from different parts.

Sample	EPP(mg/g)	NEPP(mg/g)	TPE(mg/g)
Skin	56.2±0.53 [a]	113.38±0.83. [a]	169.19±0.18 [a]
Skin roasted	41.5±0.3 [b]	102±1.07 [b]	144.37±0.57 [b]
LSD (5%)	2.1716	0.9737	0.950
Hull	3.38±0.14 [b]	5.3±0.38 [b]	8.73±0.44 [b]
Hull roasted	4.33±0.13 [a]	7.4±0.46 [a]	11.69±0.40 [a]
L.S.D. (5%)	0.3117	0.952	0.9534
Defatted flour	7.23±0.41 [b]	13.4±0.46 [a]	21.31±0.47 [a]
Roasted defatted flour	7.33±0.12 [a]	10.3±0.31 [b]	17.48±0.50 [b]
L.S.D. (5%)	0.504	0.8827	1.104

EPP= Extractable polyphenol, NEPP=Nonextractable polyphenol, TPE = Total polyphenol extract (EPP+NEPP).
Different letters in each column (between roasted and unroasted) indicates significant differences at $P<0.05$, ± = Standard deviation

Table 2 gives the values for EPP, NEPP and TPE (EPP + NEPP) for roasted and unroasted hull, skin, and defatted flour of peanuts. Results in table reveal that the NEPP was always higher than the EPP as stated by (Sáyago- Ayerdi *et al.,* 2007; Arranz *et al.,* 2009; 2010). Thus it is important to determine this part of the polyphenols that has been usually ignored. It is noteworthy to mention than NEPP have bioactive properties (Arranz *et al.*, 2009). Hydrolysable tannins are hydrolysed by week acids or weak bases to produce carbohydrate and phenolic acids. Almost all phenolic acids possess antioxidant properties as well as other bioactivities. The condensed tannins or proanthocyanidins also possess biological activities (Hongxiang *et al.,* 2004; Park *et al.,* 2011). Statistical analysis indicated a significant difference between roasted and unroasted samples at $p<0.05$ for EPP, NEPP and TPE. Highest phenolic content was found to be present in the skin part. Several authors reported peanut skin to be very rich in phytochemicals (Yu *et al.,* 2007; Win *et al.,* 2011). Roasting of the hull increased EPP, NEPP, and naturally TPE. On the contrary, roasting decreased PP compounds in the skin and defatted flour. Win *et al.* (2011) reported that roasting increased total phenolic compounds, radical scavenging activity and inhibition % of linoleic acid peroxidation in peanut kernel flour. Yu *et al.* (2005) found that among several processing methods and extraction solvents, the combination of roasting and ethanol extraction were the most efficient method in extracting phenolics with high antioxidant activity from peanut skins.

Antioxidant activity of extracts from different parts of roasted and unroasted peanuts:

The antioxidant activity (AOA) of phenolic compounds may result from the neutralization of free radicals initiating oxidation processes or from the termination of radical chain reactions. Also AOA of phenolic compounds is due to their high tendency to chelate metals. In this investigation two different methods have been used for the determination of the AOA of the extracts: the first method is the DPPH free radical scavenging activity (FRSA) and second method, is the inhibition of β-carotene co-oxidation in a linoleate model system. In the first method DPPH* is used, it is one of the free radicals widely used for testing preliminary radical scavenging activity of a compound or plant extract. The principle involved in this method is that the antioxidants (phenolic extracts) act with the stable free radical on DPPH* (having a deep violet color) and convert it to DPPH with discoloration.

Table 3: Radical Scavenging Activity of Roasted and Unroasted Different Peanut Extracts.

Sample	25µl	50µl	100µl	L.S.D.
Skin	81.53±0.16 [a/3]	85.33±0.30 [a/2]	87.33±0.51 [1]	0.3982
Roasted skin	80.36±0.21 [b/3]	84.76±0.15 [b/2]	87.10±0.27 [1]	0.4014
L.S.D. (5%)	0.4190	0.5441	0.00	-------
Hull	73.9±0.36 [a/2]	78.63±0.15 [a/1]	78.77±0.15 [a/1]	0.3059
Roasted hull	73.7±0.46 [b/3]	75.23±0.13 [b/2]	79.20±0.20 [b/1]	0.3143
L.S.D. (5%)	0.9316	0.3246	0.4006	------
Defatted flour	34.39±0.34 [b/3]	41.27±0.35 [b/2]	45.51±0.16 [b/1]	0.3467
Roasted defatted flour	36.67±0.32 [a/3]	56.47±0.46 [a/2]	60.45±0.17 [a/1]	0.3997
L.S.D. (5%)	0.7451	0.9167	0.3680	------
BHT (0.1%)	54.6 ± 0.365	72.61±0.247	77.81±0.624	

Different letters in each columns (between roasted and unroasted) indicates significant difference at ($p<0.05$), different numbers in rows (between concentrations) indicates significant difference at $p<0.05$, ± = standard deviation.

Table 3 indicates the antioxidant activity (AOA) of EPP, NEPP and TPE for skin, hull and meal of peanuts as determined by the FRSA method. Peanut skin extracts exhibited the highest FRSA followed by hull extracts then the meal extracts with values of87.33, 78.77, and45.51%, respectively, compared to 77.81% for BHT which means that the TPE of peanut skin is superior to BHT(0.1%), and rather close to hull extract but much higher than the defatted flour extract. The FRSA of the different parts of peanut were well correlated with the amount of TPE for the same parts. Statistical analysis revealed a significant difference between roasted and unroasted skin, hull and meal except for roasted and unroasted peanut skin at 100µl extract where there was no significant difference. As for the statistical analysis between different concentrations of the same extract, there was a significant difference at $p<0.05$ between the same extract at all three concentrations. Unroasted hull extract at both 50 and 100µl showed no significant difference. Roasting caused increase in FRSA of roasted defatted flour (60.45) over unroasted defatted flour (45.51). This is in agreement with Win *et al.* (2011) who reported that roasting increased total phenolic compounds, radical scavenging activity and % inhibition of linoleic acid peroxidation in peanut kernel flour.

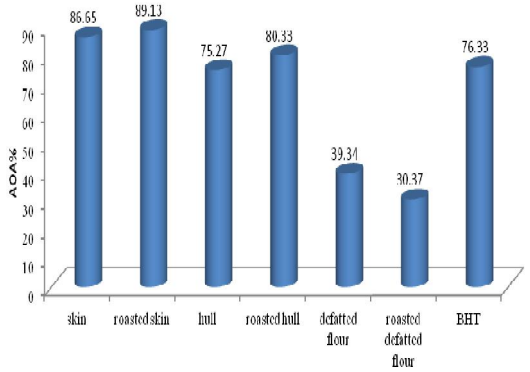

Figure 1: Antioxidant activity (AOA %) of roasted and unroasted parts of peanuts as measured by β-carotene/linoleate method

Antioxidant activity as measured by β-carotene/linoleate method is given in Figure1. It is clear from the figure that peanut skin TPE both roasted and unroasted demonstrate higher AOA than the hull and defatted flour extracts, and even higher than BHT(0.1%). This is in accordance with the results of the phenolic content and FRSA. AOA for roasted skin > skin > roasted hull > BHT >hull > defatted flour > roasted defatted flour, with values 89.13 > 86.65 > 80.33 > 76.33 > 75.27 > 39.34 > 30.37% AOA. Statistical analysis between roasted and unroasted skin, hull, and meal displayed a significant difference at $p<0.05$. Roasting resulted in an increase in AOA of the skin and hull but not the meal. Win *et al.* (2011) reported on the antioxidant activity of peanut skin, hull, roasted kernel flour phenolic extracts as indicated by FRSA% and % inhibition of linoleic acid peroxidation, and that the skin was the highest in phenolic compounds and AOA. Several authors displayed the high AOA of peanut skins (Nepote *et al.,* 2002; Yu *et al.,* 2005; Hoang *et al.,* 2008; Chukwumah *et al.,* 2009). The antioxidant activity of different peanut parts is due to the presence of a group of phenolic compounds (Yen and Duh 1995; Nepote *et al.,* 2002; Ali and Abdedaiem, 2010; Win *et al.,* 2011). Peanut phenolic compounds reported in the literature include: resveratrol in the methanolic extract of the skin (Ballard *et al.,*2009), total catechinsprocyanidin dimers, trimmers and tetramers identified in directly peeled peanut skins (Yu *et al.,* 2007), and A-type proanthocyanidins in roasted peanut skins (Monagas *et al.,* 2009). A reversed phase high performance liquid chromatography was developed for the simultaneous determination of five phenolic compounds, two stilbenes and eight flavonoidsin peanut skins extract (Francisco and resurrection, 2009b). They reported the presence of gallic, protocatechuic, epigallactocatechin, catechin, β-resorcyclic (internal standard), caffeic, procyanidin B_2, epicatechin, epigallocatechingallate, p-coumaric, ferulic, piceid, epicatechingallate, catechingallate, resveratrol and quercetin. While (Win *et al.,* 2011) found that p-hydroxybenzoic acid and resveratrol was present in skin, hull, raw kernel and roasted kernel flour of peanuts chlorogenic acid, and p-coumaric acid were present in the skin, raw and roasted kernel flour. Ferulic acid and epicatechin were present only in the skin of peanuts. Luteolin was detected only in the hull and kaempferol detected only in the raw and roasted kernel flour. This diversity in phenolic compounds would act in synergism with one another ending up with quite a strong antioxidant activity of peanuts, especially the skin.

Effect of peanut skin phenolic extract (PSE) on the oxidative stability of Flaxseed oil

The oxidative stability of oils and fats is one of the most important parameters for their quality assessment. A number of methods for such assessment have been developed. Here flaxseed oil has been subjected to accelerated oxidation at 60^{O}C for 18 days. The control sample was flaxseed oil without any addition, and flaxseed oil with BHT added at 0.01%, and the investigated sample was flaxseed oil with added 0.5% roasted peanut skin

extract (PSE). PV and p-AnV were measured every two days and their values were taken as an indication of the oxidative stability of the oil.

Table 4: Storage Stability of Flaxseed Oil (control), Flaxseed Oil + Peanut Skin Extract, and Flaxseed Oil + BHT as Measured by Increase in Peroxide Value (meq/Kg)

Storage (days)	control	Peanut skin extract	BHT
Zero	0.95 ± 0.612	0.95 ± 0.469	0.95 ± 0.425
3	15.25 ± 0.382	10.15 ± 0.672	7.25 ± 0.359
6	28.15 ± 0.536	19.09 ± 0.736	16.95 ± 0.561
9	39.95 ± 712	28.15 ± 0.355	29.95 ± 0.681
12	51.09 ± 0.666	33.75 ± 0.711	33.15 ± 0.712
15	69.09 ± 0.358	41.05 ± 0.582	43.05 ± 0.483
18	86.8 ± 0.539	50.35 ± 0.638	53.05 ± 0.569

BHT= Butylated hydroxyl toluene (0.01%)
Value is the average of four replicates ± standard deviation.

Table 5: Storage Stability of Flaxseed Oil (control), Flaxseed Oil +Peanut Skin Extract, and Flaxseed Oil + BHT as Measured by Increase in p-Anisidine value

Storage (days)	Control	Peanut skin extract	BHT
Zero	4.09 ± 0.621	4.09 ± 0.621	4.09 ± 0.621
3	10.80 ± 0.392	6.80 ± 0.298	7.60 ± 0.456
6	18.10 ± 0.0	10.30 ± 0.512	11.10 ± 0.551
9	26.70 ± 0.0	19.20 ± 0.617	15.40 ± 0.716
12	35.40 ± 0.0	20.40 ± 0.432	20.80 ± 0.501
15	45.70 ± 0.0	24.80 ± 0.339	26.10 ± 0.723
18	55.60 ± 0.0	28.50 ± 0.531	33.60 ± 0.653

BHT= Butylated hydroxyl toluene (0.01%)
Value is the average of four replicates samples ± standard deviation.

Tables 4 and 5 represent the PV and p-AnV, respectively, for the control oil, oil + PSE, and oil + BHT. It is evident from the tables that PSE and BHT both inhibited oxidation of flaxseed oil to almost close levels during the first 12 days. At day 15 and day 18, 0.5% PSE delayed oxidation of the oil more than 0.01% BHT. At zero day the three oil samples had a PV 0.95meq/Kg then the PV developed to 86.8, 50.35, 53.05 meq/Kg, at day 18 of heating for control oil, oil + PSE, oil + BHT, respectively. This result indicates 41.99% reduction in oil oxidation due to PSE addition and 38.88% reduction due to BHT addition compared to control. On the other hand, at zero day the three oils showed 4.09 p-AnV which increased during the period of the experiment to reach at end of the 18 days 55.60 for control oil, 28.50 for oil + PSE, and 33.60 for oil + BHT p-AnV. Reduction in oil oxidation when compared to control oil reached 27.10% and 22% due to oil + PSE, and oil + BHT, respectively.

The antioxidant activity of methanolic extracts of peanut hulls was tested in soybean and peanut oils that were subjected to accelerated oxidation (Duh and Yen, 1997). They reported similar results that hull extracts at all tested concentrations displayed an AOA and that at 0.48and 1.20% hull extracts delayed oil oxidation more than 0.02% BHA. Hoang *et al.* (2008) found that ethyl acetate phenolic skin extract from both conventional and high oleic acid peanuts exhibited moderate antioxidant activity in lard or rapeseed oil. This was indicated by the Schaal Oven Test. While the reducing power, FRSA, inactivation of hydroxylic and superoxide free radicals were medium, comparable to synthetic antioxidants. O'Keefe and Wang (2006) studied the effect of peanut skin extracts on quality and storage stability of beef products (ground and ground with added salt, phosphate and nitrite/erythorbate).The reduction in oxidation was pronounced in ground beef and ground beef with salts as indicated by color and TBARS. Contrary to our findings (Nepote *et al.,* 2002) tested the AOA of peanut skin on sunflower oil by applying accelerated oxidation. Their results show the peanut skin extracts did not reach the activity level of BHT.

Anticarcinogenic activity of roasted peanut skin extract (PSE)

This evaluation was carried out in the National Cancer Institute, Biology Department, Cairo. The

experiment was done by the Sulfo-Rhodamine-B stain (SRB) assay. Roasted peanut skin phenolic extract has been chosen and evaluated as a chemopreventive agent. This was established by testing the (PSE) for any cytotoxic activity against the following human tumor cell lines: Liver Carcinoma Cell Line (HEPG2); Colon Carcinoma Cell Line (HCT_{116}); Cervical Carcinoma Cell Line (HELA); and Breast Carcinoma Cell Line (MCF7).

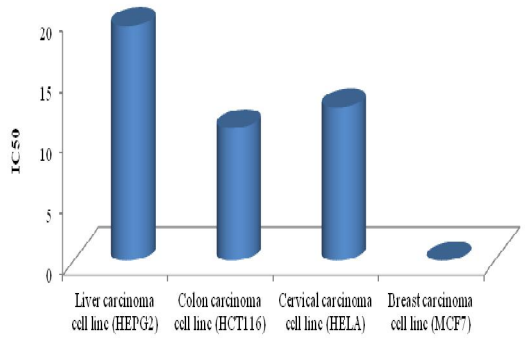

Figure 2: Anticarcinogenic activity of roasted peanut skin extract on some cell line carcinomas.

Figure 2 represent the effect of (PSE) on the human carcinoma cell lines tested and the results are indicated by the IC50, which is the dose of the compound (PSE) which kills surviving cells up to 50%. The smaller the concentration or dose the more effective is the compound. Looking at Figure 2, the following could be observed:

That (PSE) was most effective on Colon Carcinoma Cell Line (HEPG2) with an IC50 = 10.9 µg/ml. This means that at this dose of PSE, 50% of the surviving cells were killed.

PSE was next more effective on Cervical Carcinoma Cell Line (HELA)with a bit higher IC50 = 12.6µg /ml.

Liver Carcinoma Cell Line (HEPG2) needed a higher dose of (PSE) to reach IC50. IC50 = 19.3µg/ml.

PSE had no effect on Breast Carcinoma Cell Line (MCF7).

These results indicate that (PSE) possess potential anticarcinogenic properties, but as recommended by the Biology Department, National Cancer Institute, Cairo further pharmacological investigations in vitro and in vivo are required to confirm the activity of the tested extract.

Francisco and resurrection (2009b) reported the presence of five phenolic compounds, two stilbenes and eight flavonoids in peanut skin extract gallic, protocatechuic, epigallactocatechin, catechin, β-resorcyclic (internal standard), caffeic, procyanidin B_2, epicatechin, epigallocatechingallate, p-coumaric, ferulic, piceid, epicatechingallate, catechingallate, resveratrol and quercetin. While (Win *et al.,* 2011) found that p-hydroxybenzoic acid and resveratrol, chlorogenic acid, and p-coumaricacid, Ferulic acid and epicatechin were present in the skin of peanuts. Many of these compounds are reported to exhibit anticarcinogenic properties (Block *et al.,* 1992; Potter *et al.,* 2002; Soobrattee *et al.,* 2005; Srinivasan *et al.,* 2007; Actis-Goretta *et al.,* 2008; Taha *et al.,* 2012).

Conclusion

It can be thus concluded that peanut skin, although it constitutes the least part of the peanut yet it is the most valuable part due to its biological activity. It displays high antioxidant activity and is a potential anticarcinogenic agent. Roasting of the whole seed increases the antioxidant activity of the skin. Thus roasted peanut skin extracts can be used safely in the edible oil industry to delay its oxidation. It can be applied in other food industries as a natural antioxidant instead of synthetic antioxidants. Further biological studies are expected to show positive results.

Corresponding author

Suzanne M. Wagdy

Fats and Oils Department, National Research Centre, Cairo, Egypt

suzan.wagdy@hotmail.com

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