Antioxidant Activity of Peanut Skin Extracts from Conventional and High-Oleic Peanuts

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Abstract

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Peanut skins were isolated from deshelled and dried conventional and high-oleic peanuts. In order to obtain simpler mixtures of phenolics with other components of the respective extract, the samples were extracted with solvents of increasing polarity (hexane, ethyl acetate, and methanol). The amounts of extracts were as follows: methanol > hexane > ethyl acetate, and the contents of phenolic constituents in the extracts: ethyl acetate > methanol > hexane. Ethyl acetate extracts from the skins of both conventional and high-oleic peanuts were about the same. The amount of peanut skin ethyl acetate extract was higher than that of tea leaves, but lower than those of *Labiatae* plants which were also analysed. Antioxidant activities under the conditions of the Schaal Oven Test in lard and in rapeseed oil were only moderate, lower than in the case of synthetic antioxidants (butylated hydroxytoluene, butylated hydroxyanisole, ascorbyl palmitate). The reducing power, free DPP$^{\bullet}$ radical scavenging, inactivation of hydroxylic, and superoxide free radicals were medium, comparable to those of synthetic antioxidants; these activities also resembled to those in the extracts of conventional and high-oleic peanut skins.

Keywords: antioxidant activity; free radical scavenging; *Labiatae* plant extracts; natural antioxidants; peanut skins; Schaal Oven Test; synthetic antioxidants

Peanuts are important not only for the production of oil, but also for direct consumption. Shells and skins (hulls) are usually removed from the kernels before the peanut processing, and the wastes are burned or used as fertilisers, or for other purposes. Peanut skins are, however, a good source of phenolic antioxidants, which can be isolated by extraction and applied as food ingredients. Peanut oil had a lower lipid free-radical trapping ability in experiments with lecithin than did sunflower or soybean oils (Cabrini *et al.* 2001), but it had a high free radical – scavenging activity, equal to that of synthetic antioxidants such as butylated hydroxyanisole (BHA) or butylated hydroxytoleuene (BHT) (Seung *et al.* 2004). The activity was comparable with that of oils obtained from other oilseeds (Schmidt *et al.* 2003), such as soybeans (*Glycine soya*), sesam seeds (*Sesamum indicum*) or evening primrose seeds (*Oenothera biennis*). The activity of peanut-hull phenolics could be improved

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by the application of far-infrared radiation (Ram *et al.* 2005; Seung *et al.* 2006) or by ultraviolet light (Pin & Gew 1995).

Peanut lipids are rather unstable against oxidation because of a medium polyenoic acid content (about 30%) and a relatively low content of natural phenolic antioxidants. Therefore, peanut cultivars with a low polyenoic fatty acids content and a high oleic acid content were developed (Sakurai & Pokorný 2003). The oil produced from high oleic acid peanuts had a substantially higher resistance to oxidation than that from conventional peanuts (Zainuddin *et al.* 2004; Talcott *et al.* 2005a). Antioxidant activities of the two cultivar types of peanut kernels or oils need not be comparable with the antioxidant activities of phenolics obtained from the respective peanut skins. Peanut skin extracts had only a moderate effect on honey roasted peanuts during storage (Nepote *et al.* 2004), but they were active in stabilising fried potato chips under storage conditions (Rehman 2003). The extracts also possessed a pronounced antioxidant activity towards water-soluble oxidants, such as hydrogen peroxide, hydroxyl radicals, and superoxide anions (Gow & Pin 1994). Peanut skin extracts showed moderate activities under the conditions of the Schaal Oven Test (Hoang *et al.* 2007), but their free radical-scavenging activity was very good. Therefore, we compared the extracts of skins from conventional and high-oleic peanuts with the extracts from other plants (Schmidt *et al.* 2003), and the results are presented here.

MATERIAL AND METHODS

Material. Peanuts (*Arachis hypogaea*): A = cultivar Virginia, which had common content of linoleic acid (30.6% fatty acids), and was cultivated in Shandog, China; B = cultivar SunOleic, which had a low linoleic acid (6.2%) and a high oleic acid contents, was developed in Florida, USA (O'Keefe *et al.* 1993), and recultivated in Okayama, Japan. Both samples were obtained from Prof. H. Sakurai, Japan.

Commercial herbs and spices: C = black tea, produced by Black Tea Supreme (Hanoi, Vietnam); D = green tea, purchased from Lyons Green Tea (Jersey, UK); E = maté tea, purchased from Lyons Maté Tea (Jersey, UK); F = Herbes de Provence (spices of Provence, containing various herbs, such as rosemary, sage, oregano, savory, etc., purchased at a local supermarket); G = oregano stems, obtained from Biogena CB s.r.o. (Ševětín, Czech Republic); H = rosemary leaves, and I = sage leaves, both obtained from Natura s.r.o. (Děčín, Czech Republic); J = savory stems, obtained from Natura s.r.o. (Děčín, Czech Republic).

Refined rapeseed oil, winter zero-erucic variety, obtained from SETUZA a.s. (Ústí n. L., Czech Republic), peroxide value 1.15 ± 0.03 meq/kg; acid value 0.06 ± 0.01 mg/g; and dry-rendered pork lard, purchased from Schneider Masokombinát (Děčín, Czech Republic), peroxide value 0.18 ± 0.02 meq/kg, acid value 1.52 ± 0.02 mg/g. The composition of fatty acid groups were in agreement with their degree of unsaturation as shown in Table 1 for both lipidic materials. The values agreed with the literature data (Gunstone 2005; Haas 2005). The peanuts were air dried in the current of warm air, and the skins were isolated manually.

Chemicals. Ascorbyl palmitate, butylated hydroxyanisole, and butylated hydroxytoluene were obtained from Sigma Chemical Co. (St. Louis, USA); DPP (1,1-diphenyl-2-picrylhydrazyl free radical) was purchased from Sigma-Aldrich s.r.o. (Prague, Czech Republic); 2-deoxyribose from Sigma-Aldrich Chemie (Steinheim, Germany); Folin-Ciocalteu reagent was a product of Merck & Co. (Darmstadt, Germany). All other chemicals were of analytical purity, mostly from Lach-Ner s.r.o. (Neratovice, Czech Republic).

Analytical methods. The peroxide value was determined iodometrically, and the results were expressed in meq/kg. The acid value was determined volumetrically, using phenolphthalein as an indicator, and the results vere expressed in mg/g. In both cases, standard methods were used (Paquot & Hautfenne 1987).

The Schaal Oven Test was carried out gravimetrically (Gordon 2000) using storage of the samples of 25 g fat in 100 ml beakers at 60°C in the dark. The end of the induction period was determined by the change of the first derivation of the weight increase (Davídek *et al.* 1985). The

Table 1. Fatty acid composition of experimental lipid substrates (% of total fatty acis)

Fatty acid group	Pork lard	Rapeseed oil
Saturated	48.9	7.5
Monoenoic	39.8	63.4
Dienoic	10.0	19.6
Trienoic-hexaenoic	1.3	9.5

protection factor was calculated by dividing the induction period of the stabilised sample by the induction period of the blank. Original fat without antioxidants, except those present in the respective original samples. No phenolics were removed from the fats prior to the experiments.

The determination of total phenolics was carried out after Lamuela-Raventos *et al.* (1999). The stock solutions were diluted to a suitable concentration, and 0.4 ml samples were mixed with 2 ml of the tenfold diluted Folin-Ciocalteu reagent, and after 3 min, 1.6 ml of 7.5% sodium carbonate solution were added; after standing at room temperature for 30 min in the dark, the absorbance was measured at 765 nm using a Carry 100 Bio UV-Visible Spectrophotometer (Varian, Mulgrave, Victoria, Australia), and the phenolic contet was calculated using the standard curve prepared with gallic acid. The analysis was carried out in triplicates, and the results were averaged.

The reducing power was determined (Bertelli *et al.* 2004) using 0.5 ml of reducing agent (1.25 ml of 0.2M phosphate buffer, pH = 6.6, 1.25 mmol/l of 1% $K_3Fe(CN)_6$),which was incubated in a water bath at 50°C for 20 minutes. The mixture was then incubated at room temperature overnight, and the mixture was taken as the blank. Afterwards, the sample solution was mixed with 5 ml of 75% aqueous ethanol. After cooling, the samples were mixed with 1.25 ml of 10% trichloroacetic acid, and an aliquot of 1.25 ml was transferred into a fresh tube, mixed with 1.25 ml of water and 0.25 ml of 0.1% $FeCl_3{\cdot}6H_2O$ solution. The mixture was left for 10 min at room temperature, and the colouration was measured against the blank at 700 nm after 3 minutes. The reducing power was expressed in ascorbic acid equivalents.

The superoxide radical scavenging activity (Jing & Zhao 1995) was due to the radicals generated by a pyrogallol oxidation system. A 0.5 ml volume of the sample solution was added to a test tube containing 4.4 ml of the phosphate buffer (pH = 8.24, 50mM), and 40 μl pyrogallol solution (45 mmol/l in 10 mmol/l HCl) were added. The mixture was well mixed by means of a vibrator. After 2 min, 60 μl of an ascorbic acid solution (50 mmol/l) were injected to terminate the reaction. The absorbance was measured at 320 nm. The process was carried out at 25°C. The results were expressed in pyrogallol equivalents.

The hydroxyl radical scavenging activity (Aruoma *et al.* 1987) was based on the Fenton reaction, and the scavenging capacity towards hydroxyl radicals was measured using the deoxyribose method. The volume of 0.2 ml of the sample was incubated with 0.8 ml of a phosphate buffer (pH = 7.4, 50 mmol/l), and 0.4 ml of the complex solution of Fe^{3+} (0.5 mmol/l) and EDTA (0.5 mmol/l), 0.2 ml of deoxyribose (60 mmol/l), 0.2 ml of ascorbic acid solution (2 mmol/l) and 0.2 ml of hydrogen peroxide solution (10 mmol/l) were mixed. The mixture was heated at 37°C for 60 minutes. The reaction was terminated by adding 2 ml of 1% aqueous thiobarbituric acid solution, followed by 2 ml of 5% trichloroacetic acid. After boiling in a water bath for 15 min, the absorbance was measured at 532 nm, and the results were expressed in deoxyribose equivalents.

The capacity to deactivate DPP free radicals (Berset *et al.* 1997) was determined with samples dissolved in methanol and diluted to a suitable concentration. In the experiments, 2 ml of the sample solution were added to a solution of $DPP^{\bullet}$ in methanol. The absorbance was measured immediately and the after every 10 min, and was terminated after 80 minutes. The inhibition of $DPP^{\bullet}$ radical was calculated as the reduction of the initial absorbance to half the original value.

Statistical methods. The data were evaluated usung the one-way ANOVA and the regression analysis. The sofware Statsoft, Inc. (USA) modified in, STATISTICA-CZ (Software system for data analysis), version 7.1 was used; www.StatsoftCz (Statsoft, USA).

RESULTS AND DISCUSSION

The amount of the extract from peanut skins depended on the solvent used (Table 2). Hexane extracts made a medium value between methanol and ethyl acetate extracts. They contained mostly lipids and various other non-phenolic substances; tocopherols were present, in agreement with former results concerning conventional peanut skins (Hashim *et al.* 1993; Hoang *et al.* 2007). Therefore, they were not analysed for the phenolics content as it was very low in hexane extracts. The amounts of ethyl acetate extracts were smaller than those of hexane extracts, but the content of phenolic substances was very high. These consisted mainly of aglycones, e.g. ethyl protocatechuate, identified in peanut kernels (Wen *et al.* 2005), which is presumably present in peanut skins. Procyanidin dimers and trimers influenced both the colour and

Table 2. Contents of extracts and total phenolics in the dry extracts (% of extracted fraction)

Material	Solvent	Extract (%)	Phenolics (mg/g)
	HX	7.0	–
Virginia peanut skins	EA	3.6	156.8 ± 1.9
	ME	13.2	564.1 ± 1.3
	HX	5.6	–
SunOleic peanut skins	EA	2.4	362.6 ± 2.2
	ME	13.2	561.7 ± 2.9
Black tea	EA	0.8	72.0 ± 0.9
Green tea	EA	1.5	81.9 ± 2.8
Oregano	EA	2.3	56.9 ± 5.2
Rosemary	EA	11.1	92.9 ± 1.0
Sage	EA	9.1	137.2 ± 1.1
Savory	EA	6.4	57.6 ± 0.8

HX = hexane; EA = ethyl acetate; ME = methanol; standard deviation is relative value (%) – as defined by the STATISTICA software

antioxidant activities (Verstraeten *et al.* 2005). The amounts of methanolic extracts were much higher than in the case of the two previous less polar solvents. The phenolic fraction extractable with methanol exceeded both the less polar ethyl acetate-extractable and hexane-soluble fractions. The methanol-soluble fraction mostly consisted of phenolics as glycosides, almost insoluble in lipids, so its effect on the lipid stability in bulk fats would be only moderate. No pronounced difference was found between the phenolics composition of the skins from Virgina peanuts and those from SunOleic peanuts, but great differences could, nevertheless, occur between different peanut varieties (Seung *et al.* 2004). The present results are in agreement with those of Talcott *et al.* (2005b) who recorded no statistically significant differences between antioxidant activities of oils from the conventional and high-oleic peanut kernels. Unfortunately, they did not include peanut skins into their experiments.

In our experiments, great emphasis was put on medium-polar phenolics, therefore, ethyl acetate extracts of tea leaves and spices were compared with ethyl acetate extracts of peanut skins (Table 2). Only low yields were obtained from the leaves of black and green teas, and their phenolics contents were also low, compared to that of peanut skins. The antioxidant activity of tea phenolics is mainly due to the water-soluble substrates, not to ethyl acetate-soluble components (Kim *et al.* 2001). Higher amounts of extracts were obtained from green tea. This difference is natural and could be expected because about a half of original catechins are converted into tea pigments – thearubigin and theaflavin – during the fermentation, which is the essential part of the black tea production. Low values were observed in the extract of maté tea.

Plants from the *Labiatae* family, such as rosemary, sage, oregano or savory, are used as spices. Oregano, a widely used spice, contains very active antioxidants (Bendini *et al.* 2002) and has the advantage of being used as a spice also in Central Europe. Rosemary, sage, and savory resins are even more active (Yanishlieva *et al.* 2006) , but they are less common as spices in the local cuisine. All of them contain resins, which are easily obtained during their processing, and their antioxidants, possessing medium polarity, are well soluble in lipids. Therefore, they are relatively active in bulk lipids, also in the presence of tocopherols. The yields of ethyl acetate extracts were high, substantially higher than in the case of peanut skins, but the contents of total phenolics in the extracts were lower. In agreement with the literature data (Yanishlieva & Heinonen 2001), their antioxidant activity was high, and rosemary and sage resins were found as the most active among them. Spices

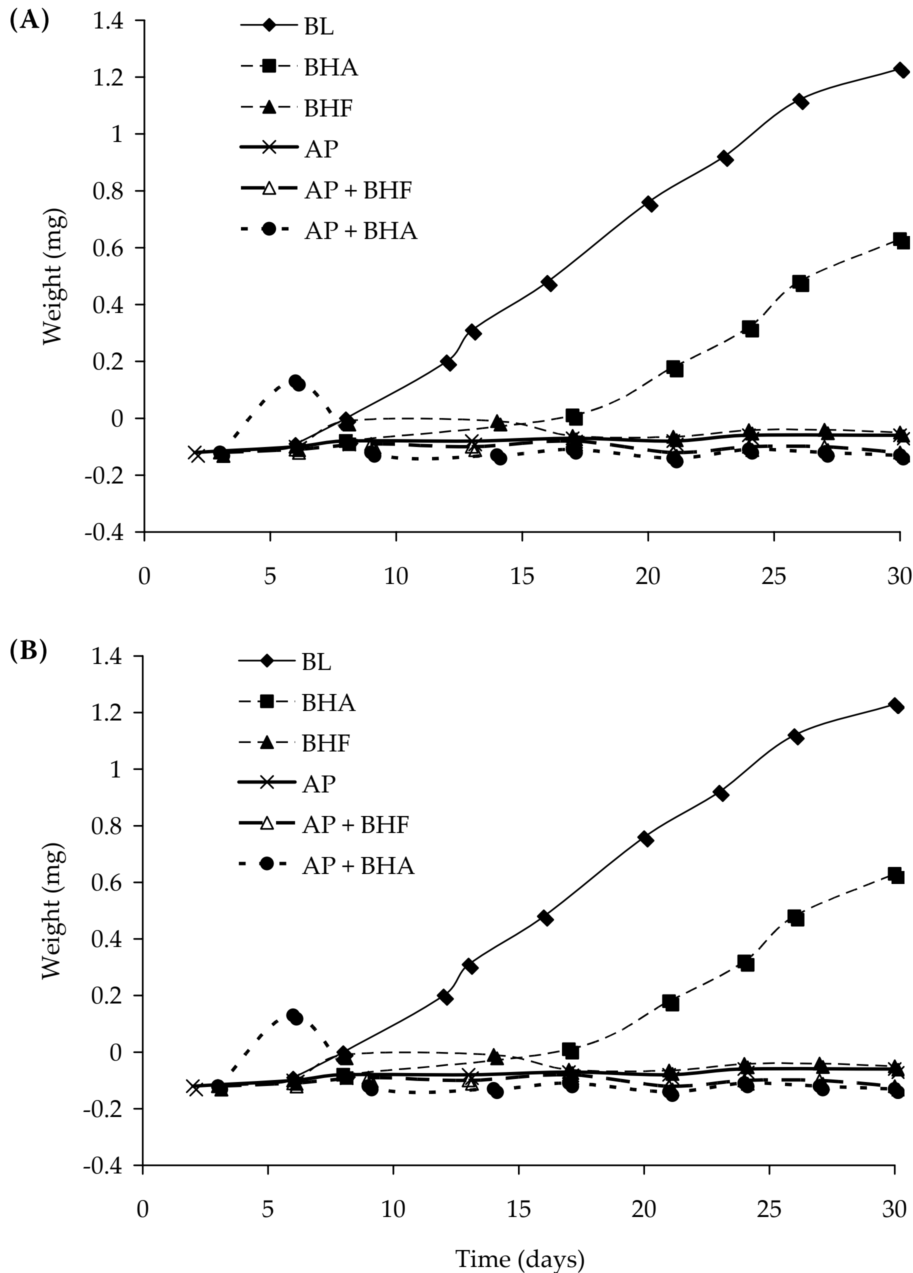

Figure 1. Antioxidant activity of synthetic antioxidants under the conditions of the Schaal Oven Test (A) in lard and (B) in rapeseed oil

BL = blank; BHA = butylated hydroxyanisole; BHT = butylated hydroxytoluene; AP = ascorbyl palmitate

of Provence were analysed for comparison, but the content of phenolic antioxidants was very low.

The effects of synthetic antioxidants (BHT and BHA) were tested only to check whether lard and rapeseed oil used as substrates corresponded to the expected stability values, and were thus suitable for the antioxidant activity testing. Peanut skin extracts were tested at the concentration level of 0.1% under the conditions of the Schaal Oven Test. The concentration of synthetic antioxidants of 0.02% is the maximum level accepted for the safety reasons. In the case of natural antioxidants, higher concentrations (0.05–0.2%) are necessary (Frankel 2007) because of their lower activities and presumed lower toxicity.

Antioxidant activities of synthetic antioxidants (Figure 1) agreed with the expectations (Frankel 2007). Phenolic antioxidants were very active in

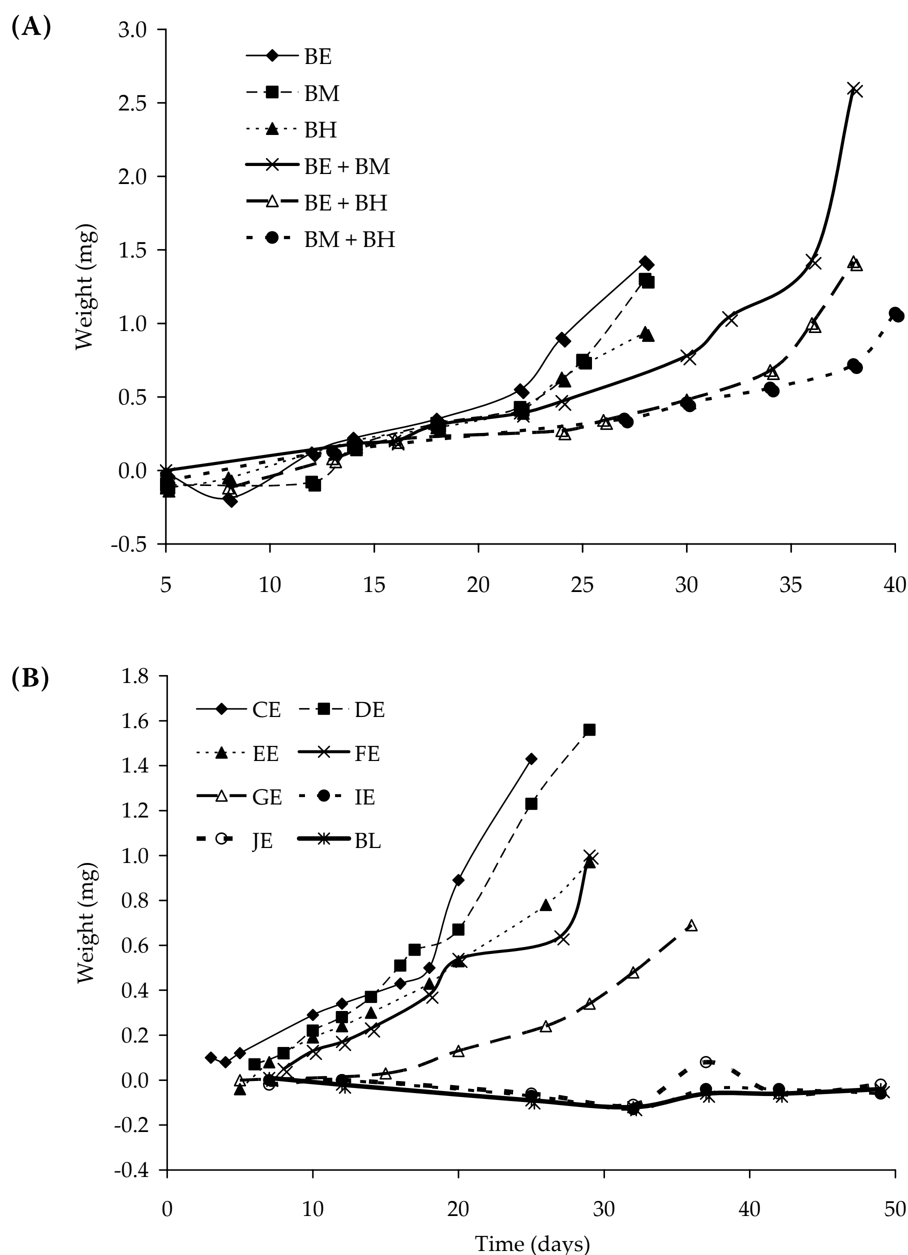

Figure 2. Antioxidant activity of natural antioxidants in lard under the conditions of the Schaal Oven Test (A) peanut skin extracts and (B) other plant antioxidants; extracts from skins of high-oleic peanuts

BE = ethyl acetate extract; BM = methanolic extract; BH = hexane extract; BL = blank: CE = black tea; DE = green tea; EE = maté tea; FE = Herbs of Provence; GE = oregano; HE = rosemary; IE = sage; JE = savory

lard (Figure 1A) as lard contains nearly no natural antioxidants (dry-rendered product may contain traces of browning products). On the contrary, ascorbyl palmitate – an important synergist – was nearly ineffective, but high activities were observed after the addition of a mixture contaning phenolic antioxidants. Rapeseed oil contains optimum amount of tocopherols as antioxidants, therefore the additions of phenolic antioxidants (BHT, BHA) were nearly inefficient, however, a mixture of BHT or BHA with ascorbyl palmitate had a pronounced effect on the oxidative stability (Figure 1B).

The results obtanied with lard stabilised with natural antioxidants are shown in Figure 2. The concentration of 0.1% was studied as it is most often used in the research as a model substance representing natural antioxidants. Blank values were the same as in the samples stabilised with

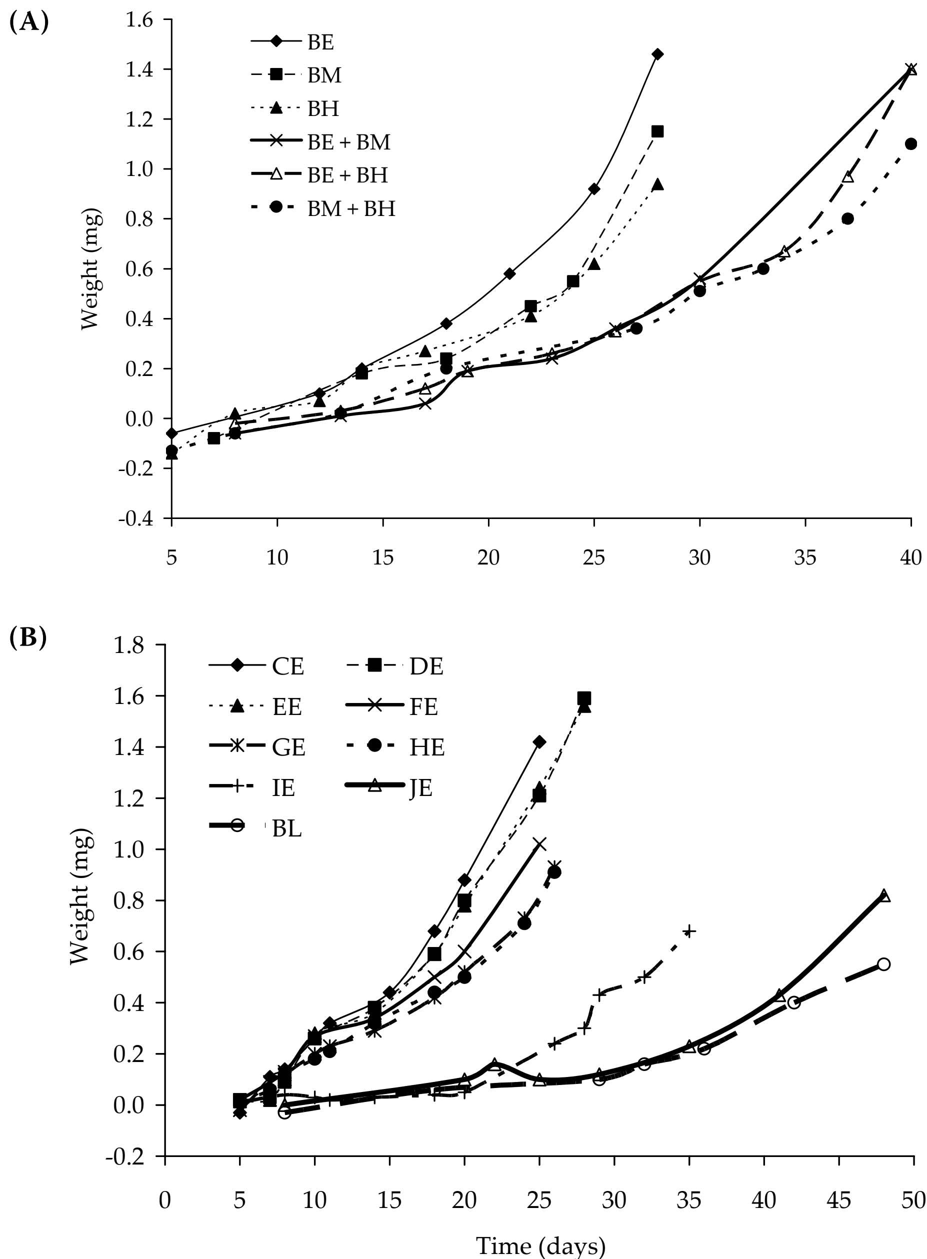

Figure 3. Antioxidant activity of natural antioxidants in rapeseed oil under the conditions of the Schaal Oven Test (A) peanut skin extracts and (B) other plant antioxidants; extracts from skins of high-oleic peanuts

BE = ethyl acetate extract; BM = methanolic extract; BH = hexane extract; BL = ethyl acetate extract: CE = black tea; DE = green tea; EE = maté tea; FE = Herbs of Provence; GE = oregano; HE = rosemary; IE = sage; JE = savory

synthetic antioxidants. To save space, only the results obtained with SunOleic skin extracts are given here (Figure 2A), the results obtained with Virginia skin extracts being very similar, only even less satisfactory, and were discussed in detail in another paper (Hoang *et al.* 2007). The ethyl acetate extract was moderately more active than the methanol extract, and the hexane extract, similarly as its combinations with other extracts, was the least efficient. The activity obviously followed the phenolics content. Antioxidant activity of SunOleic extracts was nearly negligible in lard. Synthetic phenolic antioxidants (BHA and BHT, and their mixtures with ascorbyl palmitate) were substan-

tially more active at the permitted concentration of 0.02% (see above), as expected and well known (Shiao *et al.* 1989; Satyanarayana *et al.* 2000). The activities of other plant extracts in lard are evident in Figure 2B. Green tea extract was almost inefficient, less efficient than black tea and maté tea extracts in spite of its higher content of phenolics. The extracts from *Labiataae* plants were more efficient than peanut skin extracts at the concentration of 0.1% under the same conditions.

Similarly, peanut skin extracts were nearly inefficient in rapeseed oil at the concentration level of 0.1% (Figure 3A) while rosemary and sage extracts were active (Figure 3B). In both cases, the weight started to increase after nearly the same time as with the blank, but the slope of the subsequent weight increase was slower than in the case of the blank. Therefore, the protection factor does not give correct information on the inhibitory activity of the extract. For instance, the weight increase of 50 mg was reached in blank samples after 18 days (the same blank value as in Figure 1), but in a sample with the methanol extract after 25 days, and in samples with the ethyl acetate extract after 28 days, respectively. The ethyl acetate extract showed the highest activity, and the hexane extract the lowest activity, analogously as in lard.

The weight changes of other plant extracts are shown in Figure 3B. A low activity was again observed in the tea extracts, and the highest activity in the samples stabilised with ethyl acetate extracts of *Labiatae* plants.

The summary of the protection factors obtained is given in Table 3. As it has been already stated above, the protection factors are not entirely correct indicators of stability during the whole course of oxidation. From the experiments with the Schaal Oven Test is it evident that peanut skin and other plant extracts (excepting *Labiatae* plants) are not suitable for the stabilisation of bulk fats and oils, but are more suitable for the stabilisation of lipid emulsions (Frankel 2007).

Other inhibitory activities, such as reducing power, free radical scavenging of $DPP^{\bullet}$, inactivation of $HO^{\bullet}$ radicals or superoxide radicals (Table 4) gave more satisfactory results, but the activity still was only medium. A lower activity was also observed in peanut oil as compared with other edible oils (Pellegrini *et al.* 2003). The antioxidant activity was found to be less dependent on the linoleic acid content in the lipid fraction than on the storage time, so fresh skins could be more efficient than those stored for a year or two under common storage conditions. Free radical-scavenging activity is very intensive to measure (Espin *et al.* 2000). However, it is no reliable indicator of the total activity in foods as $DPP^{\bullet}$ is a very stable free radical, compared to relatively short-lived lipid free radicals. Therefore, some activity is always evident in the case of the interaction of phenolic substances with stable radicals (Frankel

Table 3. Results of the Schaal Oven Test, expressed as protection factors

Material	Solvent	Pork lard	Rapeseed oil
Virginia peanut skins	EA	1.18	1.03
	ME	1.10	1.00
	EA+ME	1.06	1.01
SunOleic peanut skins	EA	1.47	1.04
	ME	1.59	1.04
	EA+ME	1.29	1.04
Black tea	EA	2.59	1.92
Green tea	EA	2.40	1.85
Oregano	EA	2.22	1.04
Rosemary	EA	> 10	5.14
Sage	EA	> 10	5.38
Savory	EA	3.96	1.32

EA = ethyl acetate; ME = methanol

Table 4. Other indicators of antioxidant activity of extracts

Material	Solvent	Reducing power	DPP• inactivation	HO• inactivation	Superoxide inactivation
Virginia peanut skins	EA	0.36 ± 0.02	11.2 ± 1.2	66.7 ± 2.5	2.8 ± 4.0
	ME	0.72 ± 0.01	4.7 ± 0.6	71.5 ± 1.0	12.9 ± 5.2
	EA+ME	0.57 ± 0.01	8.0 ± 1.1	70.3 ± 3.2	6.7 ± 6.4
SunOleic peanut skins	EA	0.59 ± 0.01	4.9 ± 3.2	72.0 ± 1.6	11.4 ± 6.0
	ME	0.77 ± 0.01	4.4 ± 1.6	72.5 ± 4.1	12.1 ± 3.9
	EA+ME	0.65 ± 0.01	5.2 ± 1.9	72.4 ± 6.2	12.8 ± 5.3
Synthetic					
BHA	–	0.93 ± 0.03	4.9 ± 1.5	58.7 ± 2.9	11.9 ± 8.0
BHT	–	0.59 ± 0.02	11.8 ± 0.5	50.8 ± 5.0	8.0 ± 6.1
AP	–	0.49 ± 0.01	8.3 ± 0.7	63.6 ± 7.8	92.4 ± 2.5
AP+BHA	–	0.65 ± 0.03	6.8 ± 0.9	68.5 ± 6.9	56.5 ± 3.9
AP+BHT	–	0.56 ± 0.02	10.0 ± 0.6	74.4 ± 7.1	67.5 ± 6.1

EA = ethyl acetate as solvent; ME = methanol as solvent; BHA = butylated hydroxyanisole; BHT = butylated hydroxytoluene; AP = ascorbyl palmitate; units: reducing power = asscorbic acid equivalents; DPP• inactivation = decrease of the absorbance by a half; HO• inactivation = deoxyribose equivalents; superoxide inactivation = pyrogallol equivalents

2007). There is no guarantee of superior activity in contact with less stable lipidic free radicals. The protective effect against singlet oxygen oxidation was not determined with peanut skin extracts as it was rated as very low (Neumann *et al.* 1991). Total free radical trapping activity is mainly due to tocopherols, which are concentrated in the hexane extract. As there is a plenty of information on the subject in the available literature (Kamal-Eldin & Appelqvist 1996), it was not tested in these experiments.

CONCLUSIONS

Peanut skins are an important waste product of the peanut processing. They are rich in phenolic antioxidants of medium and high polarities, but their activity in lard or rapeseed oil is only moderate, lower than that of the extracts from *Labiatae* plants. The free radical-scavenging activity of the peanut skin extract was comparable with that of other natural antioxidants. Other inhibitory activities, such as reducing power, inactivation of hydroxyl or superoxide radicals, were satisfactory. No pronounced differences were observed between ethyl acetate and methanol extracts, and between the extracts from conventional or high-oleic peanuts. Peanut skin extracts may thus be a potential source of natural antioxidants, if they become readily available at reasonable price.

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