Antioxidant Efficacy of Extracts Produced from Pickled and Dried Mustard in Rapeseed and Peanut Oils

Chang Li, Zhongfeng Tang, Meng Huang, Nengguo Tao, Bo Feng, and Shirong Huang

Abstract: Antioxidant efficacy of 70% ethanol extract (EE), 70% methanol extract (ME), and water extract (WE) produced from pickled and dried mustard (*Brassica juncea* Coss. var. *foliosa* Bailey) was evaluated in rapeseed and peanut oils by using the Schaal oven method. The protective effects of aforesaid 3 extracts in stabilizing vegetable oils were tested by measuring their peroxide values, conjugated diene values, and *p*-anisidine values during storage of 15 d at 60 °C. Results showed that the different solvent extracts produced from pickled and dried mustard, at concentrations of 0.5% and 1.0% (w/w) in vegetable oils, could significantly ($P < 0.05$) lower the peroxide value, conjugated diene value, and *p*-anisidine value of oils during storage at 60 °C. However, the extracts at various concentrations showed a less antioxidant effect than butylated hydroxytoluene (BHT) at 200 ppm. The ultraviolet spectra of different extracts exhibited a single maximum absorbance at 268 nm. The qualitative analysis of antioxidants present in the extracts was carried out by reverse phase high performance liquid chromatography (HPLC) using a C18 column. Two phenolic compounds, gallic and protocatechuric acids, were identified. The antioxidant activity of the extracts might be attributed to the presence of these phenolics. These results indicated that the pickled and dried mustard could be used as a potential source of natural antioxidants.

Keywords: antioxidant effect, lipid oxidation, pickled and dried mustard, Schaal oven method

Practical Application: The antioxidant activity of extracts produced from pickled and dried mustard toward rapeseed and peanut oils oxidation and the characterization of active phenolic compounds may be useful in developing natural antioxidants for vegetable oils. Moreover, the extracts could safely be used as potential antioxidant to suppress lipid oxidation in lipid-containing food products.

Introduction

Fats and oils undergo oxidative changes during processing and storage (Huis in't Veld 1996). These changes could lead to the development of unpleasant rancidity or off-flavors and decrease the nutritional quality of fats and oils (Frankel 1988). Furthermore, these changes can induce food poisoning when the fats and oils are severely oxidized (Gotoh and others 2006). Therefore, it's necessary to take measures to retard the oxidation process. In industrial processing, synthetic antioxidants such as butylated hydroxyanisole (BHA) or butylated hydroxytoluene (BHT) are usually used to decelerate this process and prolong the shell life of foods. However, these antioxidants are known to have toxic and carcinogenic effects on human body (Ito and others 1986; Wichi 1988). Therefore, consumers generally prefer to choose natural antioxidants over synthetic ones and the interest in natural antioxidants, especially of plant origin, has greatly increased in recent years. Many attempts have been made to prevent the oxidative deterioration of lipids by using such natural antioxidants (Chotimarkorn and others 2008; Mariod and others 2009, 2010; Mohdaly and others 2010; Konsoula and Liakopoulou-Kyriakides 2010; Zhang and others 2010).

Brassica juncea Coss. var. *foliosa* Bailey is one of the main crops cultivated in subtropical regions of China (Cao and others 2008). It is not only rich in vitamins, minerals, and dietary fibers, but also contains a high amount of flavonoids (Xie and Zhu 2000). Leaf mustard can be consumed in cooked or raw fresh vegetables, but principally, in salt-preserved or pickled forms. Pickled leaf mustard is a traditional fermented vegetable product and is widely consumed by all social groups in China. It was reported that both fresh and pickled leaf mustard were good sources of antioxidants (Wang and Zhu 2006; Fang and others 2008). However, little information is available concerning the antioxidant effect of pickled and dried mustard.

Pickled and dried mustard (PDM, also known as Meigancai in Chinese) is a very popular traditional fermented vegetable product in China. Its production procedure was mentioned in our previous work (Huang and others 2011). The pickled and dried mustards are usually cooked with meat or other dishes; they are extensively consumed by all social groups in China. Steamed pork with PDM

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is a traditional dish in China, which has a delicious taste and can enhance people's appetite. It was reported that steamed pork with PDM could be preserved for over half a year at room temperature (Xu 1996), much longer than steamed pork without PDM did. Potential antioxidant activity of the PDM was presumed for partial contribution to these properties.

Previously, we have demonstrated that PDM contains an appreciable amount of antioxidants and its various solvent (70% ethanol, 70% methanol, and water) extracts are able to directly quench free radicals, act as reducing agents, and chelate ferrous ions (Huang and others 2011). However, PDM as a source of natural antioxidants to replace synthetic antioxidants for vegetable oil protection has not been systematically investigated. Hence, the present study was designed to examine the antioxidant efficacy of extracts from PDM in retarding oxidation of vegetable oils. The final purpose of the study is to achieve future PDM applications in food industry. Thus, various solvent (70% ethanol, 70% methanol, and water) extracts of PDM were applied to rapeseed and peanut oils at levels of 0.5% and 1.0% (w/w) to examine their antioxidative activity; the development of the peroxide, *p*-anisidine, and conjugated diene values during oil oxidation was evaluated at 60 °C for 15 d. Our results indicated that all the solvent extracts exhibited good antioxidant activity, and the pickled and dried mustard could be used as a potential source of natural antioxidant ingredients in the food industry.

Materials and Methods

Materials

Pickled and dried mustards (*Brassica juncea* Coss. var. *foliosa* Bailey) were purchased from a rural market in Guangchang County, Jiangxi Province, China. They were ground in a FW100 Universal High-speed Smashing Machine (Tianjin Taisite Instrument Co., Ltd., Tianjin, China) for 2 min, but at 30 sec intervals the process was stopped for 10 sec to avoid heating the sample. The ground powder was passed through a 1.0 mm sieve and kept in a desiccator at room temperature until further use.

Refined, bleached, and deodorized (RBD) rapeseed oil, without the addition of antioxidants, was supplied by Changkang Hunan Co., Ltd., Changsha, China. Similar RBD samples of peanut oil, without added antioxidants, were obtained from Luhua Peanut Oil Co., Ltd., Laiyang, China. All other reagents were of analytical grade. Gallic acid and protocatechuic acid were of Sigma Chemical Co. (St Louis, Mo., U.S.A.). *p*-anisidine was purchased from J&K Chemical Co. Ltd. (Shanghai, China). All other chemicals (analytical grade), that is, synthetic antioxidant butylated hydroxytoluene (BHT), isooctane, chloroform, acetic acid, potassium iodide, and sodium thiosulphate used in this study were purchased from Sinopharm Group Chemical Reagent Co., Ltd. (Shanghai, China).

Extraction

Various solvent extracts of pickled and dried mustard were prepared by using a method similar to that reported previously by Liu and Yao (2007). Briefly, the powdered sample of pickled and dried mustards (10 g) was individually mixed with 150 mL of different extraction solvents: 70% ethanol (v/v), 70% methanol (v/v), and distilled water in conical flasks (500 mL). Then the mixture was sealed and left to stand in a water bath at 45 °C for 4 h. At the end of extraction, the mixture was filtered under vacuum. The residue was re-extracted as the first extraction. The extraction was repeated twice, and the combined filtrates were evaporated in a rotary evaporator under 40 °C to give a viscous dark mass. This crude extract was dissolved in water or solvent and used for assessment of antioxidant activity.

Oil storage studies

Sample preparation. Schaal oven test was conducted to evaluate the effect of antioxidants during the accelerated storage of oils. The storage tests were carried out on rapeseed and peanut oils.

RBD rapeseed oil, without any added synthetic antioxidant, such as BHT, was used for the storage studies. Oil samples were stored in uniform glass containers at 60 °C for a definite period in an incubator. The following sets of samples were included in the study. The various solvent extracts of pickled and dried mustard were added to RBD rapeseed oil at concentrations of 0.5% and 1.0% (w/w). The oil samples were stirred for 30 min at room temperature for uniform dispersion. Synthetic antioxidant (BHT) was employed at its legal limit of 200 ppm to compare the efficacy of the extracts. Rapeseed oil sample, without added antioxidants, was used as control.

Samples were analyzed after 3, 6, 9, 12, and 15 d for peroxide value, conjugated diene value, and *p*-anisidine value to trace the oxidative changes. All experiments were conducted with duplicate sets, and analyses of samples were run in triplicate and averaged. The above experiments were repeated with peanut oil.

Analysis of peroxide value. The peroxide value (PV) of all samples was measured according to GB/T 5009.37–2003 with a slight modification. Briefly, the oil samples (2.00 to 3.00 g) were dissolved in 30 mL of chloroform:glacial acetic acid (2:3, v/v). Then 1 mL saturated solution of KI was added. The mixture was shaken manually for 0.5 min and then kept in the dark for 5 min. After the addition of 100 mL distilled water, the mixture was titrated against sodium thiosulfate (0.01 M) until the yellow color almost disappeared. Then about 1 mL of starch indicator (10 g/L) solution was added. Titration was continued until the blue color just disappeared. The blank was also analyzed under similar conditions. Peroxide value (meq/kg) was calculated according to the following equation:

$$\mathrm{PV} = C \times (V - V_k) \times 12.69 \times 78.8/m$$

where C is the concentration of sodium thiosulfate (M); V and V_k are the volume of sodium thiosulfate exhausted by the sample and blank, respectively (mL); m is the mass of oil samples (g).

The changes of the induction period (IP) of oil after the addition of each extract, as a function of its concentration in oil were determined. The IP was considered as the number of hours needed for the PV of the sample to reach the value of 20 meq/kg (Wanasundara and Shahidi 1994).

Measurement of *p*-anisidine value. The *p*-anisidine value (*p*-AV) was determined according to AOCS official method Cd-18–90 (AOCS 1998).

Determination of conjugated diene value. The conjugated diene value was measured according to the method of Wettasinghe and Shahidi (1999).

Ultraviolet spectra

Ultraviolet (UV) spectra of various solvent extracts from pickled and dried mustard (in water) were recorded using a Unico UV-2820S UV/VIS spectrophotometer (Unico Instruments Inc., Shanghai, China).

HPLC analysis of PDM extract

HPLC analyses were performed with an Agilent 1200 Series HPLC instrument equipped with Chem.-Station software, a quaternary pump, an injector fitted with a 20 μL sample loop, an online degasser, and an UV-visible detector. Separation for various solvents extracts was done on Eclipse XDB reversed-phase C18 column (150 mm × 4.6 mm, i.d. Agilent, U.S.A.) with a 5-μm particle size. The elution was undertaken isocratically, with a mobile phase consisting of MeOH:H_2O (70:30, v/v) mixture. The column temperature was kept at 30 °C and the flow rate was 1.0 mL/min. The detection wavelength was 280 nm and the injection volume was 20 μL. All samples were filtered through a 0.45-μm syringe filter before analysis.

Statistical analysis

All determinations were carried out in triplicate, and data were recorded as mean ± SD of two samples of each treatment. The statistical analysis was done by the Statistical Package for Social Science (SPSS 11.5, SPSS, Inc., Chicago, Ill., U.S.A.). The two-way analysis of variance (ANOVA) was used to compare the mean values of each treatment. Significant differences ($P < 0.05$) were calculated using Duncan's multiple range tests.

Results and Discussion

Lipid oxidation is a complex process induced by oxygen in the presence of initiators such as heat, free radicals, light, photosensitizing pigments, and metal ions. Autoxidation is considered to be the main route of lipid oxidation (Laguerre and others 2007). The primary products of lipid oxidation are hydroperoxides, which are generally referred to as peroxides. However, peroxides are unstable on heating and transform rapidly to secondary oxidation products.

In practical terms, measuring the formation of primary and/or secondary oxidation products was widely used to directly assess the antioxidant capacity of a molecule toward a lipid substrate (Laguerre and others 2007). The measurement strategies based on the formation of oxidation products (primary or secondary) were relatively well adapted for studying all types of systems, including model systems, foods, or biological samples isolated from their environment.

Peroxide value is a measure of the concentration of peroxides and hydroperoxides formed in the initial stages of lipid oxidation. It was a conventionally standardized method for quantifying total hydroperoxides and hydrogen peroxide (Laguerre and others 2007). The formation of peroxides is also accompanied by an increase in UV absorbance with a maximum at about 234 nm, which is characteristic of conjugated diene systems. The degree of secondary oxidation is usually reflected by the changes in *p*-anisidine value. Therefore, it is apparent that the absolute oxidative state of oil could be estimated by measuring the three different oxidation parameters, such as peroxide value, conjugated dienes, and *p*-anisidine value. Utilization of the three parameters to assess potential of natural antioxidants in vegetable oils under accelerated storage conditions is generally accepted. The greater the levels of PV, CD, and *p*-AV, the lower will be the oxidative stability of the oils (Sultana and others 2007).

Rapeseed oil

Peroxide value is the most common measurement of lipid oxidation. It measures the miliequivalents of oxygen (hydroperoxides) per gram of oil. Table 1 shows the PV developments during the storage of rapeseed oil at 60 °C for 15 d with various solvent extracts of pickled and dried mustard at concentrations of 0.5% and 1.0% (w/w). Additional treatments included BHT at 200 ppm and a control containing no additives. In all the samples, PVs generally increased from the beginning of the storage period to the last day, showing the progression of oxidation. Rapeseed oil without the antioxidant (control) reached a maximum PV of 101.48 meq/kg after 15 d of storage, showing the highest degree of oxidation. A significant difference ($P < 0.05$) in PVs was observed between the control and rapeseed oils containing PDM extract and BHT which slowed the rate of peroxide formation. Rapeseed oils treated with extracts from PDM exhibited lower PV (less than 90 meq/kg oil) for up to 15 d as compared with the control sample (Table 1). These results indicated that PDM extracts inhibited rapeseed oil oxidation. However, for most of the storage period, the antioxidant effect of three extracts at both concentrations tested was less than that of BHT at 200 ppm. During incubation, BHT treatment resulted in a significantly lower PV than all other treatments.

The relative antioxidant efficiencies of different solvent extracts produced from pickled and dried mustard are compared in Figure 1, where an IP of rapeseed oil after the addition of each extract, as a function of the concentration of the extract in oil is presented. Experimental data showed that rapeseed oils with various solvent extracts of PDM have longer induction periods than the control sample. The 70% ethanol extract is much more effective in stabilizing rapeseed oil than the other 2 solvents extracts at the same concentration. Moreover, addition of 1.0% extracts from PDM as natural antioxidant was better at inhibiting rapeseed oil oxidation than using 0.5% of the same extract.

Conjugated diene value was also related to hydroperoxides and often used in addition or in place of PV. Table 2 represents the diene value of the experimental sets. In Table 2, the CD values of rapeseed oil with and without extracts from PDM or BHT showed a gradual increase. The diene value of the control reached 12.09 from an initial value of 4.49 after 15 d of storage, which was

Table 1–Effect of BHT and extracts produced from pickled and dried mustard on peroxide value (meq/kg oil) of rapeseed oil stored at 60 °C.

		1.0%			0.5%			
Time (d)	BHT[g,w]	EE[g,w]	ME[f,w]	WE[e,w]	EE[d,w]	ME[c,w]	WE[b,w]	Control[a]
0	6.52 ± 0.08[i]	6.55 ± 0.12	6.55 ± 0.09	6.52 ± 0.09	6.50 ± 0.07	6.56 ± 0.12	6.56 ± 0.08	6.52 ± 0.08
3	14.19 ± 0.99	8.51 ± 0.50	9.41 ± 0.29	10.00 ± 0.25	9.20 ± 0.39	12.41 ± 0.31	12.71 ± 0.20	18.67 ± 0.28
6	27.24 ± 0.64	21.75 ± 0.52	23.17 ± 0.35	29.94 ± 0.65	28.22 ± 0.49	31.81 ± 0.20	34.11 ± 1.46	40.93 ± 0.56
9	42.40 ± 0.21	45.00 ± 0.49	45.39 ± 0.49	51.74 ± 1.54	53.65 ± 1.86	53.58 ± 0.73	55.63 ± 0.71	62.52 ± 0.96
12	56.92 ± 0.47	63.38 ± 0.32	63.76 ± 1.08	65.02 ± 0.63	68.91 ± 2.12	69.82 ± 1.15	70.79 ± 1.59	83.22 ± 1.57
15	78.01 ± 1.93	80.16 ± 0.58	83.90 ± 1.42	83.22 ± 1.35	86.30 ± 0.85	86.75 ± 1.28	88.06 ± 0.87	101.48 ± 1.33

[a–g]Different letters in superscript indicate significant differences ($P < 0.05$).
[i]Values (mean ± SD) are averages of two samples, analyzed individually in triplicate ($n = 2 \times 3$).
[w]BHT = butylated hydroxytoluene; EE = 70% ethanol extract; ME = 70% methanol extract; WE = water extract.

significantly higher than the diene values of the other treatments. The three extracts with different concentrations showed higher CD values and were found to be less effective than BHT. The ethanol extract is more effective (with less CD values) in lowering the CD values of rapeseed oil than methanol extract and water extract, which showed high CD values (Table 2).

Changes in *p*-anisidine value, which represent the secondary oxidation products produced during the oxidative degradation of oil, are shown in Table 3. The formation of secondary oxidation products also increased during storage. The *p*-anisidine value of the control reached a maximum of 19.90 from an initial value of 3.41 after 15 d of storage. A significant difference was noted between the values for control and experimental samples. PDM extracts at the concentration of 0.5% and 1.0%, inhibited the formation of the secondary products in comparison with the control and the amount of secondary products formed were less than that formed in the control samples. Moreover, EE presented better effect than ME and WE did. Addition of 1.0% extract gave a better effect than using 0.5% of the same extract. However, all the PDM extracts were less effective in inhibition of secondary products than BHT.

The results mentioned above indicated that PV, conjugated diene, and *p*-anisidine values of rapeseed oil containing the extract were significantly lower than those of the control, which clearly showed the marked antioxidant effect of the PDM extract in rapeseed oil protection. Similar effect was also found in the extracts from *Anethum graveolens* L. (Singh and others 2005) and evening primrose (Niklová and others 2001).

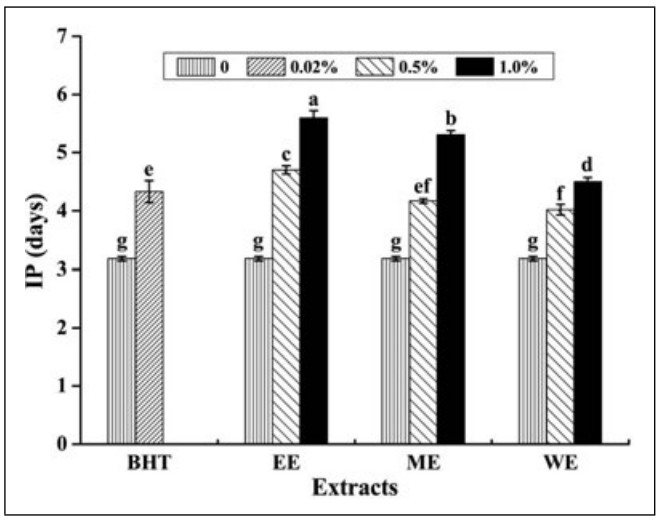

Figure 1–Changes of the induction period (IP) of rapeseed oil after the addition of various extracts and BHT at concentrations varying from 0.0% to 1.0%. Values (mean ± SD) are average of three determinations ($n = 3$). Different letters in superscript indicate significant differences ($P < 0.05$).

Peanut oil

Peroxide values of the stabilized and control peanut oils, over an incubation period of 15 d at 60 °C are shown in Table 4. It can be seen that the PVs of all the oil samples showed a gradual increase. For the control oil sample, the progressive increase in PV was significantly ($P < 0.05$) higher throughout the storage period compared to the samples containing the PDM extract or BHT. Its PV increased from an initial value of 9.16 to 90.60. The lowest increase rate of PVs was observed in the samples containing BHT. The PV of peanut oil containing the extract was significantly ($P < 0.05$) lower than that of the control, which clearly showed the marked antioxidant effect of the extract. However, PDM extract was significantly less effective than BHT at 200 ppm. It can be concluded that extracts from PDM at concentrations of 0.5% and 1.0% (w/w) were effective in stabilizing peanut oil during storage of 15 d at 60 °C. The extracts possessed good antioxidant activity and decreased the formation of peroxides in peanut oil. This can also be seen in Figure 2 which shows the induction periods of peanut oils with and without added antioxidants. The extracts from PDM can significantly extend the induction period of peanut oil. Moreover, among three solvents extracts, the 70% ethanol extract

Table 2–Effect of BHT and extracts produced from pickled and dried mustard on conjugated dienes value of rapeseed oil stored at 60 °C.

		1.0%			0.5%			
Time (d)	BHT[e,w]	EE[e,w]	ME[d,w]	WE[c,w]	EE[c,w]	ME[c,w]	WE[b,w]	Control[a]
0	4.49 ± 0.06[i]	4.50 ± 0.08	4.51 ± 0.06	4.50 ± 0.10	4.51 ± 0.07	4.48 ± 0.07	4.48 ± 0.08	4.49 ± 0.06
3	4.65 ± 0.10	5.10 ± 0.10	5.59 ± 0.11	5.20 ± 0.19	5.59 ± 0.12	5.72 ± 0.11	5.44 ± 0.16	6.32 ± 0.16
6	5.30 ± 0.14	5.71 ± 0.14	5.82 ± 0.20	6.64 ± 0.23	6.58 ± 0.20	6.64 ± 0.17	7.22 ± 0.20	7.51 ± 0.20
9	7.46 ± 0.17	7.69 ± 0.21	7.81 ± 0.21	8.21 ± 0.31	8.37 ± 0.31	8.86 ± 0.23	8.80 ± 0.26	9.36 ± 0.19
12	8.27 ± 0.26	8.57 ± 0.16	8.78 ± 0.28	9.08 ± 0.35	9.13 ± 0.22	9.41 ± 0.30	9.62 ± 0.30	10.32 ± 0.29
15	10.29 ± 0.31	11.07 ± 0.22	11.21 ± 0.40	11.59 ± 0.49	11.24 ± 0.23	10.81 ± 0.39	11.87 ± 0.48	12.09 ± 0.31

[a–e]Different letters in superscript indicate significant differences ($P < 0.05$).
[i]Values (mean ± SD) are averages of two samples, analyzed individually in triplicate ($n = 2 \times 3$).
[w]BHT = Butylated hydroxytoluene; EE = 70% ethanol extract; ME = 70% methanolic extract; WE = water extract.

Table 3–Effect of BHT and extracts produced from pickled and dried mustard on *p*-anisidine value of rapeseed oil stored at 60 °C.

		1.0%			0.5%			
Time (d)	BHT[g,w]	EE[f,w]	ME[e,w]	WE[c,w]	EE[d,w]	ME[d,w]	WE[b,w]	Control[a]
0	3.41 ± 0.07[i]	3.40 ± 0.08	3.41 ± 0.03	3.41 ± 0.07	3.40 ± 0.14	3.41 ± 0.17	3.41 ± 0.11	3.41 ± 0.07
3	4.17 ± 0.04	4.22 ± 0.06	4.45 ± 0.05	4.71 ± 0.03	4.20 ± 0.07	4.47 ± 0.06	4.93 ± 0.01	4.51 ± 0.07
6	5.70 ± 0.09	6.04 ± 0.61	6.01 ± 0.11	7.40 ± 0.02	6.20 ± 0.03	7.13 ± 0.04	7.45 ± 0.11	8.25 ± 0.10
9	8.10 ± 0.07	8.86 ± 0.03	9.04 ± 0.10	10.91 ± 0.13	10.73 ± 0.11	11.01 ± 0.08	11.72 ± 0.05	12.45 ± 0.15
12	11.07 ± 0.07	12.87 ± 0.06	13.25 ± 0.03	14.96 ± 0.10	14.35 ± 0.09	15.29 ± 0.07	15.43 ± 0.06	16.90 ± 0.05
15	11.42 ± 0.18	16.32 ± 0.35	16.40 ± 0.15	16.52 ± 0.16	17.32 ± 0.19	15.07 ± 0.15	16.98 ± 0.13	19.90 ± 0.16

[a–g]Different letters in superscript indicate significant differences ($P < 0.05$).
[i]Values (mean ± SD) are averages of two samples, analyzed individually in triplicate ($n = 2 \times 3$).
[w]BHT = Butylated hydroxytoluene; EE = 70% ethanol extract; ME = 70% methanolic extract; WE = water extract.

was the most effective at the same concentration. Again, addition of 1.0% extract gave a better effect than using 0.5% of the same extract.

The increase in CD values for peanut oil treatments was noted under accelerated (60 °C) storage (Table 5). A typical pattern in the rise of CD values was observed for all peanut oil treatments. The control had the highest CD value among all oil treatments, showing a highest degree of oxidation. Its CD value increased to 9.46 (initial value 1.84) after 15 d of storage (Table 5). While, samples treated with BHT showed lowest CD value (5.09) than all the other samples. The CD values of peanut oils treated with various solvent extracts from PDM at different concentrations varied from 6.72 to 8.44. These values are lower than those of the control stored for 15 d, indicating that PDM extract exhibited an inhibitory effect on peanut oil oxidation.

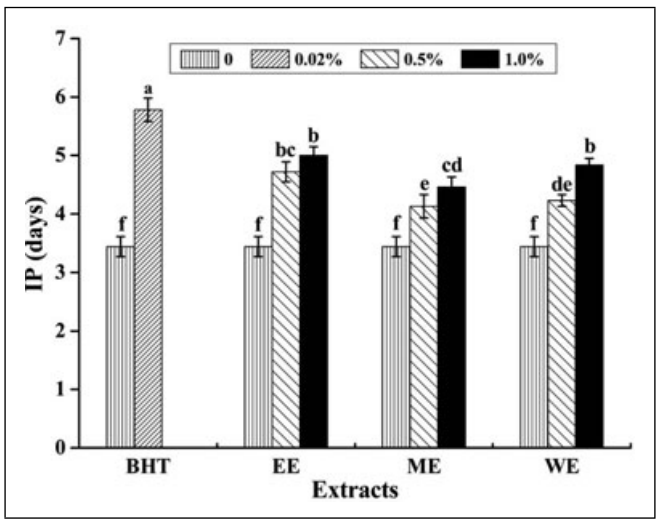

Figure 2–Changes of the induction period (IP) of peanut oil after the addition of various extracts and BHT at concentrations varying from 0.0% to 1.0%. Values (mean ± SD) are average of three determinations ($n = 3$). Different letters in superscript indicate significant differences ($P < 0.05$).

The *p*-anisidiene value of peanut oil (Table 6) without added antioxidant (control) increased from an initial value of 1.42 to 3.51 after 15 d of storage at 60 °C. While, the oil samples containing 200 ppm BHT had the lowest increase rate of *p*-anisidiene values. A significant difference was observed between the control and other treatments. The *p*-anisidiene values of peanut oils treated with various solvent extracts from PDM at different concentrations were in the range of 2.34–2.93. These results confirmed the protective action of PDM extracts against the oxidative changes of peanut oil.

From the above results of PV, CD, and *p*-AV, it is clear that the different extracts from PDM exhibited an inhibitory effect on peanut oil oxidation. The extracts from *Cortex fraxini* (Pan and others 2007), *Polygonum cuspidatum* (Pan and others 2007), and Peanut Hulls (Duh and Yen 1997) also showed the effectiveness in preventing oxidation of peanut oil.

The UV-VIS spectrum of various extracts (in term of λ_{max})

Figure 3 depicts the UV spectra of various solvent extracts from pickled and dried mustard. Results showed that the three extracts had almost the same UV spectra patterns except the difference of their absorbance. All the three extracts of PDM exhibited a single maximum absorbance at 268 nm. A similar UV spectra profile was also found in the low-molecular-weight phenolics fraction of 80% aqueous acetone crude extract from defatted almond seeds (Amarowicz and others 2005). The absorption maximum at 268 nm may be due to the presence of benzoic acids (Lambropoulos and Roussis 2007; Roussis and others 2008), vanillic acid (Amarowicz and Weidner 2001), tocols and other phenolic acids except flavonoids (Nsimba and others 2008). Absence of an absorption band at approximately 320 nm in the spectrum of the extracts denotes that concentration of phenolic acids such as ferulic, *p*-coumaric, and caffeic acids, among other phenolic constituents, was low (Amarowicz and others 2005).

Table 4–Effect of BHT and extracts produced from pickled and dried mustard on peroxide value (meq/kg oil) of peanut oil stored at 60 °C.

		1.0%			0.5%			
Time (d)	BHT[g,w]	EE[f,w]	ME[d,w]	WE[e,w]	EE[d,w]	ME[b,w]	WE[c,w]	Control[a]
0	9.16 ± 0.18[i]	9.20 ± 0.11	9.17 ± 0.16	9.18 ± 0.14	9.20 ± 0.12	9.17 ± 0.15	9.17 ± 0.16	9.16 ± 0.18
3	13.71 ± 0.50	13.48 ± 0.30	15.01 ± 0.59	13.64 ± 0.26	14.46 ± 0.68	15.87 ± 0.67	15.13 ± 0.25	17.90 ± 0.90
6	20.50 ± 0.46	23.27 ± 0.57	25.25 ± 0.60	24.01 ± 0.47	24.10 ± 0.43	26.80 ± 0.79	27.04 ± 0.57	32.23 ± 0.37
9	26.98 ± 0.55	35.89 ± 0.80	38.53 ± 1.04	36.11 ± 1.04	37.43 ± 0.81	42.19 ± 0.89	40.10 ± 1.21	46.34 ± 1.23
12	35.63 ± 0.79	49.18 ± 1.06	53.19 ± 0.97	52.30 ± 1.00	54.55 ± 0.70	57.07 ± 0.71	56.95 ± 1.45	67.97 ± 0.71
15	45.22 ± 0.77	68.32 ± 1.58	73.45 ± 1.31	69.16 ± 1.43	72.77 ± 1.65	77.37 ± 2.01	75.37 ± 1.58	90.60 ± 2.11

[a–g]Different letters in superscript indicate significant differences ($P < 0.05$).
[i]Values (mean ± SD) are averages of two samples, analyzed individually in triplicate ($n = 2 \times 3$).
[w]BHT = Butylated hydroxytoluene; EE = 70% ethanol extract; ME = 70% methanolic extract; WE = water extract.

Table 5–Effect of BHT and extracts produced from pickled and dried mustard on conjugated dienes value of peanut oil stored at 60 °C.

		1.0%			0.5%			
Time (d)	BHT[g,w]	EE[f,w]	ME[e,w]	WE[c,w]	EE[d,w]	ME[b,w]	WE[bc,w]	Control[a]
0	1.84 ± 0.09[i]	1.84 ± 0.05	1.84 ± 0.08	1.84 ± 0.10	1.84 ± 0.09	1.84 ± 0.07	1.84 ± 0.06	1.84 ± 0.08
3	2.62 ± 0.13	2.94 ± 0.17	2.81 ± 0.13	3.17 ± 0.14	2.82 ± 0.13	2.79 ± 0.14	2.35 ± 0.13	3.28 ± 0.12
6	3.77 ± 0.17	3.79 ± 0.21	3.60 ± 0.19	3.98 ± 0.18	4.20 ± 0.17	4.16 ± 0.17	4.01 ± 0.19	5.36 ± 0.16
9	4.34 ± 0.24	4.06 ± 0.18	4.61 ± 0.21	4.81 ± 0.26	4.74 ± 0.2	5.93 ± 0.22	5.07 ± 0.22	6.65 ± 0.23
12	4.90 ± 0.29	5.47 ± 0.26	6.20 ± 0.32	7.32 ± 0.31	6.28 ± 0.29	8.02 ± 0.31	6.79 ± 0.34	8.85 ± 0.34
15	5.09 ± 0.33	6.72 ± 0.31	6.78 ± 0.37	7.00 ± 0.34	6.99 ± 0.38	8.44 ± 0.38	7.58 ± 0.32	9.46 ± 0.43

[a–g]Different letters in superscript indicate significant differences ($P < 0.05$).
[i]Values (mean ± SD) are averages of two samples, analyzed individually in triplicate ($n = 2 \times 3$).
[w]BHT = Butylated hydroxytoluene; EE = 70% ethanol extract; ME = 70% methanolic extract; WE = water extract.

HPLC analysis of various solvent extracts

The HPLC analysis of PDM extracts revealed the presence of phenolic compounds. The HPLC profiles of PDM extracts were shown in Figure 4. This figure shows a representative chromatogram of the (a) EE, (b) ME, (c) WE of PDM recorded at 280 nm. By this means, in the three analyzed extracts, it was possible to identify two major phenolic compounds: gallic and protocatechuric acid. They were identified by comparing of their relative retention time with that of authentic standards. These phenolic compounds were present in all PDM extracts studied. The peak areas of both phenolic acids and thus their contents decreased by the following order: 70% ethanol extract > 70% methanol extract > water extract. This could be due to better extractability of gallic acid and its derivatives in ethanol and methanol than in water. This order was coincident with their total phenolic contents (Huang and others 2011). These results demonstrated that differences in phenolic composition of the three extracts were significantly more quantitative than qualitative. The three extracts possess similar composition. Gallic acid was reported to be more effective than BHA in inhibiting some oils oxidation (Soulti and Roussis 2007; Roussis and others 2008). Fang and others (2008) determined the phenolic acids in fresh potherb mustard (*Brassica juncea,* Coss.) and investigated the effects of pickling methods on the contents of total free phenolic acids, total phenolic acids, total phenolics, and antioxidant activities. Eight phenolic acids such as gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, vanillic acid, caffeic acid, *p*-coumaric acid, ferulic acid, and sinapic acid

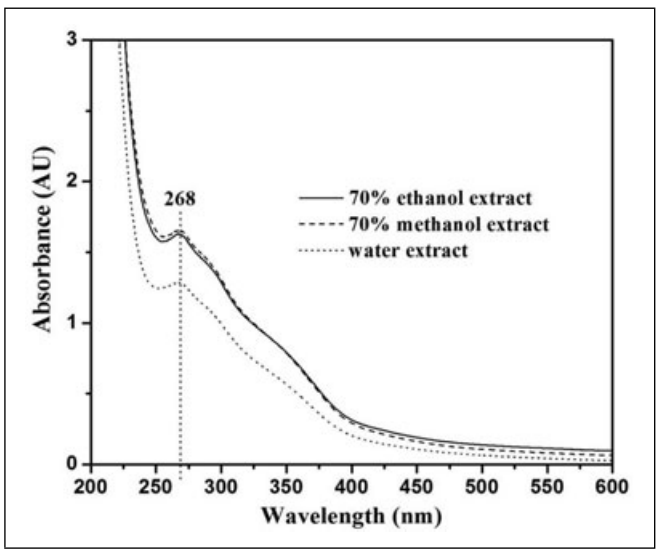

Figure 3–UV spectra of various solvent extracts of pickled and dried mustard.

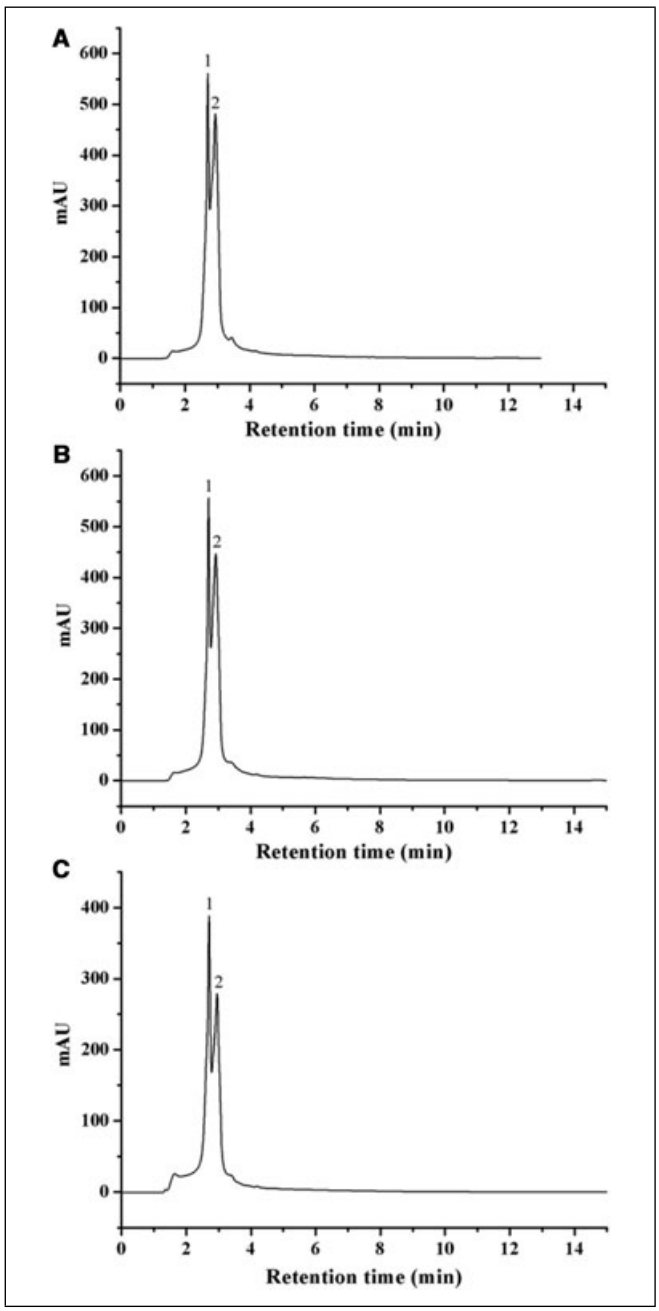

Figure 4–HPLC chromatogram of EE (A), ME (B), and WE (C) of pickled and dried mustard (detected at 280 nm). (1) Gallic acid; (2) protocatechuric acid.

Table 6–Effect of BHT and extracts produced from pickled and dried mustard on *p*-anisidine value of peanut oil stored at 60 °C.

		1.0%			0.5%			
Time (d)	BHT[f,w]	EE[e,w]	ME[d,w]	WE[c,w]	EE[d,w]	ME[c,w]	WE[b,w]	Control[a]
0	1.42 ± 0.02[i]	1.42 ± 0.01	1.42 ± 0.01	1.42 ± 0.01	1.42 ± 0.07	1.42 ± 0.03	1.42 ± 0.03	1.42 ± 0.02
3	1.78 ± 0.04	1.77 ± 0.09	1.76 ± 0.06	1.87 ± 0.02	1.74 ± 0.03	1.79 ± 0.06	1.91 ± 0.04	1.96 ± 0.08
6	1.78 ± 0.02	1.80 ± 0.09	1.84 ± 0.07	1.91 ± 0.08	1.86 ± 0.06	1.88 ± 0.04	1.98 ± 0.05	2.06 ± 0.05
9	1.88 ± 0.02	1.95 ± 0.03	1.99 ± 0.03	2.03 ± 0.04	2.04 ± 0.04	2.07 ± 0.06	2.10 ± 0.05	2.14 ± 0.04
12	2.00 ± 0.03	2.13 ± 0.03	2.15 ± 0.06	2.23 ± 0.12	2.20 ± 0.09	2.19 ± 0.05	2.30 ± 0.09	2.31 ± 0.07
15	2.18 ± 0.08	2.34 ± 0.11	2.68 ± 0.08	2.72 ± 0.05	2.42 ± 0.08	2.79 ± 0.03	2.93 ± 0.10	3.51 ± 0.12

[a–f] Different letters in superscript indicate significant differences ($P < 0.05$).
[i] Values (mean ± SD) are averages of two samples, analyzed individually in triplicate ($n = 2 \times 3$).
[w] BHT = Butylated hydroxytoluene; EE = 70% ethanol extract; ME = 70% methanolic extract; WE = water extract.

were identified in the fresh potherb mustard. However, the phenolic acids such as *p*-hydroxybenzoic acid, vanillic acid, caffeic acid, *p*-coumaric acid, ferulic acid, and sinapic acid were not identified in our investigation. This may be due to the degradation of most of the phenolic compounds by polyphenol oxidase during the pickling processes (Fang and others 2008).

Conclusion

In summary, different assays used for examining antioxidant efficacies of PDM extract revealed that the different solvent extracts, at concentrations of 0.5% and 1.0% (w/w) in rapeseed and peanut oils, could significantly ($P < 0.05$) lower the peroxide value, conjugated diene value, and *p*-anisidine value of the oils during storage at 60 °C. These results illustrate that PDM extract, at both concentrations, exhibited strong antioxidant activity in rapeseed and peanut oils during storage. The 70% ethanol extract of pickled and dried mustard at concentration of 1.0% (w/w) has the strongest antioxidant activity among the extracts used in this study and could be chosen as an efficient antioxidant in inhibiting rapeseed and peanut oils oxidation. HPLC analysis showed that the most predominant phenolic compounds in various solvent extracts of PDM were gallic and protocatechuric acids, which may contribute to the antioxidant activity of the extracts. Therefore, PDM extract in oils, fats, and other food products could be used as natural antioxidant to suppress lipid oxidation.

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