Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 104 (2007) 997–1005
Food Chemistry
www.elsevier.com/locate/foodchem

Antioxidant potential of corncob extracts for stabilization of corn oil subjected to microwave heating

Bushra Sultana [a], Farooq Anwar [a,*], Roman Przybylski [b,1]

[a] Department of Chemistry, University of Agriculture, Faisalabad 38040, Pakistan
[b] Department of Chemistry and Biochemistry, University of Lethbridge, Lethbridge, Alberta, Canada TIK3M4

Received 20 October 2006; received in revised form 14 December 2006; accepted 28 December 2006

Abstract

Antioxidant effectiveness of corncob extract with different polarity solvents (*n*-hexane, ethyl acetate, acetone, ethanol, and methanol) was assessed for total phenolics content (TPC), DPPH· radical scavenging activity and % inhibition of peroxidation in linoleic acid system. The yield of the extracts from corncob with different solvents ranged from 1.1 to 19.5 g/100 g of dry matter. TPC, DPPH· radical scavenging and inhibition of linoleic acid oxidation for corncob extracts varied significantly ($P < 0.05$) from 1.2 to 4.2, 16.0 to 42.1, and 37.3 to 89.9 g/100 g per dry matter, respectively. Methanolic extract offered the highest yield (19.5%), and also exhibited superior antioxidant activity. Corncob methanolic extract was added at concentrations of 500 and 1000 ppm to RBD (refined, bleached, deodorized) corn oil. BHT at 200 ppm served as standard beside the control. The stabilized corn oil samples subjected to microwave heating (0–21 min) were analyzed periodically. The magnitude of oxidative alterations was followed by the measurement of conjugated dienes (CD), conjugated trienes (CT), *p*-ansidine, and peroxide values. Results of different oxidation parameters revealed that methanolic extract at concentration of 1000 ppm was more effective than BHT and inhibited the rise in *p*-ansidine-, conjugated diene-, conjugated triene-, and peroxide-values up to 41.8%, 45.0%, 39.7%, and 65.0%, respectively, with respect to the control.However, at concentration of 500 ppm offered less efficient protection. The results of different antioxidant parameters investigated in the present study demonstrated that corncob is a potent source of natural antioxidants that might be explored to prevent oxidation of vegetable oils.

Keywords: Corncob; Total phenolics; DPPH radical; % Inhibition; Corn oil; Stabilization

1. Introduction

Recently, the utilization of microwave heating in, household, restaurants and fast food preparation is rapidly increasing because of its swiftness, ease of operation, and cost benefits. Microwave radiation penetrates to the internal parts of the food, causing cooking or reheating in whole mass of food. However, there are speculations on the ease of free radical formation when fatty foods are exposed to microwave energy resulting in production of objectionable compounds in microwave-cooked foods (Lee et al., 2004; Yoshida, Hirakawa, Abe, & Mizushina, 2002). The effects of microwave heating on thermo-oxidative stability of some oils and fats have been reported in the literature. Vegetable oils having greater proportions of unsaturated fatty acids are exceedingly susceptible to lipid peroxidation by means of high energy microwave radiations which might result in losses of their nutritional and organoleptic values as well as other physiological properties (Anjum, Anwar, Jamil, & Iqbal, 2006; Yoshida, Hirakawa, Tomiyama, & Miz, 2003).

Synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and propyl gallate have been used as food antioxidants. However,

* Corresponding author. Tel.: +92 41 9200161 67x3309; fax: +92 41 9200764.
E-mail addresses: bushrasultana2005@yahoo.com (B. Sultana), fqanwar@yahoo.com (F. Anwar), roman.przybylski@uleth.ca (R. Przybylski).
[1] Tel.: +1 403 317 5055; fax: +1 403 329 2057.

0308-8146/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2006.12.061

the use of these synthetic antioxidants is negatively perceived by consumer due to potential toxicity and their connotation as chemicals in food (Jeong et al., 2004). Natural antioxidants have fascinated an immense deal of interest because of their health effects and positive image against degenerative diseases and certain cancers. Therefore, owing to consumer concerns about the safety of synthetic antioxidants, the declaration for natural antioxidants has been greater than before ((Iqbal & Bhanger, 2007).

Potential sources of natural antioxidant have been searched in many plant sources such as vegetables, fruits, leaves, oilseeds, cereal crops, barks, and roots, spices and herbs, and crude plant drugs (Rababah, Hettiarachy, & Horax, 2004). Besides role of natural antioxidants as preventative components towards diseases, they also prevent oxidative deterioration of vegetable oils and fats during processing, distribution, and storage (Vagi et al., 2005). Effectiveness of different natural antioxidants in stabilizing vegetable oils has been previously studied (Anwar, Bhanger, & Yasmeen, 2003; Jung, Lee, Hun, Kyung, & Chung, 2001; Shaker, 2006; Siddiq, Anwar, Manzoor, & Fatima, 2005).

Currently, an increased attention has been focused on the industrial wastes for the search of natural antioxidants (Bandoniene, Pukalskas, Venskutomis, & Grudiene, 2000; Zia-ur-Rehman, 2006). Jayaprakasha, Singh, and Sakariah (2001) reported that grape seed extract exhibited good antioxidant activity in preservation of food products. Methanolic extract prepared from peanut hulls protected potato chips (Zia-ur-Rehman, Salariya, & Habib, 2003). Chatha, Anwar, Manzoor, and Bajwa (2006) investigated the stabilization of sunflower oil with rice bran extracts. Anwar, Jamil, Iqbal, and Sheikh (2006) also examined antioxidant efficacy of wheat bran and some other agricultural wastes towards protection of sunflower oil under ambient and accelerated storage.

The presence of natural antioxidants in cereals and legumes has been extensively assessed. In many instances, the actual antioxidant activity was not observed. However, phenolic acids content have been measured as potential source of antioxidants and neutraceutical components (White & Xing, 1997). Corn or maize (*Zea mays* L.) after wheat and rice is the most important cereal produced in the world. Corn grain is an outstanding feed for livestock offering high energy, low amount of fiber and high digestibility. It is an important forage crop, being used green, made into silage, or dried for fodder. Corn produces a number of important industrial products such as starch, corn oil, alcohols, acetaldehyde, acetone, glycerol, acetic, citric and lactic acids, which are usually obtained by the wet-milling, extraction and fermentation processes.

Corncob is one of the most plentiful and important agriculture waste accounts for up to 50% of the total corn seed production. Immature cobs are boiled and eaten as corn on the cob or the grains may be removed and eaten as vegetable, or it may be canned. More mature cobs are roasted. The cobs are used for fuel, smoking pork products, and are also as source for charcoal. Corncobs were not assessed for components with antioxidative activity, therefore present study was designed to evaluate the antioxidant potential and efficacy of corncob extracts to preserve corn oil which was subjected to microwave heating.

2. Materials and methods

2.1. Materials

Samples of refined-bleached-deodorized (RBD) corn oil, without additives and corncobs were received from Rafhan Maize Products, Pvt. Ltd., Faisalabad, Pakistan. The following chemicals were used for the following experiments: Folin-Ciocalteu's phenol reagent (2 N), gallic acid (98.0%), 1,1,-diphenyl-2-picrylhydrazyl (90.0%), linoleic acid (99.0%), synthetic antioxidant butylated hydroxytoluene (BHT) (99.0%) were of Sigma Chemical Co. (St Louis, MO, USA). All other chemicals (analytical grade) i.e. anhydrous sodium carbonate ferrous chloride, ammonium thiocyanate, potassium dihydrogen phosphate, dipotassium hydrogen phosphate, iso-octane, chloroform, acetic acid, potassium iodide, and sodium thiosulphate used in this study were purchased from Merck (Darmstadt, Germany), unless stated otherwise.

2.2. Equipment and apparatus

The following instruments were used: spectrophotometer (U-2001, Hitachi, Ibaraki, Japan), commercial blender (TSK-949, Westpoint, France), hot air oven (IM-30, Irmeco, Germany), orbital shaker (Gallenkamp, UK), rotary vacuum evaporator(EYELA, N-N Series, Rikakikai Co. Ltd. Tokyo, Japan), microwave oven (DW-180 G, Concave Reflux System, Dawlance, Busan, Korea).

2.3. Preparation of corncob extract

The corncobs were dried in a hot air oven at 65 °C and then ground into a fine powder using a commercial blender. The material that passed through 80-mesh sieve was used for extraction purposes. Sample (20 g) of corncob powder was extracted with 200 mL of each of the five solvents: hexane, ethylacetate, acetone, ethanol and methanol, overnight at room temperature in an orbital shaker. The extracts were separated from the residue by filtering through Whatmann No. 1 filter paper. The residues were extracted twice with the same solvent and extracts combined. Then, the combined extracts were concentrated to dryness under reduced pressure at 45 °C, using a rotary evaporator. The dry extracts were weighed to calculate the yield and stored in a refrigerator (−4 °C), until used for analyses.

2.4. Determination of total phenolics content (TPC)

Amount of TPC were assessed using Folin-Ciocalteu reagent as described by Chaovanalikit and Wrolstad

(2004). Briefly, 50 mg of dry mass of corncob extract was mixed with 0.5 mL of Folin-Ciocalteu reagent and 7.5 mL deionized water. The mixture was kept at room temperature for 10 min, and then 1.5 mL of 20% sodium carbonate (w/v) added. The mixture was heated in a water bath at 40 °C for 20 min and then cooled in an ice bath; absorbance was measured at 755 nm using a spectrophotometer. Amounts of TPC were calculated using gallic acid calibration curve within range of 10–100 ppm ($R^2 = 0.9986$). The results were expressed as gallic acid equivalents (GAE) per dry matter. All samples were analyzed thrice and results averaged.

2.5. DPPH· scavenging assay

Free radical scavenging activity of corncob extracts was assessed using procedure described by Iqbal, Bhanger, and Anwar (2005). Briefly, to 1.0 mL of corncob extract containing 25 μg/mL of dry matter in methanol, 5.0 mL of freshly prepared solution of 1,1′-diphenyl-2-picrylhydrazyl (DPPH) at concentration 0.025 g/L was added. Absorbance at 0, 0.5, 1, 2, 5 and 10 min was measured at 515 nm using a spectrophotometer. The remaining amounts of DPPH· radical was calculated from calibration curve. Absorbance measured at fifth minute was used for comparison of radical scavenging activity of corncob extracts.

2.6. Determination of antioxidant activity in linoleic acid system

The antioxidant activity of corncob extracts was also determined by measuring % of oxidation of linoleic acid system (Iqbal et al., 2005). Extracts, 5 mg of dry matter, were added to a solution of linoleic acid (0.13 mL), 99.8% ethanol (10 mL) and 10 mL of 0.2 M sodium phosphate buffer (pH 7). The mixture was make up to 25 mL with distilled water and incubated at 40 °C up to 360 h. Extent of oxidation was measured by peroxide value applying thiocyanate method as described by Yen, Duh, and Chuang (2000). Briefly, 10 mL of ethanol (75% v/v), 0.2 mL of aqueous solution of ammonium thiocyanate (30% w/v), 0.2 mL of sample solution and 0.2 mL of ferrous chloride ($FeCl_2$) solution (20 mM in 3.5% HCl; v/v) added sequentially. After 3 min of stirring, the absorption was measured at 500 nm using a spectrophotometer. A control contained all reagents with exception of extracts. Synthetic antioxidants butylated hydroxytoluene (BHT) was used as positive control. Percent of inhibition of linoleic acid oxidation were calculated with the following equation: 100 − [(Abs. increase of sample at 360 h/Abs. increase of control at 360 h) × 100], to express antioxidant activity.

2.7. Stabilization of oil

The crude concentrated methanolic extract of corncob was added to RBD corn oil at concentrations of 500, and 1000 ppm. The oil samples were stirred for 30 min at 50 °C for uniform dispersion. Synthetic antioxidant (BHT) was employed at its legal limit of 200 ppm to compare the efficacy of extract. Stabilized and control oil samples (100 mL) were placed in dark brown airtight glass bottles with narrow neck and heated in a microwave oven at a frequency of 2450 MHz (medium power setting, capable of generating 500 W). All oil samples were prepared in triplicate. Corn oil sample, without antioxidant, was used as control. Oil samples were taken after every 3 min interval up to 21 min. The oxidative deterioration level was assessed by the measurement of peroxide value (PV), conjugate dienes (CD), conjugate trienes (CT) and *p*-ansidine values.

2.8. Determination of oxidative stability of corn oil

2.8.1. Peroxide value (PV)

Determination of peroxide value (PV) of stabilized and the control corn oil samples were measured following AOCS Official method Cd 8-53 (AOCS, 1997).

2.8.2. Conjugated dienes (CD), conjugated trienes (CT)

Specific extinctions at 232 and 268 nm (i.e. conjugated dienes and conjugated trienes, respectively) were determined using a spectrophotometer. Oil samples were diluted with *iso*-octane to bring the absorbance within limits following the standard method of IUPAC method II. D. 23 (IUPAC, 1979).

2.8.3. p-Ansidine value

p-Ansidine value of the oil samples was determined following IUPAC method II. D. 26 (IUPAC, 1979). The oil samples dissolved in *iso*-octane were allowed to react with *p*-ansidine solution in acetic acid (0.25% w/v) for 10 min and absorbance was measured at 350 nm using a spectrophotometer.

2.9. Statistical analysis

Two samples of corncob for each treatment/solvent extraction were taken. Each sample was analyzed individually in triplicate and data are reported as mean ($n = 2 \times 3$) ±SD ($n = 2 \times 3$). Analysis of variance was performed by ANOVA using Minitab 2000 Version 13.2 statistical software (Minitab Inc.USA). Significant differences ($P < 0.05$) of means were calculated using Duncan's multiple range tests (Steel & Torrie, 1980). The relationship between different antioxidant assays was described as Pearson product-moment correlation coefficient (r). Differences were considered statistically significant if $P < 0.05$.

3. Results and discussion

3.1. Yield of components with antioxidative properties

The yield of extracts from corncobs using different solvents in Table 1 is shown and ranging from 1.1% to

Table 1
Yield of crude extract of corncob using different solvents

Solvent	Yield (g/100 g)
Methanol[a]	19.5 ± 0.6
Ethanol[b]	12.3 ± 0.6
Acetone[c]	1.1 ± 0.1
Ethylacetate[b]	11.3 ± 0.7
n-Hexane[c]	1.1 ± 0.1

Values (mean ± SD) are average of duplicate samples, analyzed individually in triplicate ($n = 2 \times 3$), ($P < 0.05$).
Different letters in superscript indicate significant differences.

19.5%. A significant difference ($P < 0.05$) in the yield of extracts with different solvent was observed. The highest amount was extracted with methanol (19.5%), followed by ethanol (12.3%), ethyl acetate (11.3%), acetone (1.1%), and *n*-hexane (1.1%). Significant ($P < 0.05$) differences in the yield of extracts of corncob with different solvents might be attributed to the polarity of solvent used and availability of different extractable components.

The amount of materials that can be extracted from a plant depends on the nature and amount of solvent used, mixing during extraction procedure and it is possible sample-to-sample variation in extracted materials (Hsu, Coupar, & Ng, 2006). Therefore, an appropriate extraction method should be developed to extract maximum quantity of antioxidative compounds before its exploration for the possible applications in food industry. Attributable to polar nature of natural antioxidants, the yield of the antioxidative components goes on increasing with increasing polarity of the solvent. Methanol and ethanol are generally employed for the extraction of antioxidant components from plant materials due to their polarity and good solubility of many antioxidative components in these two solvents (Siddhuraju & Becker, 2003; Zhou & Yu, 2004). In the present study of corncob the highest yield of extract was gained with methanol, revealing the greater efficacy of methanol to acquire maximum extract yield from plant materials. Our results of extraction of corncob were in accord to the earlier reports (Iqbal & Bhanger, 2007; Siddhuraju & Becker, 2003), showing a high efficacy of methanol for extraction of antioxidative extracts.

3.2. Total phenolic contents (TPC)

TPC were determined by using Folin-Ciocalteu reagent (FCR). Folin-Ciocalteu reagent react nonspecifically with phenolic compounds as it can be reduced by a number of nonphenolic compounds e.g., vitamin C, Cu (II), etc. Although exact reaction of the reagent with reducing species is not known, but it is considered that a complex is formed between phospho-molybdic tungstate and reducing species, phenolate ion, changing color from yellow to blue where absorbance at 755 nm is measured (Haung, Ou, & Prior, 2005).

The amount of TP determined in different solvent extracts of corncob is shown in Fig. 1. The contents of TP ranging from 1.2 to 4.2 GAE (g/100 g per dry matter) differed significantly ($P < 0.05$) within different solvent extracts of corncob. Methanol as polar solvent extracted significantly ($P < 0.05$) higher amounts of TP (4.2%), followed by ethanol (3.2%), ethylacetate (3.7%), acetone (2.8%) and hexane (1.2%). Methanol is efficient and the most widely used to extract antioxidative components including phenolic acids and other phenolic components (Siddhuraju & Becker, 2003). Although, ethanol and ethyl acetate also extracted reasonable amounts of TPC, however, due to comparatively lower their polarity, were less effective. Conversely, hexane being non-polar in nature was the least effective for the extraction of phenolics. Singh, Murthy, and Jayaprakasha (2002) extracted antioxidative compounds from pomegranate peels and seeds and found

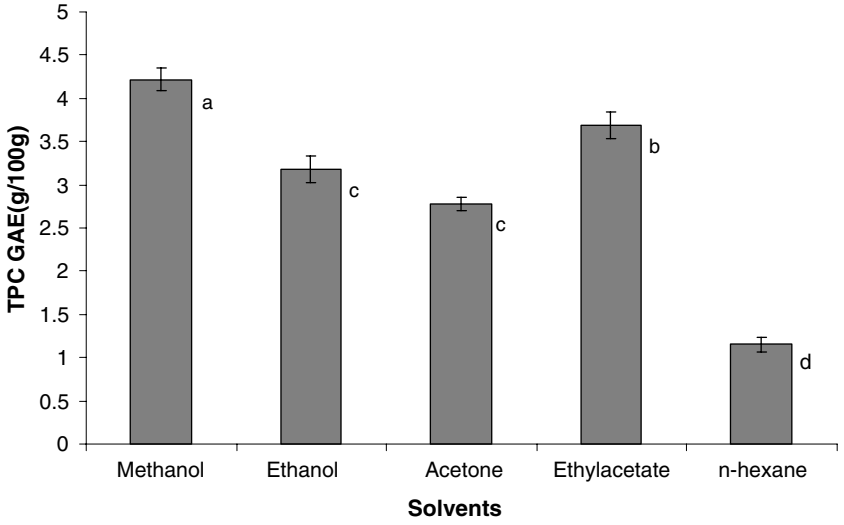

Fig. 1. Total phenolic contents (TPC) of different solvents extract of corncob. Values (mean ± SD) are average of duplicate samples, analyzed individually in triplicate ($n = 2 \times 3$), ($P < 0.05$). Different letters in superscript indicate significant differences.

that methanol gave maximum antioxidant yield. Similar results were observed in the present investigations as the most effective antioxidative compounds were extracted with methanol.

Many phenolics had been determined in different parts of corn plant, however, their concentration was found to be higher in corn flour (White & Xing, 1997). Amount of TP (4.2%) determined in methanolic extract of corncob in the present investigation was found to be lower than pomegranate peels (46.2%) (Negi, Jayaprakasha, & Jena, 2003), however, greater than some other agriculture wastes like rice bran (0.36%) (Iqbal et al., 2006), wheat bran (0.10%) (Zhou & Yu, 2004), and banana peels (0.91%) (Someya, Yoshiki, & Okubo, 2002). It has been proven that antioxidant activity of plant extracts is mainly ascribed to the concentration of the phenolic compounds present in the plants (Heim, Taigliaferro, & Bobilya, 2002). Several studies showed good correlation between the total phenols and antioxidant activity (Haung et al., 2005; Javanmardi, Stushnoff, Locke, & Vivanco, 2003; Silva, Souza, Rogez, Rees, & Larondella, 2006).

3.3. DPPH· radical scavenging activity

Using DPPH· radical, the free radical scavenging ability of the corncobs extracts was evaluated considering that DPPH· radical is commonly used for the assessment of antioxidant activity in vitro and is foreign to biological systems (Zhou & Yu, 2004). DPPH· is a very stable organic free radical with deep violet color which gives absorption maxima within 515–528 nm range. Upon receiving proton from any hydrogen donor, mainly from phenolics, it loses it chromophore and became yellow. As the concentration of phenolic compounds or degree of hydroxylation of the phenolic compounds increases their DPPH radical scavenging activity also increases, and can be defined as antioxidant activity (Sanchez-Moreno, Larrauri, & Saura-Calixto, 1999). Because these radicals are very sensitive to the presence of hydrogen donors, the whole system operates at very low concentration; with it, it can allow a large number of samples to be tested in a short time (Iqbal et al., 2006; Zhou & Yu, 2004).

Absorbance in this assay was recorded at 0.5–10 min time intervals from initiation of the reaction. Observed scavenging activity was similar at the beginning of the reaction and changed with the increase of the reaction time until stabilized at 10th min. Practically the significant ($P < 0.05$) differences between different extracts were observed at 5th minute of the reaction (Fig. 2).

Remaining amount (%) of DPPH· radical at 5 min after initiation of the reaction was 16.0%, 21.2%, 34.2%, 31.4%, and 42.1% for methanol, ethanol, acetone, ethylacetate, and hexane extracts, respectively. Free radical scavenging activity of methanolic extract from corncob established in this study was greater than previously reported for wheat bran, rice bran (Yu, Perret, Harris, Wilson, & Haley, 2003; Iqbal et al., 2005).

Methanolic extract having highest amount of TP also exhibited good antioxidant activity in terms of measurement of $DPPH^-$ scavenging activity. These findings are in close agreement with previous findings of Singh et al. (2002) who found a strong correlation between the contents of TP and DPPH· scavenging activity of methanolic extract from pomegranate peels.

3.4. Inhibition of linoleic acid oxidation by corncob extracts

Inhibition of linoleic acid oxidation was also used to assess the antioxidant activity of the corncob extracts. Linoleic acid is a polyunsaturated fatty acid, upon oxidation peroxides are formed which oxidize Fe^{2+} to Fe^{3+}, the later forms complex with thiocyanate ion which have maximum

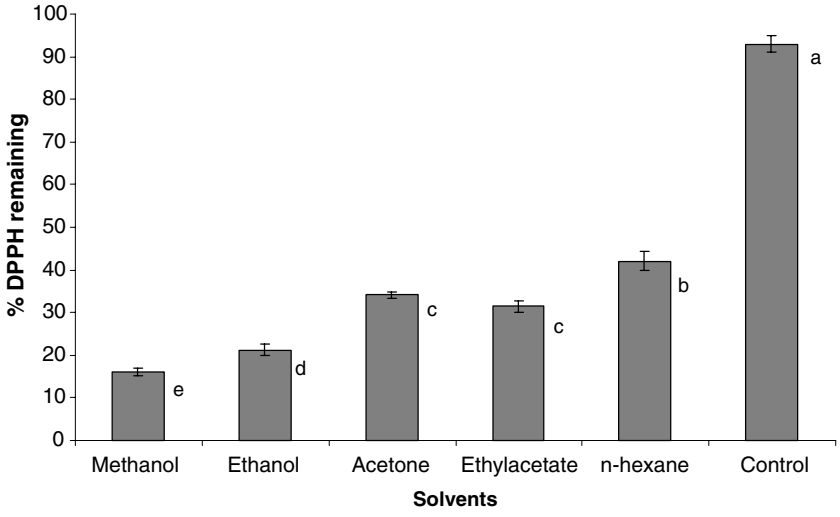

Fig. 2. Antioxidant activity of different solvents extract of corncob determined as $DPPH^-$ radical scavenging activity. Values (mean ± SD) are average of duplicate samples analyzed individually in triplicate ($n = 2 \times 3$), ($P < 0.05$.) Different letters in superscript indicate significant differences.

of absorption at 500 nm. Higher the absorbance higher concentration of peroxides formed during reaction, consequently lower will the antioxidant activity.

Inhibition of linoleic acid oxidation as affected by different extracts is shown in Fig. 3 and ranged from 37.3% to 89.9%, whereas BHT provided inhibition at the level of 84.7%. Inhibition of linoleic acid oxidation showed significantly ($P < 0.05$) different effectiveness for analyzed extracts of corncob. Methanolic extract exhibited the highest inhibition of linoleic acid oxidation (89.9%) followed by ethanol (60.5%), ethyl acetate (59.2%), acetone (55.7%), and *n*-hexane (37.3%). The efficacy of methanolic extract of corncob to inhibition of oxidation is comparable with that of BHT. The effectiveness of oxidation inhibition by methanolic extract from corncob was somewhat lower than pomegranate peel extract (Singh et al., 2002), however, comparable with rice bran extract (Iqbal et al., 2006).

Results of the present study showed that among all the solvent extracts; methanolic extract of corncob had the highest yield of TPC, which also demonstrated the highest antioxidant activity as measured by DPPH· scavenging and inhibition of linoleic acid oxidation. Whereas, hexane extract demonstrated the least antioxidant activity probably because of its low polarity. Mainly, lipidic components are soluble in hexane which are present at low concentration as compared to other antioxidative compounds. Previous reports also revealed that methanolic extracts of plant materials offer more effective antioxidants (Chatha et al., 2006; Siddhuraju & Becker, 2003).

3.5. Correlation between different antioxidants assays

Relationship between the results of different antioxidant assays was established by correlation analysis (Table 2). Antioxidant activity of phenolic compounds is often associated with their redox properties, which allow them to act as antioxidative agents (Singh et al., 2002; Siddhuraju et al., 2002). Results showed that amount of TP well correlated with DPPH· radical scavenging activity ($r = 0.825$, $P<0.05$) and % inhibition of linoleic acid oxidation ($r = 0.862$, $P = <0.05$). Furthermore, DPPH· radical scavenging activity showed good correlation with inhibition of linoleic acid ($r = 0.895$, $P < 0.05$). Overall, correlation analysis indicated that results of three assays used in this study were correlated with each other, verifying that antioxidant activity of the plant extracts is mainly dependent on the amount of total phenolics.

3.6. Antioxidant potential of methanolic extract of corncob for stabilization of corn oil

On the basis of preliminary evaluation of antioxidant activity of extracts of corncob from different solvents, methanolic extract showing the highest antioxidant activity was further evaluated towards stabilization of corn oil. Results of different oxidation parameters adopted as an

Table 2
Correlation analysis between results of different antioxidant assays implicated to different solvent extracts of corncob

Antioxidant activity parameter	TPC	DPPH· scavenging ability	% Inhibition of linoleic acid oxidation
TP C	–	$r = 0.825$	$r = 0.862$
DPPH· scavenging ability	$r = 0.825$	–	$r = 0.895$
% Inhibition of linoleic acid oxidation	$r = 0.862$	$r = 0.895$	–

Correlation analysis between different antioxidant assays in individual solvent extract ($n = 2 \times 3$) were described as Pearson product-moment correlation coefficient (r). Differences were considered statistically significant if $P < 0.05$.

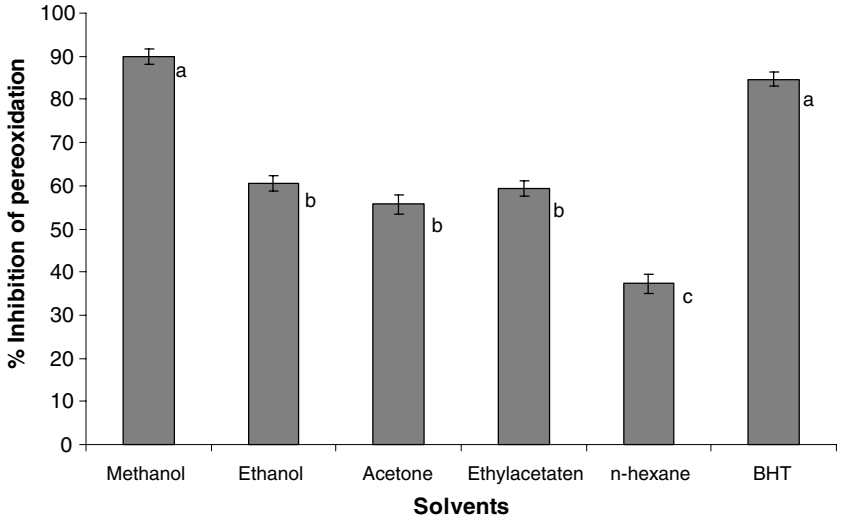

Fig. 3. Antioxidant activity of different solvents extract of corncob determined as inhibition of linoleic acid oxidation. Values (mean ± SD) are average of duplicate samples, analyzed individually in triplicate ($n = 2 \times 3$), ($P < 0.05$) Different letters in superscript indicate significant differences.

indicator of oxidative deterioration of the corn oil are presented here:

3.6.1. Peroxide value (PV)

Peroxide value is an indicator of the extent of primary oxidation products formation in oil (Shahidi & Wanasundara, 1997). Peroxide value of the stabilized and control corn oil, over an incubation period of 21 min in microwave oven is shown in Table 3. It is noticeable from results that addition of synthetic antioxidant at 200 ppm concentration and methanolic extract at 500 and 1000 ppm inhibited oxidation significantly in corn oil ($P < 0.05$), compared to the control. The effectiveness at 1000 ppm of corncob methanolic extract was better than BHT (65.4% vs. 53.7%). The highest PV was observed for control sample while lower for extracts from corncobs. Lower values of PV for corn oils with corncob antioxidant extracts as compared to the control showed the effectiveness of the corncob in preventing oxidation of oils.

Peroxide value is often used as measure of oxidation statues of oil, fat and fatty foods, hence, efficacy of an antioxidant extract to prevent or delay the onset of oxidation could be predicted by the rate of these primary oxidation components formation. Oil stabilized with synthetic or natural antioxidants usually shows lower PV and extended induction periods (Anwar et al., 2003). Zia-ur-Rehman et al. (2003) reported the antioxidant activity of ginger extracts in stabilizing sunflower oil under accelerated storage conditions in a concentration dependent manner. Anwar et al. (2006) investigated the efficacy of different natural extracts in retarding the peroxides formation in sunflower oil subjected to ambient and accelerated aging.

3.6.2. Conjugated dienes and trienes (CD & CT)

Conjugated dienes (CD) and trienes (CT) contents of corn oil samples stabilized with methanolic extract, BHT and control are shown in Tables 4 and 5. The CD and CT contents increased parallely to increase of heating time with greater rate for control. The oil samples stabilized with corncob extract showed lower levels of CD and CT compared to control, indicating antioxidant potential of the corncob extracted components. The lowest level of CD accumulation was found for corn oil samples stabilized with methanolic corncob extract at 1000 ppm, followed by BHT (200 ppm) and corncob extract at 500 ppm. The results of the CD in present study revealed the antioxidant activity of corncob extract at 1000 ppm was better than BHT at its legal amounts. Different trend was observed while measuring CT, the antioxidant activity of corncob extract applied at both concentrations, 1000 and 500 ppm were less efficient than BHT.

Measurement of CD and CT is a good parameter for the determination of oxidative stability of the oils (Shahidi & Wanasundara, 1997). Lipids containing methylene-interrupted dienes or polyenes show a shift in their double bond position during oxidation. The resulting conjugated dienes exhibit intense absorption at 232 nm; similarly conjugated trienes absorb at 268 nm. The increase in CD and CT contents is proportional to the uptake of oxygen. Greater the levels of CD and CT lower will be the oxidative stability of the oils (Chatha et al., 2006). Iqbal and Bhanger (2007) described the antioxidant activity of garlic extracts in sunflower oil, assessed under accelerated conditions, using CD and CT as indicators of oxidative degradation. Siddiq et al. (2005) lso investigated the antioxidant efficacy of methanolic extract of *Moringa oleifera* leave for the

Table 3
PV of stabilized and control corn oil (meq/kg)

Incubation period (microwave heating in min.)	Control[d]	BHT (200 ppm)[b]	Corncob extract	
			500 ppm[c]	1000 ppm[a]
0	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1
3	2.3 ± 0.2	0.8 ± 0.2	1.1 ± 0.1	0.8 ± 0.1
6	3.2 ± 0.1	1.0 ± 0.1	2.0 ± 0.3	1.0 ± 0.3
9	5.0 ± 0.4	2.8 ± 0.2	3.2 ± 0.1	1.9 ± 0.1
12	6.9 ± 0.2	3.2 ± 0.1	3.8 ± 0.2	2.0 ± 0.2
15	14.2 ± 0.7	5.0 ± 0.2	6.0 ± 0.1	3.2 ± 0.1
18	19.7 ± 0.6	6.2 ± 0.4	8.2 ± 0.4	5.4 ± 0.4
21	17.2 ± 0.4	8.0 ± 0.2	9.5 ± 0.3	6.0 ± 0.2

Values (mean ± SD) are average of duplicate samples, analyzed individually in triplicate ($n = 2 \times 3$), ($P < 0.05$).
Different letters in superscript indicate significant differences.

Table 4
Conjugated dienes ($\varepsilon_{1\ cm}(\lambda_{232\ nm})$) of stabilized and control corn oil

Incubation period (microwave heating in min.)	Control[d]	BHT (200 ppm)[b]	Corncob extract	
			500 ppm[c]	1000 ppm[a]
0	1.2 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.2 ± 0.1
3	4.7 ± 0.3	1.9 ± 0.3	2.7 ± 0.3	1.1 ± 0.2
6	7.0 ± 0.4	2.9 ± 0.2	3.8 ± 0.2	1.3 ± 0.2
9	8.4 ± 0.2	3.6 ± 0.1	4.7 ± 0.3	2.3 ± 0.3
12	11.5 ± 0.4	4.9 ± 0.4	6.9 ± 0.2	3.8 ± 0.1
15	16.2 ± 0.7	8.7 ± 0.5	8.9 ± 0.5	5.6 ± 0.2
18	16.6 ± 0.5	9.1 ± 0.4	10.6 ± 0.3	7.3 ± 0.3
21	15.9 ± 0.6	11.3 ± 0.2	12.8 ± 0.3	8.7 ± 0.2

Values (mean ± SD) are average of duplicate samples, analyzed individually in triplicate ($n = 2 \times 3$), ($P < 0.05$).
Different letters in superscript indicate significant differences.

Table 5
Conjugated trienes ($\varepsilon_{1\ cm}(\lambda_{268\ nm})$) of stabilized and control corn oil

Incubation period (microwave heating in min.)	Control[c]	BHT (200 ppm)[a]	Corncob extract	
			500 ppm[b]	1000 ppm[a]
0	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1
3	1.8 ± 0.2	1.0 ± 0.1	1.2 ± 0.2	0.9 ± 0.1
6	2.2 ± 0.1	1.4 ± 0.2	2.0 ± 0.1	1.1 ± 0.2
9	3.2 ± 0.2	1.7 ± 0.4	2. 5 ± 0.2	1.9 ± 0.1
12	3.9 ± 0.3	2.7 ± 0.2	3.0 ± 0.1	2.6 ± 0.3
15	4.7 ± 0.2	3.3 ± 0.3	3.8 ± 0.2	3.3 ± 0.1
18	6.0 ± 0.3	3.8 ± 0.2	4.2 ± 0.2	3. 9 ± 0.2
21	7.0 ± 0.2	4.0 ± 0.3	5.0 ± 0.1	4.2 ± 0.2

Values (mean ± SD) are average of duplicate samples, analyzed individually in triplicate ($n = 2 \times 3$), ($P < 0.05$).
Different letters in superscript indicate significant differences.

Table 6
p-Anisidine value of stabilized and control corn oil

Incubation period (microwave heating in min.)	Control[d]	BHT (200 ppm)[a]	Corncob extract	
			500 ppm[c]	1000 ppm[a]
0	0.6 ± 0.1	0.6 ± 0.2	0.6 ± 0.1	0.6 ± 0.2
3	1.4 ± 0.2	0.9 ± 0.3	1.0 ± 0.1	0.9 ± 0.1
6	2.0 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	1.1 ± 0.2
9	3.9 ± 0.4	2.7 ± 0.2	3.0 ± 0.3	2.3 ± 0.1
12	5.7 ± 0.3	3.1 ± 0. 2	3.5 ± 0.2	3.3 ± 0.3
15	6.8 ± 0.2	4.5 ± 0.3	5.0 ± 0.1	4.0 ± 0.2
18	7.4 ± 0.3	4.8 ± 0.2	5.5 ± 0.3	4.5 ± 0.3
21	8.2 ± 0.3	5.2 ± 0.2	6.9 ± 0.3	4.8 ± 0.2

Values (mean ± SD) are average of duplicate samples analyzed individually in triplicate ($n = 2 \times 3$), ($P < 0.05$).
Different letters in superscript indicate significant differences.

stabilization of sunflower oil under accelerated aging, using the measurement of CD and CT contents.

3.6.3. *p*-Ansidine value

p-Anisidine value is extensively used to measure the secondary oxidation products, mainly non-volatile carbonyls, formed during lipid oxidative degradation. Aldehydes in oil and the *p*-ansidine reagent react with each other under acidic conditions, resulting in the formation of yellowish colored product, concentration of which is determined spectrophotometrically by measuring absorbance at 350 nm. Greater the absorbance greater will be the concentration of aldehydes, and lower will be the oxidative stability of the oil (Anwar, Manzoor, & Bajwa, 2004).

Table 6 depicts the *p*-ansidine values for corn oil samples stabilized with methanolic extract, BHT, and control. *P*-ansidine values showed the same trend as CD, increasing with incubation time. In control sample formation of carbonyls was at the higher rate as in samples with added extracts ($P < 0.05$). When compared with the control, corncob extract at 1000 ppm was found to be even more effective in retarding the formation of carbonyl components (a reduction up to 41.8%) than BHT (a reduction of 37.3%), which predicted its high antioxidant potential. However, at 500 ppm level this extract was less effective than BHT. Utilization of *p*-anisidine measurement to assess potential of natural antioxidants in vegetable oils under accelerated storage conditions is generally accepted (Chatha et al., 2006).

4. Conclusion

By coupling the results of different assays for evaluating antioxidant activity of corncob extracts from different solvent with those of oxidation parameters of the stabilized corn oil, we can conclude that methanolic extract showed the best antioxidant activity. Overall outcome of the present investigation depicted thatcorncob is a good source of potent antioxidants that can be used in the nutraceutical and functional food applications, and to protect vegetable oil. Discussed study offer some preliminary information about antioxidants potential in this source, but to develop useful antioxidants more work is required to further establish composition of components involved and how to isolate them in appropriate form for particular application.

References

Anjum, F., Anwar, F., Jamil, A., & Iqbal, M. (2006). Composition and oxidative stability of sunflower seed oil. *Journal of American Oil Chemist Society, 83*(9), 770–777.

Anwar, F., Bhanger, M. I., & Yasmeen, S. (2003). Antioxidant activity of some natural extracts in corn oil. In N. Murata, M. Yamada, I. Nishida, H. Okuyama, J. Sekiya, & W. Hajime (Eds.), *Advanced research of plant lipid* (pp. 24–27). Netherlands: Kluwer Publishers.

Anwar, F., Manzoor, M., & Bajwa, J. R. (2004). Antioxidant activity of solvent extracts of strawberry (*F. Ananassa*) using various antioxidant assays. *Pakistan Journal of Analytical Chemistry, 5*(2), 28–37.

Anwar, F., Jamil, A., Iqbal, S., & Sheikh, M. A. (2006). Antioxidant activity of various plant extracts under ambient and accelerated storage of sunflower oil. *Grassay y Aceites, 57*(2), 189–197.

AOCS (1997). *Official methods and recommended practices of the Americal Oil Chemical Society* (5th ed.). *AOCS Official method Cd 8-53*. Champaign, IL, USA: AOCS Press.

Bandoniene, D., Pukalskas, A., Venskutomis, P. R., & Grudiene, D. (2000). Preliminary screening of antioxidants of some plants extracts in rape seed oil. *Food Research International, 23*(9), 785–791.

Chaovanalikit, A., & Wrolstad, R. E. (2004). Total anthocyanins and total phenolics of fresh and processed cherries and their antioxidant properties. *Journal of Food Science, 69*(1), 67–72.

Chatha, S. A. S., Anwar, F., Manzoor, M., & Bajwa, J. R. (2006). Evaluation of the antioxidant activity of rice bran extracts using different antioxidant assays. *Grassay Aceites, 57*(3), 328–335.

Haung, D., Ou, B., & Prior, R. L. (2005). The chemistry behind antioxidant capacity assays. *Journal of Agriculture and Food Chemistry, 53*, 1841–1856.

Heim, K. E., Taigliaferro, A. R., & Bobilya, D. J. (2002). Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. *The Journal of Nutritional Biochemistry, 13*, 572–584.

Hsu, B., Coupar, I. M., & Ng, K. (2006). Antioxidant activity of hot water extract from the fruit of the Doum palm, *Hyphaene thebaica*. *Food Chemistry, 98*, 317–328.

Iqbal, S., & Bhanger, M. I. (2007). Stabilization of sunflower oil by garlic extract during accelerated storage. *Food Chemistry, 100*(1), 246–254.

Iqbal, S., Bhanger, M. I., & Anwar, F. (2005). Antioxidant properties and components of some commercially available varieties of rice bran in Pakistan. *Food chemistry, 93*, 265–272.

Iqbal, S., Bhanger, M. I., Akhtar, M., Anwar, F., Ahmed, K. R., & Anwer, T. (2006). Antioxidant properties of methanolic extracts from leaves of *Rhazya stricta*. *Journal of Medicinal Food, 9*(2), 270–275.

International Union of Pure and Applied Chemistry (IUPAC) (1979). Standard methods for the analysis of oils, fats and derivatives. In C. Paquot (Ed.), *IUPAC method II. D. 23* (6th ed.). Oxford, England: Pergamon Press.

Javanmardi, L., Stushnoff, C., Locke, E., & Vivanco, J. M. (2003). Antioxidant activity and total phenolic contents of Iranian *Ocimum* accessions. *Food Chemistry, 83*, 547–550.

Jayaprakasha, G., Singh, R., & Sakariah, K. (2001). Antioxidant of grape seed (*Vitis vinifera*) extracts on peroxidation models in vitro. *Food Chemistry, 37*, 285–290.

Jeong, S. M., Kuo, S. Y. D., Kim, R. S., Jo, C., Nam, K. C., Ahn, D. U., et al. (2004). Effect of heat treatment on the antioxidant activity of extracts from citrus peels. *Journal of Agriculture and Food Chemistry, 52*, 3389–3393.

Jung, U. K., Lee, S. K., Hun, S., Kyung & Chung, G. H. (2001). Antioxidant effects of natural lecithin on borage oil. *Food Science and Biotechnology, 10*(3), 1–6.

Lee, Y. C., Kim, I. H., Chang, J., Rhee, Y. K., Oh, H. I., & Park, H. K. (2004). Chemical compositions and oxidative stability of safflower oil prepared with expeller from safflower seeds roasted at different temperatures. *Journal of Food Science, 1*(69), 33–38.

Negi, P. S., Jayaprakasha, G. K., & Jena, B. S. (2003). Antioxidant and antimutagenic activities of pomegranate peel extracts. *Food Chemistry, 80*, 393–397.

Rababah, T. M., Hettiarachy, N. S., & Horax, R. (2004). Total phenolics and antioxidant activities of feurgreek, green tea, black tea, grape seed, ginger, rosemary, gotu kola, and ginkgo extracts, vitamin E, and ter-butylhrdroquinone. *Journal of Agriculture and Food Chemistry, 52*, 5183–5186.

Sanchez-Moreno, C., Larrauri, J. A., & Saura-Calixto, F. (1999). Free radical scavenging capacity and inhibition of lipid oxidation of wines, grape juices and related polyphenolic constituents. *Food Research International, 32*, 407–412.

Shahidi, F., & Wanasundara, U. N. (1997). Measurement of lipid oxidation and evaluation of antioxidant activity. In *Natural antioxidants, chemistry, health effects and applications* (pp. 1–10). IL, USA: AOCS Press Champaign.

Shaker, E. S. (2006). Antioxidant effect of extracts from red grape seed and peel on lipid oxidation in oils of sunflower. *LWT, 39*(8), 883–892.

Siddhuraju, P., & Becker, K. (2003). Antioxidant properties of various extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera lam.*) leaves. *Journal of Agriculture and Food Chemistry, 51*, 2144–2155.

Siddiq, A., Anwar, F., Manzoor, M., & Fatima, M. (2005). Antioxdant activity of different solvent extracts of *Moringa oleifera* leaves under accelerated storage conditions of sunflower oil. *Asian Journal of Plant Sciences, 4*(6), 630–635.

Silva, E. M., Souza, J. N. S., Rogez, H., Rees, J. F., & Larondella, Y. (2006). Antioxidant activities and polyphenolic contents of fifteen selected plant species from the Amazonian region. *Food Chemistry, 101*(3), 1012–1018.

Singh, R. P., Murthy, K. N. C., & Jayaprakasha, G. K. (2002). Studies on antioxidant activity of pomegranate (*Punica granatum*) peel and seed extracts using in vitro models. *Journal of Agriculture and Food Chemistry, 50*, 81–86.

Someya, S., Yoshiki, Y., & Okubo, K. (2002). Antioxidant compounds from bananas (*Musa cavendish*). *Food Chemistry, 79*, 351–354.

Steel, R. G. D., & Torrie, J. H. (1980). *Principles and procedures of statistics*. London: McGraw Hill, p. 245.

Vagi, E., Rapavi, E., Hadolin, M., Peredi, K. V., Balazs, A., Blazovics, A., et al. (2005). Phenolic and triterpenoid antioxidants from *Origanum majorana* L. herb and extracts obtained with different solvents. *Journal of Agriculture and Food Chemistry, 53*, 17–21.

White, P. J., & Xing, Y. (1997). Antioxidants from cereals and legumes. In F. Shahidi (Ed.), *Natural antioxidant, chemistry, health effects and applications* (pp. 25–45). IL, USA: Champaign.

Yen, G. C., Duh, P. D., & Chuang, D. Y. (2000). Antioxidant activity of anthraquinones and anthrone. *Food Chemistry, 70*, 307–315.

Yoshida, H., Hirakawa, Y., Abe, S., & Mizushina, Y. (2002). The contents of tocopherols and oxidative quality of oils prepared from sunflower (*Helianthus annuus* L.) seeds roasted in a microwave oven. *European Journal of Lipid Science and Technology, 104*, 116–122.

Yoshida, H., Hirakawa, Y., Tomiyama, Y., & Miz, Y. (2003). Effect of microwave treatment on the oxidative stability of Peanut (*Arachis hypogeae*) oils and the molecular species of their triacylglycerols. *Journal of Lipid Science and Technology, 105*, 351–358.

Yu, L., Perret, J., Harris, M., Wilson, J., & Haley, S. (2003). Antioxidant properties of bran extracts from "Akron" wheat grown at different locations. *Journal of Agriculture and Food Chemistry, 51*, 1566–1570.

Zhou, K., & Yu, L. (2004). Effects of extraction solvent on the wheat bran antioxidant activity estimation. *LWT, 37*, 717–721.

Zia-ur-Rehman Salariya, A. M., & Habib, F. (2003). Antioxidant activity of ginger extract in sunflower oil. *Journal of Science of Food and Agriculture, 83*, 624–629.

Zia-ur-Rehman (2006). Citrus peel extract – a natural source of antioxidant. *Food Chemistry, 99*(3), 450–454.