

Antioxidant potential of extracts from different agro wastes: Stabilization of corn oil

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RESUMEN

Potencial antioxidante de extractos de residuos agroalimentarios: estabilización de aceite de maíz.

En este estudio se determinó el potencial antioxidante de extractos de metanol al 80%, de distintos residuos de granada, manzana, frutos cítricos, maíz, trigo y arroz. Los rendimientos de los extractos variaron en un amplio rango (8.89 a 29.9 g/100 g de materia seca). La cantidad total de compuestos fenólicos, de flavonoides y de flavonoles, así como la capacidad para secuestrar radicales y la inhibición de la oxidación del ácido linoleico varió significativamente ($P < 0.05$). El extracto de piel de granada contenía las cantidades más elevadas de compuestos fenólicos, de flavonoides y de flavonoles y exhibió la capacidad antioxidante más elevada, seguido del de piel de manzana, piel de cítricos, piel de plátano, mazorca de maíz, salvado de trigo, salvado de arroz, cáscara de trigo y cáscara de arroz. Igualmente, se estableció la actividad antioxidante de los extractos usando aceite de maíz como sustrato. Muestras de aceite estabilizadas con los distintos extractos (600 mg/kg) fueron sometidas a oxidación acelerada (60 °C en horno durante 30 días con ciclos de calentamiento de 8 h/día) y periódicamente se determinaron los siguientes índices: dienos y trienos conjugados, índice de *p*-anisidina e índice de peróxidos. Finalmente, se estudiaron las correlaciones entre los resultados de los distintos ensayos y los niveles de compuestos con acción antioxidante, destacando los extractos de pieles de fruta por su mayor contenido en compuestos fenólicos, flavonoides y flavonoles, e igualmente por su potencia para aumentar la estabilidad oxidativa del aceite de maíz.

PALABRAS CLAVE: Aceite de maíz – Extracción – Fenoles y flavonoides totales – Flavonoles – Inhibición (%) – Parámetros de oxidación – Radical DPPH.

SUMMARY

Antioxidant potential of extracts from different agro wastes: Stabilization of corn oil

The antioxidant potential of 80% methanolic extracts of some agro wastes (pomegranate peel, apple peel, banana peel, citrus peel, corncob, wheat husk, wheat bran, rice bran, and rice hull) was assessed. The yields of the extracts varied over a wide range (8.83 to 29.9 g/100g of dry weight). TPC, TFC, total flavonols (kaempferol, quercetin, myricetin;

HPLC method), DPPH radical scavenging and inhibition of linoleic acid peroxidation for the extracts varied significantly ($P < 0.05$) ranging from 0.16-36.40, 0.026-4.89 g/100g of dry matter, 1.06-2622.41 mg/Kg of dry matter, 11.2-52.6, and 64.8-96.4%, respectively. Pomegranate peel extract, offering the highest TPC, TFC, and total flavonols exhibited superior antioxidant activity followed by apple peel, citrus peel, banana peel, corncob, wheat bran, rice bran, wheat husk, and rice hull. Furthermore, the antioxidant effectiveness of the extracts was assessed using corn oil (CO) as the oxidation substrate. CO samples stabilized with extracts at a concentration of 600 ppm (w/w) were subjected to accelerated aging (60 °C, oven heating, 30 days; 8 h heating cycle per day), and analyzed periodically for the extent of oxidative alterations following the measurement of conjugated dienes (CD), conjugated trienes (CT), *p*-anisidine and peroxide values. The correlation between the results of different antioxidant assays and oxidation parameters investigated in the present study indicated that fruit peel extracts, exhibiting higher TPC, TFC, flavonol and scavenging power, were also more potent for enhancing the oxidative stability of corn oil.

KEY-WORDS: Corn oil – DPPH radical – Extraction – Flavonols – HPLC – Inhibition(%) – Oxidation parameters – Total phenolics and flavonoids.

1. INTRODUCTION

Lipid peroxidation that results from the reaction between unsaturated fatty acids and molecular oxygen is a severe problem for the oil and fat industry. It not only deteriorates the quality of fats and fatty foods and brings about chemical spoilage, it also produces free radicals and reactive oxygen species (ROS) which are reportedly associated with carcinogenesis, mutagenesis, inflammation, aging and cardiovascular diseases (Sikwese and Duodu, 2007; Wang *et al.*, 2003). Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertiary butylhydroquinone (TBHQ) have been widely used for the preservation and protection of oil-based products against oxidation, however, their use is discouraged due to the perceived carcinogenic potential (Siddhuraju, 2007; Sultana *et al.*, 2007).

Currently, the applications of plant-derived natural antioxidants such as phenolic acid, flavonoids and tocopherols in foods, as well as preventive and therapeutic medicine, is gaining recognition because of their anticarcinogenic potential and other health-promoting effects (Choi *et al.*, 2007; Fan *et al.*, 2007; Iqbal *et al.*, 2007; Siddhuraju, 2007). Such natural antioxidant substances are believed to play a potential role in interfering with the oxidation process by reacting with free radicals, chelating catalytic metals and scavenging oxygen in foods and biological systems (Kim, 2005). The antioxidant activity of this manifold group of plant-based natural antioxidants has been studied *in vitro* and *in vivo*, and depends on the individual structure, position and degree of hydroxylation (Shahidi, 1997). Following the new trends, in recent years, the use of plant extracts as antioxidant additives for improving the oxidative stability and shelf-life of vegetable oils and fats has also attracted a great deal of scientific interest (Anwar *et al.*, 2007; Siddiq *et al.*, 2005).

Several types of plant materials such as vegetables, fruits, leaves, oilseeds, cereal crops, barks, roots, spices and herbs have been sought out for their potential antioxidants (Choi *et al.*, 2007; Iqbal *et al.*, 2007; Kim, 2005; Sikwese and Duodu, 2007; Sultana *et al.*, 2007; Wang *et al.*, 2003). However, relatively less information is available on the antioxidant principles of the agro wastes which are usually discarded in huge quantities. As a result, in the last few years, increased attention has been focused on the antioxidant prospective of food processing products and agro-industrial wastes (Iqbal *et al.*, 2007; Moure *et al.*, 2001; Zia-ur-Rehman, 2006). The determination of antioxidants in peels, brans, seed coats and oilseed residues etc. has been gaining importance with the recognition that these parts of the fruits and seeds are often rich sources of natural antioxidants.

Pakistan, being an agricultural country is blessed with medicinally or economically important flora and agricultural crops. There are a number of agro wastes, fruit processing products and cereal residual sources for bioprospecting and screening of antioxidant components. This study was an attempt to investigate different indigenously available agro wastes as potential sources of natural antioxidants and to appraise their efficacy for stabilizing corn oil.

2. EXPERIMENTAL

2.1. Materials

Samples of refined-bleached-deodorized (RBD) corn oil, without citric acid or other additives and corncob (*Zea mays*, var. neelam) were procured from Rafhan Maize Products, Pvt. Ltd., Faisalabad, Pakistan; rice bran and rice hull (*Oryza sativa*, var. super kernel) from Ahmed Rice Mill, Chiniot,

Pakistan; wheat bran and wheat husk (*Triticum sativum*, var. inqalab) from Ayub Agriculture Research Institute, Faisalabad, Pakistan; peels of citrus (*Citrus reticulata*, var. kinow), apple (*Malus pumila*, var. skysuper), banana (*Musa sapientum*, var. basrai) and pomegranate (*Punica granatum*, var. qandhari) were obtained from local fruit market sources of Faisalabad, Pakistan. The varieties of agricultural materials were checked, further identified and authenticated by Professor Dr. Muhammad Ashraf, Dean Faculty of Basic Sciences, Department of Botany, University of Agriculture, Faisalabad, Pakistan. HPLC grade flavonol standards (kaempferol, quercetin, myricetin), (±) catechin, TBHQ (99.0%), BHT (99.0%), Folin-Ciocalteu phenol reagent (2 N), gallic acid (98.0%), 1, 1-diphenyl-2-picrylhydrazyl (DPPH) (90.0%) and linoleic acid (99.0%) were of Sigma Chemicals Co. (St. Louis, MO, USA). All other chemicals (analytical grade) i.e. anhydrous sodium carbonate, sodium hydroxide, sodium nitrite, ferrous chloride, ammonium thiocyanate, aluminum chloride, 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 otherwise stated.

2.1.1. Dry matter determination

Owing to the varying water contents of different plant materials, all calculations were made on dry mass basis. For the determination of dry matter, all samples were dried under a fan at ambient temperature using the passive air-drying technique for 1-2 weeks, until constant weight was achieved.

2.2. Preparation of Extracts and Evaluation of Antioxidant Activity

2.2.1. Extraction of plant materials

Air-dried plant materials were ground into a fine powder using a commercial blender ((TSK-949, Westpoint, France), except for rice bran and wheat bran, which were processed as such. The material that passed through an 80-mesh sieve was used for extraction purposes. Powdered material (20g) for each sample was extracted with 200 mL of 80% methanol, overnight at room temperature using an orbital shaker (Gallenkamp, UK). The extracts were separated from the residue by filtering through Whatman No. 1 filter paper. The residues were extracted twice with the same solvent and extracts pooled. The pooled extracts were concentrated to dryness under reduced pressure at 45 °C, using a rotary evaporator (EYELA, N-N Series, Rikakikai Co. Ltd. Tokyo, Japan). The dry extracts were weighed to calculate the yield and stored in a refrigerator (-4 °C), until used for further analyses.

2.2.2. Determination of total phenolics content (TPC)

The amount of TP were assessed using Folin-Ciocalteu reagent (Chaovanalikit and Wrolstad, 2004). Briefly, 50 mg of crude extract were 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 (U-2001, Hitachi Instruments Inc. Tokyo, Japan). Amounts of TP were calculated using a gallic acid calibration curve within the range of 10-100 ppm ($R^2 = 0.9986$). The results were expressed as gallic acid equivalents (GAE) g/100g of dry matter. All samples were analyzed thrice and results averaged.

2.2.3. Determination of total flavonoids (TF)

TF were determined following the procedure of Dewanto *et al.*, (2002). One milliliter of aqueous extract containing 0.01g/mL of dry matter was placed in a 10 mL volumetric flask, then 5 mL of distilled water were added followed by 0.3 mL of 5% NaNO_2 . After 5 min, 0.6 mL of 10% AlCl_3 were added. After another 5 min 2 mL of 1M NaOH were added and the volume made up with distilled water. The solution was mixed and absorbance was measured at 510 nm using a spectrophotometer (U-2001, Hitachi Instruments Inc. Tokyo, Japan). TF amounts were expressed as (\pm) catechin equivalents g/100g of dry matter. All samples were analyzed thrice and results averaged.

2.2.4. DPPH. Scavenging assay

The free radical scavenging activity of extracts was assessed using procedure the reported earlier (Sultana *et al.*, 2007; Iqbal *et al.*, 2007). Briefly, 5.0 mL of a freshly prepared solution of 1, 1'-diphenyl-2-picrylhydrazyl (DPPH) at concentration 0.025g/L were added to 1.0 mL of extract containing 25 $\mu\text{g/mL}$ of dry matter in methanol. Absorbance at 0, 0.5, 1, 2, 5 and 10 min was measured at 515 nm using a spectrophotometer (U-2001, Hitachi Instruments Inc. Tokyo, Japan). The remaining amounts of DPPH radical were calculated from the calibration curve. Absorbance measured at the 5th minute was used for comparison of the radical scavenging activity of the extracts.

2.2.5. Determination of antioxidant activity in linoleic acid system

The antioxidant activity of extracts was also determined by measuring the oxidation of the linoleic acid system (Sultana *et al.*, 2007; Iqbal *et al.*, 2007). 5 mg of each extract were added separately 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 made up to 25 mL with distilled water and incubated at 40 °C up to 360 hours. The extent of oxidation was measured by the peroxide value applying the thiocyanate method as described by Yen *et al.*, (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) were added sequentially. After 3 min of stirring, the absorption was measured at 500 nm using a spectrophotometer. A control contained all reagents with the exception of extracts. The synthetic antioxidant butylated hydroxytoluene (BHT) was used as a positive control. The percent of inhibition of linoleic acid oxidation was calculated with the following equation: $100 - [(Abs. \text{ increase of sample at } 360 \text{ h} / Abs. \text{ increase of control at } 360 \text{ h}) \times 100]$, to express antioxidant activity.

2.3. Sample Extraction for HPLC

The extraction/hydrolysis of flavonols was carried out following the method of Tokusoglu *et al.*, (2003) with slight modifications. Briefly, acidified methanol (25 mL) containing 1 % (v/v) HCl and 0.5 mg mL^{-1} TBHQ was added to each ground (80-mesh sieve) plant material (5 g). HCl (1.2 M, 5 mL) was added and the mixture was stirred at 90 °C under reflux for 2 hours to obtain aglycons of flavonol glycosides. The extract was cooled to room temperature and centrifuged (EBA 20, Hettich, Germany) at 1538 g (5000 rpm) for 10 min. Upper layers were taken and sonicated for 5 min, to remove the air. The final extracts were filtered through a 0.45- μm (Millipore) filter before injecting into HPLC.

2.4. HPLC Separation

A flavonol (kaempferol, quercetin, myricetin) analysis was performed using a high-performance liquid chromatograph. An HPLC (model LC-10A, Shimadzu, Japan), equipped with two LC-10 AS pumps, SCL-10A system control unit, Rheodyne injector, CTO-10A column oven, SPD-10A UV-Vis detector and data acquisition class LC-10 software were used. A 20- μL volume of the filtered sample was injected into an analytical Supelco (Supelco Inc., Supelco Park, Bellefonte, USA) ODS reverse phase (C18) column (250 \times 4.6 mm; 5 μm particle size). Two solvent systems A: contained 3% trifluoroacetic acid and B: contained acetonitrile and methanol (80:20 v/v) were used. The chromatographic separation was performed by isocratic elution of the mobile phase (mixture of solvent A and B (50:50 v/v) which was filtered under vacuum through a 0.45 μm membrane before use) at a flow rate of 1.0 mL min^{-1} at 30 °C. Detection was performed at a wavelength of 360 nm.

The identification of flavonols (kaempferol, quercetin, myricetin) was carried out by comparing their retention times with those of authentic standards (Sigma Chemicals Co., St. Louis, MO, USA). Quantitative determination was carried out using calibration curves of the standards. By analyzing dilution series of pure standards solutions ranging from 0.01 to 0.08 mg L⁻¹, minimum quantities were determined for the flavonols. The mean value of the signal-to-noise ratio ($n = 8$) generated from the solution that just caused more than 3 times S/N ratio was used to calculate the detection limit (based on S/N = 3) of the corresponding flavonol. The detection limit for kaempferol, quercetin and myricetin was 0.04, 0.05, 0.05 mg L⁻¹, respectively.

2.5. Evaluation of Antioxidant Efficacy of Extracts Using Corn Oil as Oxidation Substrate

2.5.1. Stabilization of oil

The crude concentrated methanolic extracts of each plant material were separately added to preheated (50 °C) RBD corn oil (CO) at concentrations of 600 ppm (w/w). The CO 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 extracts. Stabilized and control oil samples (100 mL) were placed in dark brown airtight glass bottles with narrow necks and subjected to accelerated storage in an electric hot air oven (IM-30, Irmeco, Germany) at 60 °C (8 hours heating cycle per day) for 30 days. All oil samples were prepared in triplicate. A CO sample, without antioxidant, was used as the control. Oil samples were taken after every 5-day interval. The oxidative deterioration level was assessed by the measurement of peroxide value (PV), conjugate dienes (CD), conjugate trienes (CT) and *p*-anisidine values.

2.5.2. Measurement of oxidation parameters of corn oil

Determination of the peroxide value (PV) of stabilized and control corn oil samples was made following the AOCS Official method Cd 8-53 (AOCS, 1997). Specific extinctions at 232 and 268 nm were determined using a spectrophotometer (U-2001, Hitachi, Instruments Inc., Tokyo, Japan). Samples were diluted with *iso*-octane to bring the absorbance within limits (0.2-0.8), and spectra were recorded at 232 and 268 nm, and ($\epsilon^{1\%}_{1\text{cm}(\lambda)}$) which was calculated following an IUPAC method II D.23 (IUPAC, 1987). The determination of the *p*-anisidine value was made following an IUPAC method II. D. 26 (IUPAC, 1987). The oil samples dissolved in *iso*-octane were allowed to react with the *p*-anisidine solution in acetic acid (0.25 % w/v) for 10 minutes to produce colored complex and the

absorbance values were noted at 350 nm, using a spectrophotometer (U-2001, Hitachi Instruments Inc., Tokyo, Japan).

2.6. Statistical Analysis

Three samples of each plant material were taken. Each sample was analyzed individually in triplicate and data are reported as mean ($n = 3 \times 3$) \pm SD ($n = 3 \times 3$). An analysis of variance ANOVA was performed using Minitab 2000 Version 13.2 statistical software (Minitab Inc. Pennsylvania, USA). Significant differences ($P < 0.05$) of means were calculated using Duncan's multiple range tests. The relationship between different antioxidant assays was described as the Pearson product-moment correlation coefficient (r). Differences were considered statistically significant if $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Extract yields and antioxidant activity

The extract yield of antioxidative components from different agro wastes varied over a wide range, 8.83 to 29.9% (Table 1). The highest amounts were extracted from pomegranate peel (29.9%), followed by banana peel (24.6%), corncob (21.6%), citrus peels (21.5%), rice bran (20.5%), apple peel (16.4%), wheat bran (12.7%), wheat husk (9.41%), and rice hull (8.83%). Okonogi *et al.*, (2007) investigated the extract yield of banana and pomegranate peels with 95% ethanol and found it to be 7.66 and 6.21%, respectively. The literature revealed the amount of extractable components using methanol from citrus peels as 19.87% (Zia-ur-Rehman, 2006) and rice bran 13.60% (Chatha *et al.*, 2006) and that of wheat bran with ethanol was 10.6% (Liyana-Pathirana and Shahidi, 2007). Our present analysis revealed somewhat higher extract yields of the investigated plant materials.

Variations in the yield of extracts from different plant materials might be attributed to the availability of different extractable components, defined by the chemical composition of the plant, nature of the soil and agro-climatic conditions (Hsu *et al.*, 2006). Among other parameters, effectiveness of the extracting solvent to dissolve endogenous compounds might also be a contributing factor.

The amount of total phenolics (TP) determined in 80% methanolic extracts of different agro wastes is shown in Table 1. The contents of TP were determined by means of the Folin-Ciocalteu reagent (FCR) assay, commonly known as the total phenols (or phenolic) assay. The total phenol assay is often used for the assessment of the antioxidant activity of plant extracts due to its ease, simplicity, and reproducibility, apart from undefined chemical nature of FCR. Several researchers have used this assay to determine total phenolics and often established a good linear correlation between the "total phenolic profile" and antioxidant activity

Table 1
Yield of extract (g/100g of dry matter), total phenolic contents GAE (g/100g of dry matter), total flavonoids CE (g/100g of dry matter), percent DPPH radical remaining and percent inhibition of peroxidation of different agro wastes

Agro waste	Yield of extract	Total phenolic contents	Total flavonoid contents	DPPH· remaining	Inhibition of peroxidation
Pomegranate peels	29.9 ^f ± 0.8	36.4 ^c ± 0.7	4.89 ^a ± 0.15	11.2 ^a ± 0.8	96.4 ^e ± 1.9
Apple peels	16.4 ^c ± 0.9	3.9 ^b ± 0.6	2.21 ^b ± 0.13	15.6 ^b ± 0.5	88.2 ^d ± 3.7
Banana peels	24.6 ^e ± 0.4	1.1 ^{ab} ± 0.3	0.62 ^c ± 0.03	23.6 ^c ± 1.8	82.7 ^c ± 4.1
Wheat husk	9.4 ^a ± 0.6	0.3 ^a ± 0.2	0.05 ^d ± 0.05	46.2 ^e ± 1.9	68.2 ^{ab} ± 3.6
Wheat bran	12.7 ^b ± 0.5	0.4 ^a ± 0.2	0.04 ^d ± 0.04	28.9 ^{cd} ± 0.8	70.7 ^{ab} ± 3.5
Corn cob	21.6 ^d ± 1.2	1.5 ^{ab} ± 0.4	0.05 ^d ± 0.02	26.1 ^c ± 1.4	72.5 ^b ± 2.8
Rice bran	20.5 ^d ± 0.7	0.4 ^a ± 0.2	0.03 ^d ± 0.04	31.6 ^d ± 1.1	69.5 ^{ab} ± 2.6
Rice hull	8.8 ^a ± 0.6	0.2 ^a ± 0.1	0.03 ^e ± 0.03	52.6 ^f ± 0.7	64.8 ^a ± 3.9
Citrus peel	21.5 ^d ± 0.5	2.4 ^{ab} ± 0.3	1.92 ^b ± 0.12	21.8 ^c ± 1.4	86.9 ^{cd} ± 1.8

Values (mean ± SD) are the average of triplicate samples, analyzed individually in triplicate (n = 3 x 3), (P < 0.05): Different letters in superscript indicate significant differences. GAE, Gallic acid equivalents. CE, Catechin equivalents.

(Huang *et al.*, 2005). The contents of TP, ranging from 0.2 to 36.40 GAE (g/100g of dry matter), differed significantly (P < 0.05) among various plant materials. Of the different residual sources investigated in the present analysis, fruit peels contained relatively higher amounts of TP. Our results are consistent with a previous report (Li *et al.*, 2005) which reveals that peel tissues usually contain larger amounts of phenols, anthocyanins and flavonols.

Within peels, the highest TP were found in pomegranate (36.40%), followed by apple (3.90%), citrus (2.4%), and banana (1.1%). The results of TP for pomegranate peels in the present work were found to be higher than those reported by Li *et al.*, (2005), however, lower than that of Negi *et al.*, (2003), while that of apple were higher than the earlier report (Leontowicz *et al.*, 2002), although comparable with the results of Wolfe and Liu, (2003). Citrus and banana peels also contained appreciable amounts of TP, which were relatively higher than earlier reports (Anagnostopoulou *et al.*, 2006; Someya *et al.*, 2002).

The TP contents of rice bran and wheat bran were found to be 0.4 and 0.4%, respectively. Iqbal *et al.*, (2005) reported the TP of four varieties of rice bran indigenous to Pakistan to range from 0.251-0.359 g/100 g. The TP of wheat bran in the present investigation were found to be comparable with earlier reports (0.212-0.37 g/100g) (Iqbal *et al.*, 2007), however, the current level was greater than that reported by Zhou and Yu, (2004) (0.063-0.263 g/100g). The lowest contents of TP (0.2 and 0.3%) were observed in the samples of rice hull, and wheat husk, respectively. Corn cob also offered an appreciable amount of TP (1.5%) which were found to be lower than fruit peels but higher than rice hull, rice bran, wheat bran, and wheat husk.

Total flavonoid contents (TFC) also varied significantly (P < 0.05) among different agriculture waste materials ranging from 0.03- 4.89 CE (g/100 gm of dry matter) (Table 1). All fruit peels exhibited greater concentrations of flavonoids with the

highest contents in pomegranate peels (4.89), followed by apple (2.21), and citrus (1.92) peels, however, a lower amount was observed in banana (0.62). On the other hand, total flavonoid contents were found to be quite low in cereal by-products ranging from 0.03 – 0.05 CE (g/100 gm of dry matter), which also contained lower amounts of TP. Significant (p < 0.05) variations in TP and TF amounts among different plant materials might be attributed to factors such as natural chemical composition, maturity at harvest, soil state and conditions of post-harvest storage (Huang *et al.*, 2005). However, a good correlation (r = 0.901, p < 0.05) between TF and TP was observed in the present analysis (Table 4).

The results of DPPH· scavenging activity are shown in Table 1. DPPH· radical is commonly used for the assessment of antioxidant activity. The DPPH· radical with deep violet color gives intensive absorption within the range of 515-528 nm. This test is based on the ability of the DPPH· (1, 1-diphenyl-2-picrylhydrazyl) to react with hydrogen donor species such as phenolics. Upon receiving a proton, it loses its color and becomes yellow. As the concentration of phenolic compounds or degree of hydroxylation of the phenolic compounds increases, their DPPH radical scavenging activity also increases, the antioxidant efficacy of a typical plant material can be predicted. Because these radicals are very sensitive to the presence of hydrogen donors, the whole system operates at a very low concentration; with it, a large number of samples can be tested in a short time (Roginsky and Lissi, 2005). Absorbance in this assay was recorded at 0.5 to 10 min time intervals from the initiation of the reaction. Observed scavenging activity was similar at the beginning of the reaction and changed with the increase in reaction time until stabilized after 10 min. Significant (P < 0.05) differences among different extracts were observed at the 5th minute of reaction (Table 1). The DPPH scavenging activity of each sample was reported as the percent of DPPH· remaining, with a higher value of percent of DPPH·

remaining associated to a weaker antioxidant activity.

All extracts possessed the free radical scavenging properties but to varying degrees. The remaining amount (%) of DPPH[•] radical at 5 min after initiation of the reaction ranged from 11.2 to 52.6%. Results from the present analysis show that the pomegranate peel extract exhibited maximum scavenging activity followed by apple peel, citrus peel, banana peel, corncob, wheat bran, rice bran, wheat husk, and rice hull. The large amount of phenolics contained in peel extract may account for its strong antioxidant activity. In the investigations of antioxidant activities of certain fruit peel extracts including pomegranate, and banana, pomegranate peel exhibited the highest DPPH[•] scavenging activity (Okonogi *et al.*, 2007). The antioxidant activity of pomegranate peel extracts might be attributed to the presence of polyphenols, such as ellagic tannins, ellagic acid and gallic acid (Gil *et al.* 2000). Apple peel also exhibited good radical scavenging activity, which is in accordance to the previous findings of Abrosca *et al.*, (2007) who reported that apple peel extract caused a 78% reduction in DPPH from the initial DPPH.

The radical scavenging activity of wheat bran (var. inqalab) (28.9% DPPH remaining) in the present study was found to be slightly lower than the var. Punjab-9 (24%), however, it was comparable with that of var. Bhakkar 2002 (30%) (Iqbal *et al.*, 2007). DPPH radical scavenging activity of wheat bran in the present analysis was found to be greater than that reported by Cheng *et al.* (2006) (35.6% DPPH remaining) but lower than that reported by Zhou and Yu, (2004) (16.87% DPPH remaining), while that of rice bran (31.6% DPPH remaining) was found to be lower than the literature reports (Iqbal *et al.*, 2005) (20.6-30.6% DPPH remaining).

It has been proven that the free radical scavenging activity of plant extracts is mainly ascribed to the concentration of phenolic compounds present in the plants (Moure *et al.*, 2001). Several studies showed good correlation between the total phenolic contents and antioxidant activity of plant extracts (Huang *et al.*, 2005). Significant linear correlation of TP ($r = -0.552$, $P < 0.05$), TF ($r = -0.720$, $P = 0.03$), and total flavonol contents ($r = -0.725$, $P < 0.02$) (table. 4) with DPPH scavenging activity were also observed in the present work.

The inhibition of linoleic acid oxidation as exhibited by different plant extracts (Table 1) ranged from 64.8 - 96.4%, whereas BHT provided inhibition at the level of 84.7%. The sample with the greatest level of % inhibition of peroxidation was the pomegranate peel extract (96.4%), followed by apple (88.2%), citrus (86.9%), and banana (82.7%) extracts. On the other hand the lowest inhibition of peroxidation was offered by wheat husk (68.2%) and rice hull (64.8%).

The variation in the level % inhibition of different plant materials can be explained by their varying

total phenolic concentration. Correlation analyses ($r = 0.686$; $r = 0.909$, $r = 0.920$ at $p = 0.05$) (Table. 4) in the present investigation also indicated a good relationship between TP, TF, and total flavonol contents with antioxidant activity determined in terms of the measurement of percent inhibition of peroxidation. Lower levels of % inhibition of peroxidation offered by the samples (wheat bran, rice bran, wheat husk, rice hull and corn cob) could be explained by the lower concentrations of phenolic compounds in these materials. Major phenolics generally present in these samples are hydroxybenzoate and hydroxycinnamate that are considered to exhibit low antioxidant activity as compared to anthocyanins and flavonoids (Kahkonen *et al.*, 1999). A few reports in the literature revealed that the antioxidant activity of some of these plant materials is based on their inhibition of peroxidation. The literature revealed that different varieties of rice bran inhibited lipid peroxidation up to 90% (Iqbal *et al.*, 2005). This variation may be due to differences in rice bran varieties, solvent used or the extraction procedure adopted in both of these studies.

3.1.1. Flavonol contents in agro wastes

The data for flavonol (myricetin, quercetin, kaempferol) contents of different agro wastes are presented in Table 2. The results show a wide variation in the levels of individual flavonols (myricetin, quercetin, kaempferol) among different agriculture wastes. Myricetin was the dominant flavonol detected in most of the samples, except rice bran and corncob and its concentration varied from 32.85 to 2495.89 mg kg⁻¹. The highest levels were found in pomegranate peel (2495.89), followed by citrus peel (1102.39), banana peel (666.68), apple peel (493.44), and rice hull (32.59). However, myricetin was not detected in rice bran, wheat bran, wheat husk and corncob.

The levels of quercetin and kaempferol in different agricultural waste materials varied widely ranging from 24.11-415.09 and 1.06-102.33 mg kg⁻¹ of dry matter, respectively. The highest quercetin levels were observed in apple peel (415.09), followed by wheat husk (107.34), corncob (102.51), wheat bran (88.75), and pomegranate peel (24.11); while the highest kaempferol levels were detected in pomegranate peel (102.33), followed by wheat bran (52.32), apple peel (51.54), and rice bran (1.06). On the other hand, quercetin and kaempferol were not detected in the present analysis of citrus peels which is in contrast to the findings of Wang *et al.*, (2007) who reported that the quercetin and kaempferol contents in different varieties of citrus ranged from 61-573 and 9-1035 mg/kg of dry matter, respectively. Rice hull, wheat husk, citrus and banana peels did not exhibit any amounts of quercetin and kaempferol either.

Overall, the results of HPLC indicate that all fruit peels contain good levels of total flavonols as

Table 2
Flavonol contents (mg /kg of dry matter) of different agro wastes

Agro waste	Myricetin ^A	Quercetin ^B	Kampeferol ^C	Total flavonols
Rice (bran) ^f	ND	ND	1.06 ± 0.04	1.06
Rice (hull) ^f	32.6 ± 1.0	ND	ND	32.6
Wheat (bran) ^e	ND	88.8 ± 2.7	52.3 ± 2.1	141.1
Wheat (husk) ^f	ND	107.3 ± 3.2	ND	107.3
Corn cob ^e	ND	102.5 ± 4.1	ND	102.5
Citrus (peel) ^b	1102.4 ± 44.1	ND	ND	1102.4
Banana (peel) ^d	666.7 ± 20.0	ND	ND	666.7
Pomegranate (peel) ^a	2495.9 ± 25.0	24.1 ± 0.7	102.4 ± 4.1	2622.4
Apple (peel) ^c	493.4 ± 9.9	415.1 ± 8.3	51.5 ± 1.1	960.0

Values (mean ± SD) are average of triplicate samples, analyzed individually in triplicate (n = 3 x 3), (P < 0.05); Small and capital alphabets in superscript indicate significant differences among agro wastes and flavonols, respectively. ND, Not detected.

compared with cereal by-products. The presence of high amounts of flavonols in fruit peels is in agreement with the previous reports which revealed that fruit peels had higher levels of phenols, anthocyanins and flavonols (Li *et al.*, 2006). A correlation analysis (Table 4) of flavonol contents with those of TP and TF (r = 0.909 and r = 0.987 at p < 0.05, respectively) also showed that samples which have greater amounts of TP and TF also exhibit a greater proportion of flavonols.

It is worth noting that samples which demonstrated greater antioxidant activity are all rich in phenolics, suggesting that these compounds might be contributing to this activity. Pomegranate is a rich source of anthocyanins (a glycosylated form of anthocyanidins). Because of their ortho-dihydroxy structure, cyanidin glycosides offer good antioxidant activity. Therefore, the higher antioxidant activity of pomegranate might be attributed to its cyanidin glycoside contents (Karadeniz *et al.*, 2005). The antioxidant activity of apples is also associated with their concentration of anthocyanins and flavonoids (Wolfe and Liu, 2003). Anthocyanins are the main phenolic compounds present in the peel of a red apple (Karadeniz *et al.*, 2005). The antioxidant activity of citrus peel may be attributed to the presence of phenolic compounds such as flavonones, flavone glycosides, polymethoxylated flavones, hydroxy cinnamate and other miscellaneous phenolic glycosides and amines (Zia-ur-Rehman, 2006). However, some authors have suggested that the antioxidant activity of a plant material may be attributed to their gallo-derivative contents. This is the case with bananas, which are high in galocatechins (Someya *et al.*, 2002).

3.2. Antioxidant potential of agro waste extracts for the stabilization of corn oil

After preliminary assessment of antioxidant efficacies of different extracts, the effectiveness of the extracts towards the stabilization of corn oil was also examined. The magnitude of oxidative alteration in the stabilized corn oil was followed by

the measurement of conjugated dienes, conjugated trienes, peroxide values and *p*-anisidine values.

Peroxide value is an indicator of the extent of primary oxidation products in oil (Anwar *et al.*, 2007; Chatha *et al.*, 2006). The peroxide values of the stabilized and control (without extract) corn oil, over an incubation period of 30 days at 60 °C is shown in Figure 1. It is evident from the results that the addition of both the synthetic antioxidant at a concentration of 200 ppm and methanolic extracts from different plant materials at 600 ppm inhibited the oxidation in corn oil significantly (P < 0.05) as compared with the control. The results indicate (Table 3) that the addition of antioxidant extracts from plant materials decreased the rise in PV by 42.5-70.0% with respect to the control. The rate of decline in PV was highest in the oil sample stabilized with pomegranate peel extracts (70.0%), followed by apple peel (64.6%), citrus peels (61.6%), banana peels (58.4%), corncob (55.8%), BHT (55.3%), wheat bran (53.6%), rice bran (51.3%), wheat husk (48.8%), and rice hull (42.5%) (Table 3).

Peroxide value is often used as a measurement of the oxidative deterioration 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 formation of these primary oxidation products. Oil stabilized with synthetic or natural antioxidants usually shows lower PV and extended induction periods (Anwar *et al.*, 2007; Chatha *et al.*, 2006; Siddiq *et al.*, 2005). The decline in PV of soybean oil stabilized with a crude methanolic extract from rice bran at the end of a incubation period (at 60 °C) of 15 days was 32.7% (Devi *et al.*, 2007). Zia-ur-Rehman, (2006) reported the antioxidant activity of citrus peels extracts for the stabilizing of corn oil stored at 25 °C for a period of 6 months. The results of Zia-ur-Rehman, (2006) indicated a rapid decline in the PV (89.9-93.9%) of oil samples stabilized with citrus peel extracts at different concentrations. Chatha *et al.*, (2006) reported the decline in PV (22.2-45.6%) of sunflower oil using 100 and 80% acetone and methanolic extracts from rice bran.

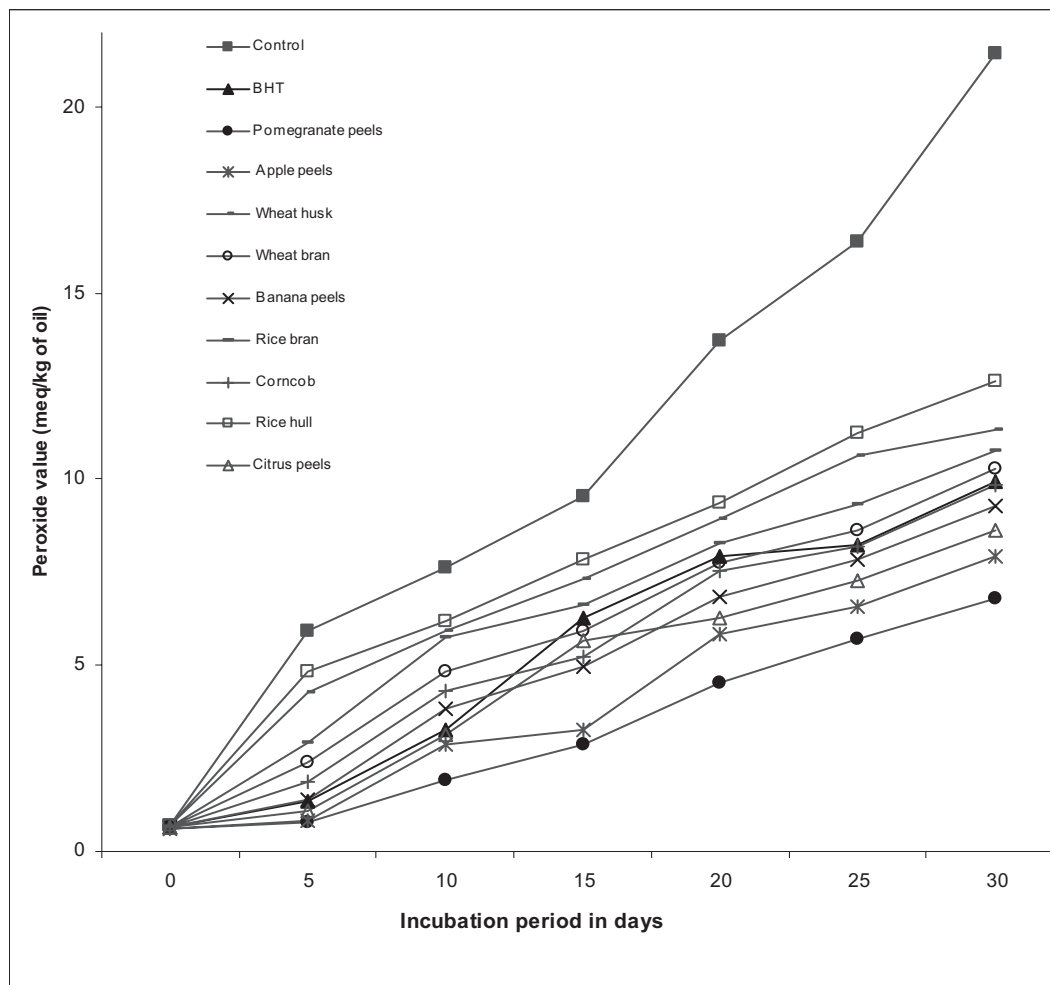


Figure 1 Peroxide value (meq/kg of oil) of stabilized and control corn oil.

The difference in the results of these studies with our investigation may be due to the nature of substrate oil, variations in storage conditions and concentration of the extract used. However, in all such cases lower values for PV for oils with antioxidant extracts as compare to the control

showed the effectiveness of the extracts in preventing the oxidation of the oil.

Conjugated diene (CD) and triene (CT) contents of corn oil samples stabilized with methanolic extract of different plant materials, BHT and control are shown in Figures 2 and 3. The CD and CT

Table 3 Alteration in oxidation parameters of stabilized corn oil at the end of incubation period

	PV		CD		CT		PA	
	Increase from initial	% decline	Increase from initial	% decline	Increase from initial	% decline	Increase from initial	% decline
BHT	9.27	55.3	6.0	62.3	5.94	47.8	8.61	33.3
Pomegranate peels	6.22	70.0	4.49	71.8	4.66	59.1	5.74	51.2
Apple peels	7.33	64.4	4.97	68.8	4.99	56.2	4.2	43.9
Wheat husk	10.63	48.8	9.32	41.5	7.11	37.5	4.83	23.0
Wheat bran	9.62	53.6	6.7	57.9	6.24	45.2	6.63	27.9
Banana peels	8.62	58.4	5.61	64.8	5.7	49.9	6.21	35.8
Rice bran	10.1	51.3	8.61	45.9	6.54	42.5	5.53	25.3
Corncob	9.17	55.8	5.97	62.5	5.97	47.5	6.43	33.4
Rice hull	11.95	42.5	9.83	38.3	7.6	33.2	5.73	19.2
Citrus peels	7.97	61.6	5.56	65.1	5.26	53.8	5.18	39.8

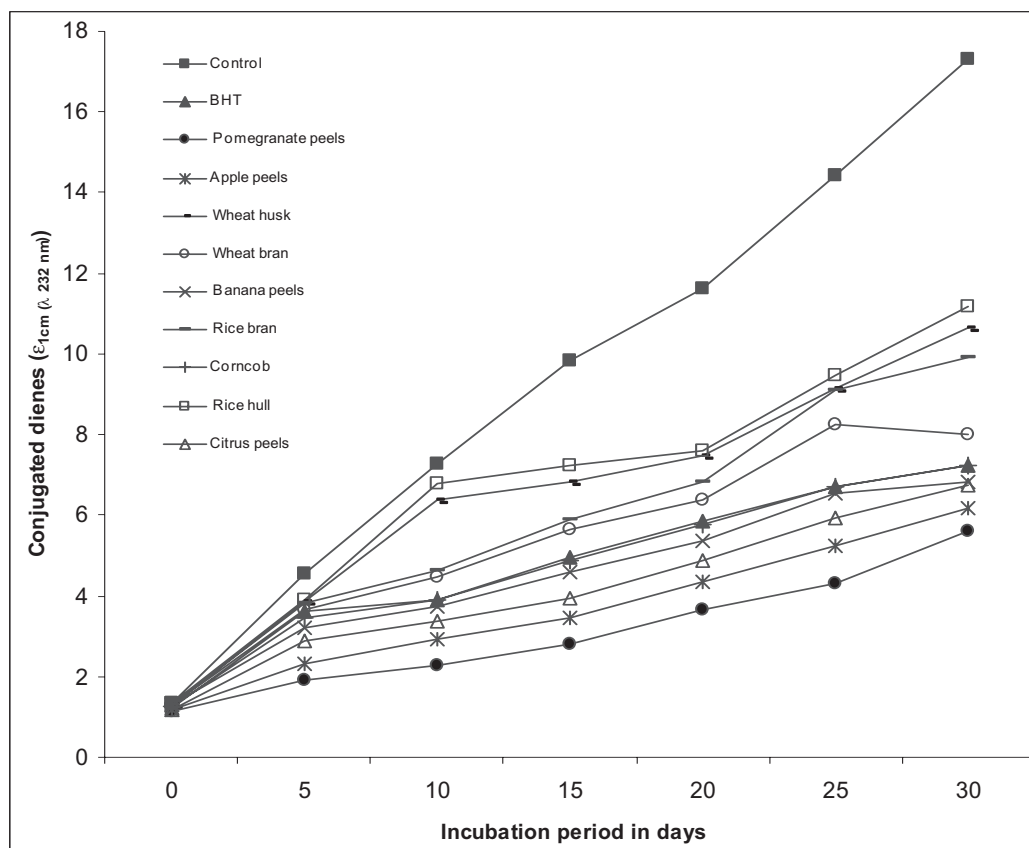


Figure 2
Conjugated dienes ($\epsilon_{1\text{cm}} (\lambda 232 \text{ nm})$) of stabilized and control corn oil.

contents increase paralleled the increase in incubation period with the highest rates for the control. The oil samples stabilized with plant extract showed lower levels of CD and CT as compared to the control, indicating the antioxidant potential of the extracts from the investigated plant materials. A decline in the rise of CD (38.3-71.8%) and CT (33.2-59.1%) in corn oil samples (Table 3) stabilized with plant extracts as compared with that of the control might be attributed to the presence of natural antioxidant components within these extracts. The lowest level of CD was noted for corn oil samples stabilized with the extract of pomegranate peel, followed by apple peels, citrus peels, banana peel, corncob, wheat bran, rice bran, wheat husk, and rice hull.

Devi *et al.*, (2007) reported a decline in the rise of conjugated dienes in soybean oil up to 38.1 % as compared to a control using a methanolic extract from rice bran. Chatha *et al.*, (2006) also concluded that the addition of 100 and 80 % acetone and methanolic extracts from rice bran reduced the formation CD, CT in sunflower oil stabilized under ambient conditions (room temperature) up to levels of 39.2-54.9% and 11.1-36.1%, respectively. Anwar *et al.*, (2007) also investigated the stabilization of sunflower oil under ambient aging using *Moringa oleifera* leaf extracts by estimating CD and CT contents, thus evaluating the antioxidant potential of *Moringa* leaves. The antioxidant activity of garlic

extracts in sunflower oil was also assessed under accelerated conditions, using CD and CT as indicators of oxidative degradation (Iqbal and Bhangar, 2007).

Measurement of CD and CT are reliable parameters for predicting the oxidative stability of oils. 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 oxygen intake. The greater the levels of CD and CT, the higher the magnitude of oxidative degradation will be, thus indicating a lower oxidative stability of the oils (Anwar *et al.*, 2007; Siddiq *et al.*, 2005; Chatha *et al.*, 2007).

Figure 4 depicts the level of *p*-anisidine values for corn oil samples stabilized with methanolic extracts, BHT, and the control. The periodical analysis of the oil samples with and without antioxidant extracts as a function of storage time revealed a decline in *p*-anisidine values (33.2-59.1%) (Table 3) as compared to the control. In the control sample, formation of carbonyls was significantly ($P < 0.05$) higher as compared to samples with added extracts. Pomegranate peel extract was found to be the most effective in retarding the formation of carbonyl components as measured by the *p*-anisidine value (59.1% decline), while rice hull displayed the least

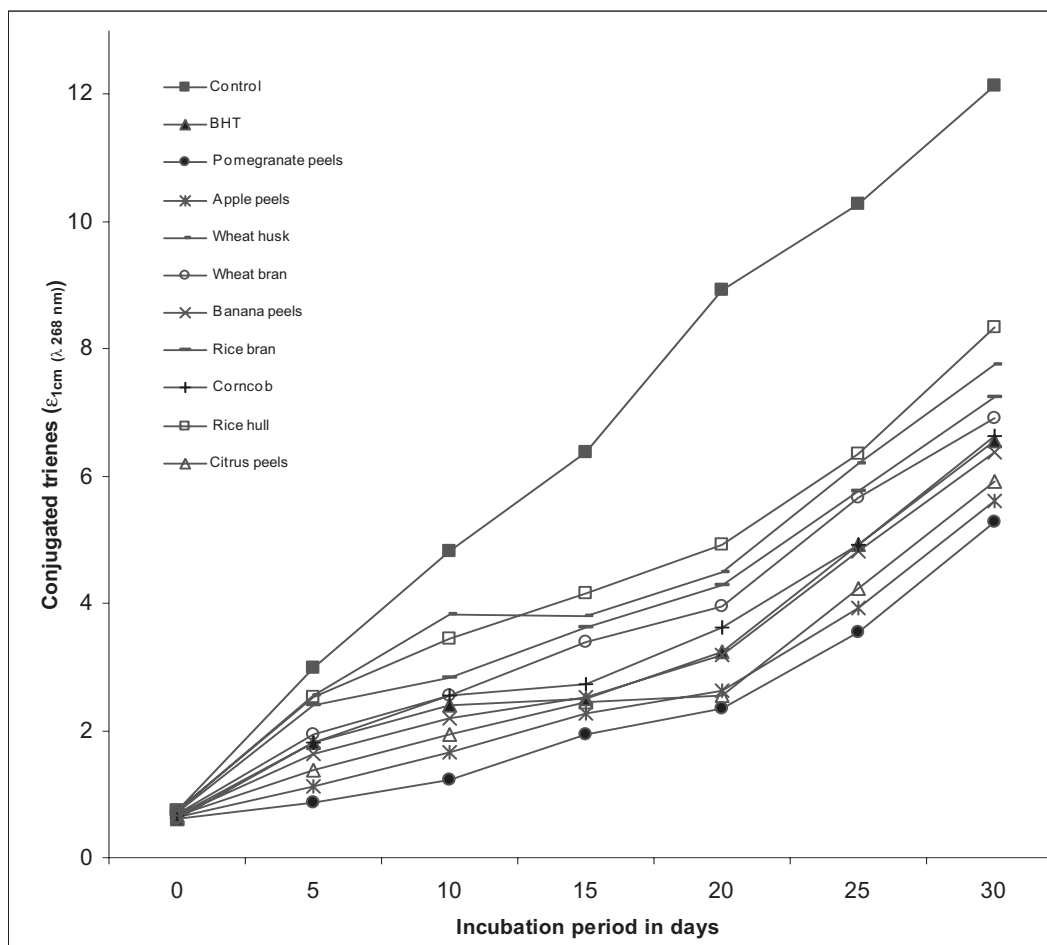


Figure 3
Conjugated trienes ($\epsilon_{1\text{cm}} (\lambda_{268\text{ nm}})$) of stabilized and control corn oil.

efficiency (33.2% decline) (Table 3). Devi *et al.*, (2007) reported a decline in *p*-anisidine values in soybean oil stabilized (at 60 °C) with crude methanolic extracts of rice bran up to 24.0% at the end of an incubation period of 15 days. In another study (Chatha *et al.*, 2007), the decline in *p*-anisidine value of sunflower oil stabilized (ambient storage) with 100 and 80% acetone and methanolic extracts of rice bran ranged from 20.6-51.1%. The extent of the decline in *p*-anisidine value in the present study was 33.2-59.1% (Table 3). The measurement of *p*-anisidine value to assess the potential of natural antioxidants in vegetable oils under accelerated storage conditions is generally accepted (Chatha *et al.*, 2007). The measurement of *p*-anisidine value is extensively used to assess secondary oxidation products, mainly non-volatile carbonyls (2-alkenal and 2, 4 alkadienal) formed during the lipid oxidative degradation of oils and fats. Aldehydes in oil and the *p*-anisidine reagent react with each other under acidic conditions, resulting in the formation of a yellowish colored product, whose concentration is determined spectrophotometrically by measuring absorbance at 350 nm. The greater the absorbance, the greater the concentration of aldehydes will be, thus indicating the poor oxidative state of the oil (Anwar *et al.*, 2007).

3.3. Correlation between the results of antioxidant assays and oxidative parameters

The relationship between the results of antioxidant assays with that of the oxidative parameters established by the Pearson product-moment correlation coefficient (*r*) is shown in Table 4. A moderate correlation i.e. -0.677 , -0.507 , -0.592 , and -0.711 between total phenolic contents and peroxide, conjugate dienes, conjugate trienes and *p*-anisidine values, respectively, was demonstrated; however, a fairly strong relationship was established between total flavonoid contents and these oxidation parameters. A strong correlation was also observed i.e. 0.962 , 0.957 , 0.980 , and 0.933 between the results of the DPPH radical scavenging assay and peroxide, conjugate dienes, conjugate trienes and *p*-anisidine values, respectively. The correlation between % inhibition of peroxidation and oxidation parameters was also strong (Table 4). From the results of the correlation analysis it could be declared that the antioxidant components of plant extracts are accountable for the enhancement of the oxidative stability of the vegetable oil under investigation.

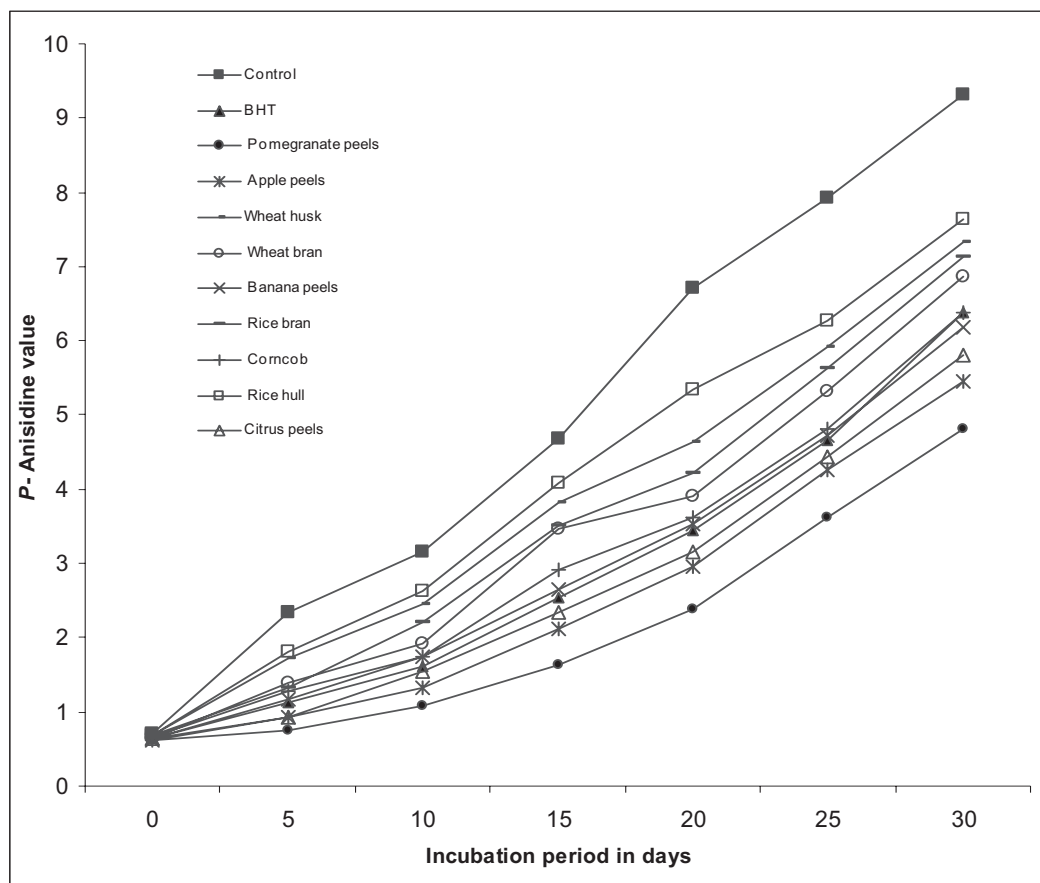


Figure 4
Para-anisidine value of stabilized and control corn oil.

4. CONCLUSION

From the results of present investigations it is reasonable to say that the methanolic extracts of different plant materials have exhibited varying degrees of total phenolic contents and antioxidant

activities. HPLC data indicate that fruit peels contain greater contents of flavonols (myricetin, quercetin, kaempferol). The correlation between the results of antioxidant assays and the oxidative parameters of corn oil demonstrated that fruit peel extracts which have higher antioxidant activity are

Table 4
Correlation between the results of different antioxidant assays and oxidation parameters of stabilized corn oil

	TPC	TFC	Total flavonols	DPPH remaining of peroxidation	% inhibition
TPC	-	0.901, <i>P</i> = 0.000	0.909, <i>P</i> = 0.000	-0.552, <i>P</i> = 0.003	0.686, <i>P</i> = 0.000
TFC	0.901, <i>P</i> = 0.000	-	0.987, <i>P</i> = 0.000	-0.720, <i>P</i> = 0.000	0.909, <i>P</i> = 0.000
DPPH Remaining % inhibition of peroxidation	-0.552, <i>P</i> = 0.003	-0.720, <i>P</i> = 0.000	-0.725, <i>P</i> = 0.002	-	-0.833, <i>P</i> = 0.000
Peroxide value	0.686, <i>P</i> = 0.000	0.909, <i>P</i> = 0.000	0.920, <i>P</i> = 0.000	-0.833, <i>P</i> = 0.000	-
Conjugated dienes	-0.677, <i>P</i> = 0.045	-0.803, <i>P</i> = 0.002	-0.759, <i>P</i> = 0.021	0.962, <i>P</i> = 0.000	-0.962, <i>P</i> = 0.000
Conjugate trienes	-0.507, <i>P</i> = 0.164	-0.606, <i>P</i> = 0.041	-0.542, <i>P</i> = 0.078	0.957, <i>P</i> = 0.000	-0.878, <i>P</i> = 0.002
<i>P</i> -anisidine value	-0.592, <i>P</i> = 0.093	-0.736, <i>P</i> = 0.034	-0.677, <i>P</i> = 0.043	0.980, <i>P</i> = 0.000	-0.945, <i>P</i> = 0.000
	-0.711, <i>P</i> = 0.032	-0.834, <i>P</i> = 0.002	-0.782, <i>P</i> = 0.0032	0.933, <i>P</i> = 0.000	-0.976, <i>P</i> = 0.000

Correlation analysis between different antioxidant assays and oxidative parameters (*n* = 3 x 3) were described as Pearson product-moment correlation coefficient (*r*). Results are statistically significant at *P* < 0.05. TPC, Total Phenolic Contents; TFC, Total Flavonoid Contents.

more potent towards enhancing the oxidative stability of corn oil. Hence, the agro materials investigated can be explored as a potential antioxidant source of natural origin; however, it is recommended that the cytotoxicity of the investigated extracts be investigated before their applications in vegetable oils, functional foods or nutraceuticals.

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