

Phenolic Composition and Antioxidant Activity of Some Agro-industrial Wastes

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ABSTRACT

The present study investigated the content and profile of phenolic compounds, antioxidant activity of methanolic extracts of selected agro- industrial waste (AIW) i.e. date palm racemes (*Phoenix dactylifera*, DPR), Prickly pear peels (*Opuntia ficus-indica*, PPP) and empty pea pods (*Pisum sativum* L, EPP). Non phenolic constituents of DPR were also analyzed by GC-MS. The oxidative stability of sunflower oil using these methanolic extracts was also investigated. The major phenolic compounds were rutin in PPP, myrecetin in EPP and quercetin in DPR. Amongst the three methanolic extracts, DPR revealed the highest scavenging activity with IC₅₀ values of 21.6µg/ml, whereas EPP revealed the lowest scavenging activity with IC₅₀ of 94.5µg/ml. A significant difference in lipid oxidation indices was noticed between the control and the sunflower oil samples containing DPR, PPP and EPP extracts.

Keywords: Date palm racemes, Prickly pear peels, empty pea pods, antioxidant

INTRODUCTION

Nutraceutical compounds derived from vegetable and fruit wastes such as natural phenolics are in great demand as a food supplement. They have been contributed with a number of beneficial promoting properties such as antioxidant (Naidu *et al.*, 2000). The antioxidant activity can probably be attributed to the major phenolic compounds, such as myrecetin, *p*-coumaric and catechin. Therefore, the polyphenols in waste might be used as a cheap source of natural compounds, with prospective applications in the food industry. Substitution of synthetic food additives with safe and effective natural additives may be of further benefit to the food (Barbosa-Pereira *et al.*, 2014). They have been added to prolong the duration period of the oil and prevent oxidation deterioration. Butylated hydroxytoluene (BHT) and butylated hydroxy anisole (BHA) are the most widely used chemical antioxidants. (National Toxicology Program, 2011). The BHT has a toxic effect on the lungs and increases the incidence of liver tumors. Also, BHA can induce the formation of stomach tumors.

The International Agency for Research on Cancer (IARC) stated that BHA has possible human

carcinogenic effects (IARC, 1986). The BHT can proceed as a tumor promoter in certain situations. Therefore, there is a strong need to find out alternative antioxidants from natural sources to prevent food deterioration. Edible oils containing high levels of unsaturation are liable to oxidation deterioration. The efficiency of natural antioxidants has been evaluated in oil oxidation process. Nowadays, researchers have made a significant develop in substituting synthetic antioxidants with natural ones from agro-industrial wastes to stabilize oils and fats during processing and storage, thus increasing their shelf life (Hamadou *et al.*, 2013). Recently, the number of researches on residual sources of antioxidants has grown substantially (Zeyada *et al.*, 2008, Sachindra *et al.*, 2010, Shibani *et al.*, 2012 & AL-Rawahi *et al.*, 2014). They noticed that natural antioxidants attained from agro-industrial wastes decelerated the rancidity process of oil to a higher extent than acted by synthetic antioxidants. Therefore, the aim of the present study was to assess the oxidative stability of sunflower oil using the methanolic extract of some Egyptian AIW i.e date palm racemes (DPR), prickly pear peels (PPP) and empty pea pods (EPP) as natural antioxidants.

MATERIALS AND METHODS

Materials:-

Agro- industrial waste samples (AIW):

Empty pea pods (EPP) were obtained from food processing company (Giverx), Alexandria, Egypt during January, 2014. Date palm racemes (DPR) of Zagloul date palm tree (*Phoenix dactylifera*) were obtained from a private farm located in Semoha district, Alexandria, Egypt at the end of October, 2014. Prickly pear fruits (*Opuntia ficus indica*) were obtained from Alexandria local market, Alexandria, Egypt at the 2nd week of August, 2014. The fruits were pear-shaped, green to yellow in colour and medium in size, 12-14 fruit per kg. These fruits were used to prepare prickly pear peels (PPP). Upon arrival at the laboratory, the aforementioned raw materials were thoroughly washed with tap water followed by distilled water to remove dirt from surfaces and drained. The DPR and EPP were divided into small pieces (strips) of approximately 2 cm length. Prickly pear fruits were peeled using a sharp knife. The obtained peels were sliced into small pieces of 1 cm². All the prepared samples were spreaded in a single layer on stainless steel trays and dehydrated at 55° C for 12 h in a thermostatically controlled hot air oven drier with air van (Memmert / UF). The dehydrated samples were ground electrically in an electrical grinder (FW- 400) to pass through 150-µm sieve according to Zeyada *et al.* (2008). The dehydrated flour belonging to each sample was packed in air-tight Kilner Jar and kept at 4°C.

Chemical reagents

Folin- Ciocalteu reagent, Gallic acid, 1,1-Diphenyl-2-picrylhydrazyl (DPPH), Butylated hydroxy toluene (BHT) food grade, HPLC grade methanol, acetonitrile, The derivatizing reagent (N-Methyl-N-trimethylsilyl- Trifluoroacetamide (MSTFA) and N-Trimethylsilylimidazole (TMSI), anhydrous Na₂SO₄, Sodium carbonate, Acetic acid, Chloroform, *p*-Anisidine and certified reference materials were purchased from Sigma Aldrich.

Sunflower oil

Refined, bleached and deodorized sunflower oil (RBD) used in the present study without any additives were obtained from Sila Company, Fayoum, Egypt.

Methods:

Preparation of the methanolic extracts:

The methanolic extracts containing antioxidant compounds were extracted according to the method described by Adegoke & Gopala Krishna (1998). Fine powder of each dried sample (100 g) was extracted using methanol (500 ml), with constant stirring for 24 hr at 25±2°C. The sample extracts were filtered with filter paper (Whatmann No. 1). The residue was susceptible to re-extract for maximizing the antioxidant extract. The filtrate was evaporated under vacuum in a rotary evaporator (BUCHI- R-215, Switzerland) at 45 °C to dryness, and then weighed to determine the extracted yield of each sample.

Determination of total phenolics content

The total phenolics content in the AIW methanolic extracts was determined according to Lim *et al.* (2006) with the Folin- Ciocalteu reagent. Concentrations of 10, 20, 30, 40 and 50 µg/ml of gallic acid were prepared and 0.5ml of each sample extract (1mg/ml) was presented into test tubes and mixed gently with 2.5ml of 10% Folin- Ciocalteu reagent and 2ml of sodium carbonate (7.5%), then allowed to stand at room temperature for 30 min. The absorbance reading was measured spectrometric ally (Jenway VIS/UV-6305, England) at 760 nm. The total phenolics content was expressed as gallic acid equivalent (GAE) in mg per g sample. For the gallic acid, the curve was established by plotting concentration (µg/ml) versus absorbance (nm) $y = 0.0066x + 0.039$, $R^2 = 0.9991$, where y is the absorbance and x is the concentration.

Determination of antioxidant activity:

Antioxidant activity of each extract was assayed by DPPH (1,1-Diphenyl-2-picrylhydrazyl) scavenging capacity method described by Brand-Williams *et al.* (1995). The methanolic extract was lyophilized and diluted from 200µg/ml to 10 µg/ml. One ml (0.135 mM) of DPPH solution was mixed with 1.0 ml of the extract (in methanol). The mixture was mixed thoroughly using a vortex and incubated at 25±2°C in the dark for 30 min. The absorbance at 515 nm was measured by the spectrophotometer (Shimadzu UV-1650PC). The half maximal inhibitory concentration (IC₅₀) values represented the concentration of sample requisite to scavenge 50% of DPPH free radicals is calculated. The capacity for neutralizing DPPH radicals was detected by the following equation:

(%) = $100 - [(As - Ab) \times 100 / Ac]$ while As is the absorbance in the of the sample in the DPPH reagent, Ac is the absorbance value of the control (containing only DPPH) and Ab is the absorbance value of the sample solution without DPPH.

Ultra-high pressure liquid chromatography (UPLC MS/MS) analysis:-

Instrumentation

The liquid Chromatographic (LC) system is comprised from LC (Agilent 1200) instrument and triple quad MS detector (6460). The separation occurred in capillary column ($2.1 \times 100\text{mm} \times 1.8 \mu\text{m}$, Eclipse plus Agilent), G1216C at a flow of 0.4 ml/min, with a two mobile phase (eluent A = water; eluent B = Acetonitrile). The eluent gradient used for all extracts was as follows: 3 min, A: 40%, B: 60%; 5.5 min, A: 90%, B: 10%; 9 min A: 90%, B: 10%; 9.5 min, A: 15%, B: 85%; 10 min, A: 15%, B: 85%. Sample injection volume was 20 μl .

Mass spectrometry

Agilent Jet stream fragmentation technology (ESI-MS/MS) was used. The chromatographic conditions applied to identify phenolic compounds were selected according to the method described by Anubhuti & Priti (2013), the temp was 350°C; Gas flow, 10 L min⁻¹; Nebulizer, 50 psi; Sheath gas temp 400°C with flow rate 10 L/min. Negative ionization mode capillary voltage 3000 V and charging 1500 were used (Demiray *et al.*, 2009). Data was represented by triple quad Mass Hunter software (LC-MS).

Gas chromatography with mass spectrometry (GC-MS) analysis:-

Silylated DPR methanolic extract compounds were prepared according to the method demonstrated by Blau & Halked (1993). The mixture of MST-FA + 1% TMCS (200 μl) was added to 10 mg of sample extract and sonicated at 70°C for 30 minute.

Chromatographic analysis:

The extracts were analyzed by Agilent gas chromatograph (6890) coupled Agilent mass spectrometer (5973). The separation occurred in capillary column HP-5 MS. The injection temperature of extract was 280°C in splitless mode and 300°C for interface. The mass detector operated by both "scanning" and "SIM" mode. Chromatographic program conditions were: initial temperature of 80°C (1 min) heating to 250°C, at a rate of 20°C/min (1 min), heating to 300°C (5 min) at a rate

of 6°C/min. The integration was done using the Chemstation- GCMS software.

Oxidative stability of sunflower oil (SFO).

The methanolic extract of each of DPR, PPP and EPP at different concentrations (100, 200, 400, 600 and 800 ppm) were added separately to 50g SFO sample. At the same time, BHT as a synthetic antioxidant (200 ppm) was added to the SFO sample as the control. Sunflower oil and different additives were placed in 100 ml beakers and thoroughly mixed with ultrasonic waves (Zeyada *et al.*, 2008). Beakers were transferred to a drying oven adjusted at $60 \pm 2^\circ \text{C}$ for up to 18 days. Samples were withdrawn at 0, 3, 6, 9, 12, 15 and 18 days for determining the following parameters.

Peroxide value (PV) was determined iodometrically and the results were expressed in meq O₂ / kg oil according to the AOCS procedure (1989).

p-Anisidine value (p-AV) which is a measure of aldehyde content in oils was determined according to the IUPAC (1987) standard method.

Conjugated dienes (CD) and conjugated trienes (CT) hydroperoxides were measured according to the method reported by Vieira & Regitano-Darce (1999). The absorbance readings were recorded in UV region at 232 for measuring CD and 268 nm for CT.

Statistical analysis: Data were statistically analyzed and differences among means were implemented by Least Significant Differences method at ($\alpha = 0.05$) probability level (L.S. D 0.05) according to Peterson (1985).

RESULTS AND DISCUSSION

Phenolics content (TPC) and antioxidant activity:

The results in Table (1) show the TPC and antioxidant activity of AIW methanolic extracts. The TPC of DPR was 81.7 ± 5.7 mg GAE/g. It was significantly ($P \leq 0.05$) higher than that of PPP (45.1 ± 3.6 mg GAE /g) and EPP (23.4 ± 2 mg GAE/g), respectively. This value was significantly higher than that found in several medicinal plants like *G. multifolial* (12.36 mg GAE/g DW), *G. villosa* (20.81 mg GAE/g DW) and *M. edule* (70.07 mg GAE/g DW) (Laouini *et al.*, 2012). Table (1) also shows that DPR revealed the highest scavenging activity with IC₅₀ value of 21.6 $\mu\text{g}/\text{ml}$ comparing with other natural phenolic extracts and BHT as the control

Table1: Total phenolics content (TPC) and calculated IC₅₀ (µg/ml) values of some agro-industrial waste methanolic extracts

Agro-industrial waste	Total phenolic content mg GAE/ g dry extract*	Calculated IC ₅₀ (µg/ml) values
DPR	81.7 ± 5.7	21.6
PPP	45.1 ± 3.6	55.3
EPP	23.4 ± 2	94.5
BHT	-	27.01

*Mean of three determinations ± S.D.

EPP: Empty pea pods

GAE: Gallic acid equivalent.

DPR: Date palm racemes

BHT; Butylated hydroxytoluene. PPP: Prickly Pear Peels.

whereas EPP revealed the lowest scavenging activity with IC₅₀ value of 94.5µg/ml. This indicates that the DPR can be considered as a promising antioxidant source, and has the proficiency to substitute synthetic antioxidants with those that are naturally originated compounds. However calculated IC₅₀ value of PPP was 55.3µg/ml. El- Houfi(2015) found that the DPPH inhibition % of the methanolic extract of PPP was 60.81 %. Thus, PPP as a by-product can indeed be used as a good and cheap source of beneficial antioxidant. The results in Table (1) also shows that the IC₅₀ of the methanolic extract

of EPP was 94.5µg/ml. The DPPH free radical scavenging activity (DPPH inhibition %) of the methanolic extract of EPP was 31.53% (EL-Houfi, 2015).

Identification of phenolic compounds using UPLC/ MS /MS.

In the present study, separation and identification of phenolic compounds in the different extracts using UPLC/MS/MS were studied. Multiple Reaction Monitoring technique (MRM) was used as a mode of data acquiring to tentatively identify 10 major phenolic compounds (rutin, myrecetin, quercetin, luteolin, catechien and chlorogenic, ellagic, caffeic, ascorbic, *p* – coumaric acid) in the tested samples according to the chromatographic conditions described in Table (2). These compounds are known for their broad spectrum of activities as antioxidants.

The data in Table (2) show that 4 phenolic compounds were presented in the DPR. Rutin, quercetin, ellagic acid and luteolin were tentatively identified at MS pre ions *m/z* signal 609, 301, 301 and 285 and MS/MS product ion fragment *m/z* signal 109, 179, 229 and 133, respectively.

The present data are considered as the first attempt to identify phenolic compounds in PPP by using UPLC/MS/MS. All previous studies have been focused on the fruit pulp (edible portion), while little is known about the peels and seeds because they

Table2: Tentative identification of the phenolic compounds in the methanolic extracts using UPLC/ MS /MS.

Compounds	Family	Agro-industrial wastes *			***Chromatographic conditions			
		area **			Pre Ion	Pro Ion	Frag (V)	CE (V)
		1	2	3				
		DPR	PPP	EPP				
Rutin	Flavonols	3497	5106	-	609	109	145	35
Chlorogenic acid	Phenolic acids	-	-	-	353.3	191.3	90	12
Myrecetin	Flavonols	-	-	1800	317	151	120	20
Quercetin	Flavonols	1390	154	-	301	179	125	12
Ellagic acid	Phenolic acids	4084	247	1600	301	229	120	20
Catechien	Flavanol		-	-	289	245	125	10
Luteolin	Flavones	69075	6593	1759	285	133	45	20
Caffeic acid	Phenolic acids	-	-	-	178	135	30	20
Ascorbic acid	Phenolic acids	-	274	1200	175	115	75	4
<i>p</i> -Coumaric acid	Phenolic acids	-	-	840	163	119	90	10

*1. Date Palm racemes, 2. Prickly pear peels, 3. Empty pea pods

**The area under the peak is proportional to the amount of compound which has passed the detector, and this area was calculated automatically by the computer linked to the display.

*** Frag: Fragmentation energy. CE: Collision energy; Pre Ion: Precursor Ion; Pro Ion: product Ion.

discarded as food wastes. Table (2) shows that 5 phenolic constituents were tentatively identified in the PPP methanolic extract (Rutin, quercetin, ellagic acid, luteolin, and ascorbic acid) EL Houfi (2015) identified and quantified 11 peaks in the methanolic extract from prickly pear peels by HPLC. He revealed that caffeic and chlorogenic acid were the dominant hydroxycinnamic acids, whereas salicylic followed by gallic acids were dominant hydroxybenzoic acids. Catechin, ellagic acid and homogentistic acid were also determined. The content of phenolic compounds in EPP are shown in Table (2). Myrecetin, ellagic acid, luteolin, ascorbic acid and *p*-coumaric acid were tentatively identified by MS information for pre ions at *m/z* signal 317, 301, 285, 175 and 163, respectively, and followed by MS/MS spectrum of product ions at *m/z* signal 151, 229, 133, 115 and 119. Hydroxycinnamic acid derivatives, caffeic and ferulic acids were the dominant, whereas gallic and vanillic acids were the dominant hydroxybenzoic acid derivatives. In addition, homogentistic acid was the abundant phenolic in the methanolic extract of pea pod (EL Houfi, 2015).

Phenolic composition of DPR by GC-MS.

The DPR extract showed the highest total phenolics content (81.7 ± 5.7 mg GAE/g) and the highest scavenging activity with the smallest IC_{50} value (21.6 μ g/mL). Many studies showed that there is a correlation between TPC and antioxidant activity (Babbar *et al.*, 2011). On the other hand, AL-Harithi *et al.* (2015) stated that the relation between antioxidant activity and TPC was figured out. This evidently reveals that other non-phenolic components existing are showing antioxidant activity. The methanolic DPR extract was subjected to GC-MS analysis for more chemical composition that might be responsible for the scavenging activity of this extract.

The GC-MS report tentatively identified 23 compounds in the methanolic DPR extract. Table (3) shows the chemical composition of the methanolic DPR extract that possess antioxidant activity. D-(-)-lactic acid, L-malic acid were among the major components in this extract. There are several studies had proven their antioxidant activities (Ana *et al.*, 2014 & Florentina *et al.*, 2017).

Different amino acids were also identified in the methanolic DPR extract using GC-MS. L- alanine, L-threonine, serine, L-aspartic acid and L- proline were among these amino acids. L-alanine motivates countenance of the antioxidant defense proteins such

as ferritin in endothelial cells and heme oxygenase-1 (HO-1). Increased ferritin expression and hemeoxygenase activity enhance endothelial dysfunction recommending an antiatherogenic effect of L-alanine (Grosser *et al.*, 2004). L-threonine can improve the expression of heat shock protein and counteract apoptosis and cellular injury (Baird *et al.*, 2013).

Other organic acids identified were gallic, succinic, erythronic and phthalic acids. Gallic acid, a trihydroxybenzoic acid (3,4,5-trihydroxybenzoic acid) and its derivatives are strong natural antioxidants existing in many vegetables and fruits (Farahmandfar *et al.*, 2014). α -tocopherol has been proposed to be the most effective lipid-soluble natural radical scavenging antioxidant (Packer, 2001).

Five fatty acids were identified at the DPR methanolic extract, two essential fatty acids, linoleic and α -linolenic acid. The essential fatty acids (ω 3 and ω 6) showed significant antioxidant activity and metal chelating characterization as well as significant ($P < 0.05$) reducing power potentials (Sarikurku *et al.*, 2016).

Pentadecanoic acid (C 15:0) is a saturated fatty acid uncommon in nature being found at the level of 1.2% in the dairy product from cows. Margarine acid (heptadecanoic acid C17:0), is a saturated fatty acid occurs as a rare component of the natural animal or vegetable fat (Beare-Rogers *et al.*, 2001). Stearic acid (octadecanoic acid C18:0) is mostly prevalence saturated fatty acids existent in nature. Arachidic acid (eicosanoic acid C20:0) is another saturated fatty acid detected in DPR extract. Because of its surfactant-like properties, arachidic acid is used in the manufacture of pharmaceuticals, soaps, cosmetics, and food packaging. Caprylic acid, capric acid, n-pentadecanoic acid, heptadecanoic, stearic acid, arachidic acid, linoleic acid, α -linolenic acid, α -tocopherol (vitamin E), campesterol and stigmasterol are among other compounds have been tentatively identified in the DPR extract analyzed by GC-MS. Caprylic acid, (C8:0) and Capric acid (C10:0) are antioxidants used in health care facilities (Avery *et al.*, 2015).

Campesterol and stigmasterol are phytosterol that parallel cholesterol in structure, while are present exclusively in plants. These compounds are recognized for potential effects on blood cholesterol levels; however, investigate into their prospective role in antioxidant effect, has established comparably little attention. It has been proven that

Table 3: Composition of the methanolic DPR extract by GC-MS

Compounds	RT(min)	Corr. % max	Ion (m/z, abundance between parenthesis)
D-(-)-Lactic acid	4.76	6.23	147 (100), 191 (89), 190 (70), 174 (38)
l-Alanine	5.1	1.35	73 (100), 147 (60), 119(20)
Caprylic acid	5.55	0.16	73(100),75(70),187(28),201(25)
Gallic acid	6.216	0.67	105 (100),135 (62), 217(32)
Succinic acid	6.623	1.3	147 (100), 73 (92) 44 (32), 55 (28);
L-threonine	7.175	1.28	73(100), 117 (28), 147 (73), 219 (22)
Serine	6.980	1.94	204 (100), 218 (60), 147 (32)
Decanoic acid	7.6	0.97	117(100),129(65), 132(38)
DL-Malic acid	7.821	14.84	147 (100), 233 (46.5), 245 (22)
L-Aspartic acid	8.033	2.48	232 (100), 100 (28.8), 147 (20), 218 (15.8)
L- proline	8.11	6.71	156 (100), 157 (70), 230 (50.0), 258 (42.3)
Erythronic acid	8.3	0.44	147(100),205(29),292(36)
n-pentadecanoic acid	10.463	3.33	73(100),117(70), 229 (60)
Dibutyl phthalate	10.677	1.68	149(100),150(84),205(30),223(36)
Linoleic Acid	11.466	5.53	67(100),81(87),109(37),150(26)
Octadecanoic acid (stearic acid)	11.636	5.61	74(100),87(80),143(30),298(15)
alpha.-Linolenic acid	12.375	25.39	75(100), 79(90),108(70),117(63)
Heptadecanoic acid	11.746	5.4	73(100), 117(88),145(34),327(40)
Arachidic acid	13.173	0.92	73(100), 75(88),117(73),106(51)
α.-Tocopherol (vitamin E),	21.592	4.4	237 (100), 502 (93), 238 (27)
Campesterol	23.512	45.23	129(100), 343(88),145(34),282(20)
Stigmasterol	24.064	22.97	83(100),129(80),255(35),282(20),394(25)

campesterol and stigmasterol act as physical stabilizers, antioxidant agents and radical scavengers (Yoshida & Niki, 2003).

Stability of Sunflower oil

The antioxidant efficiencies of the DPR, PPP and EPP methanolic extracts in sunflower oil (SFO) were evaluated by determination peroxide value (PV), *p*-anisidine value (*p*-AV), conjugated dienes (CD) and conjugated trienes (CT) as indices of lipid oxidation.

Peroxide value (PV)

Peroxide value (PV) is one of the most common tests for measuring the concentration of peroxides and hydro-peroxides formed in the primary stage of lipid oxidation. A continuous rising in PV with increasing the storage period was observed in all the tested samples (Fig. 1). Initially, the amount of peroxides formed was modest, while it elevated after 9 days of storing then further increment with expansion storing time. Peroxide value range was

(1.45 ± 0.026 to 15.2 ± 0.06 meq O₂ / kg), (1.7 ± 0.09 to 20.5 ± 0.13 meq O₂ / kg) and (1.8 ± 0.02 to 23.5 ± 0.26 meq O₂ / kg) for the methanolic extract of DPR, PPP and EPP, respectively. Control oil sample with no additives showed the highest PV of 50 ± 0.2 meq O₂/kg after 18 day. The difference in PV among the control and oil samples containing the natural methanolic extracts or BHT (synthetic antioxidant) was significant. The results obtained in the present study are in harmony with the results of Mohdaly *et al.*, (2010) who stated that the AIW methanolic extracts can be recommended as a potent source of antioxidants for the stabilization of unsaturated oils. The PV of SFO containing 200 ppm of the methanolic extracts of DPR, PPP and EPP as well as the BHT after 18 days of storing was found to be 10.3 ± 0.11 , 16.3 ± 0.1 , 17.7 ± 0.26 and 11.3 ± 0.07 meq O₂ / kg, respectively. The DPR methanolic extract at a concentration of 200 ppm exhibited the superiority stabilization efficiency, even comparable with those of synthetic antioxidants. The data obtained from the present study are in agreement with those obtained

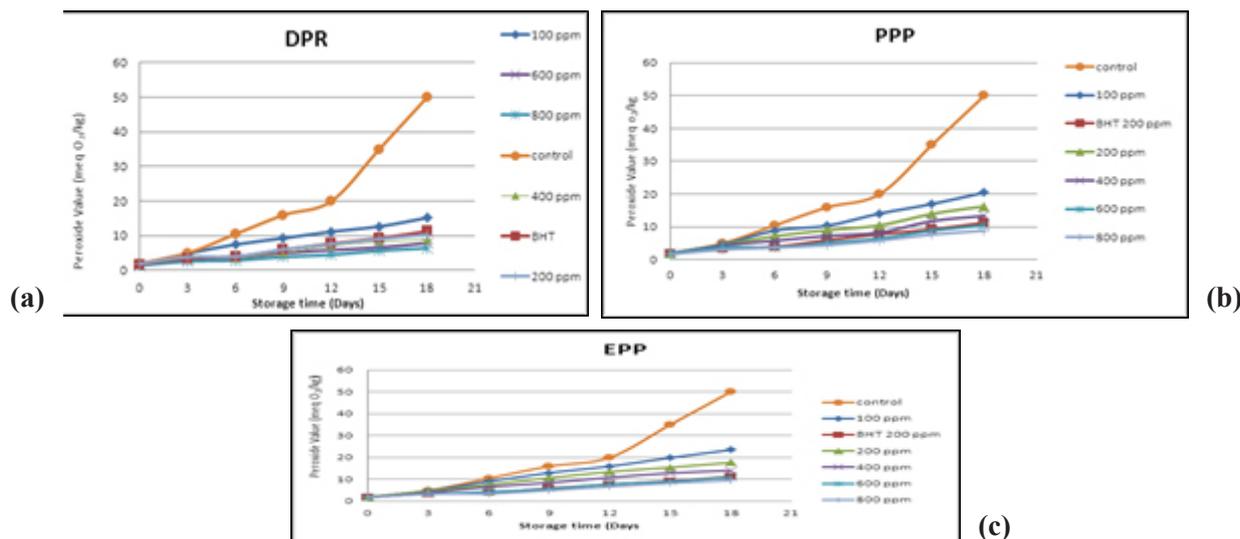


Fig. 1: Peroxide value (PV) of treated SFO samples: (a) DPR, (b) PPP and (c) EPP methanolic extracts under accelerated storage conditions ($60 \pm 2^\circ \text{C}$).

by Ozcan & AL Juhaimi (2015). They concluded that date by-product methanolic extract can be used as an oxidation inhibitor agent in different oil products

The *p*-anisidine value (*p*-AV):-

The P-AV is a measure for quantification oxidation products produced in the advanced stages of edible oil oxidation (De Abreu *et al.*, 2010). Fig. (2) shows the significant ($P < 0.05$) changes recorded for *p*-AV under accelerated conditions as affected by adding methanolic extract of DPR, PPP and EPP compared with BHT used as synthetic antioxidant. Addition of the different concentrations of the methanolic extracts and BHT lead to decrease in *p*-AV

comparing with the control sample. The highest concentration (800 ppm) of the methanolic extracts affords the highest resistance to form carbonyl products such as aldehydes and ketones. Under accelerated condition, BHT in SFO samples reduced *p*-AV by approximately 42.5% comparative to the control, whereas *p*-AV was decreased by adding of the methanolic extracts depending on the concentration used. From these results, it can be concluded that the amount of secondary lipid oxidation compounds was significantly decreased by increasing the concentration of the methanolic extract in SFO. Bioactive compounds present in DPR extract appeared the highest restrictive effect on the carbonyl products

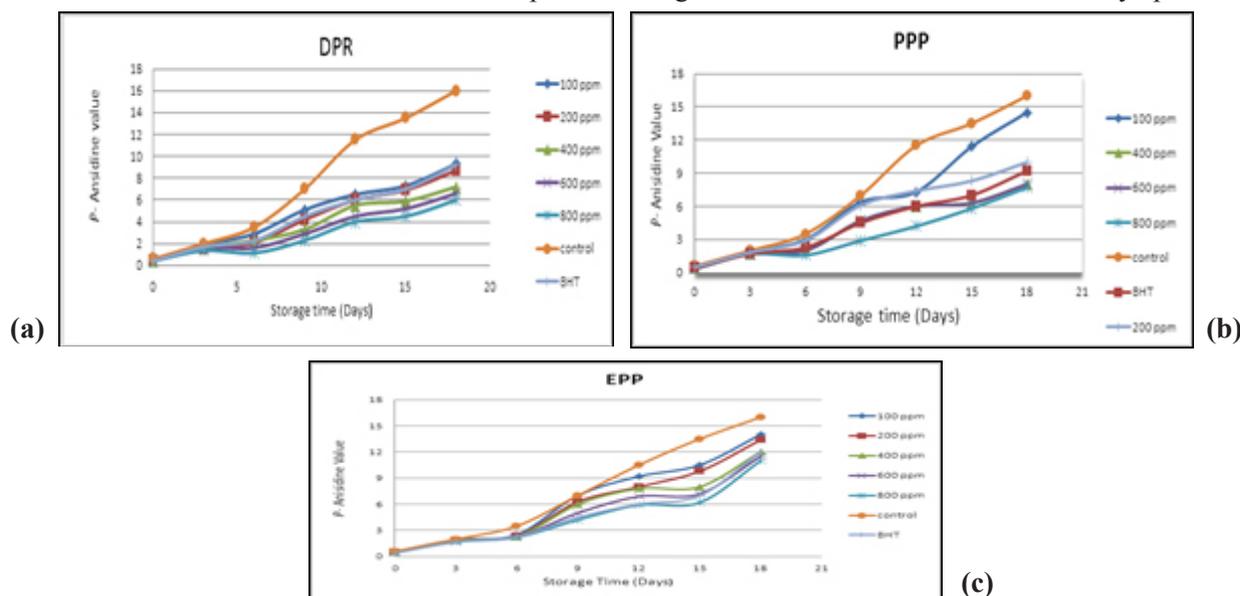


Fig. 2: *p*- Anisidine value (*p*-AV) of treated sunflower oil samples: (a) DPR, (b) PPP and (c) EPP methanolic extract under accelerated storage conditions ($60 \pm 2^\circ \text{C}$).

formation and exhibits the lowest value of p-AV (2.81) at 800 ppm followed by PPP (3.45). The results revealed that the higher phenolics content of DPR has the higher inhibition effect on producing secondary oxidation products (Fig. 2) and has great proficiency to prevent sunflower oil oxidation deterioration at concentrations 400ppm, 600 and 800 ppm used in the present study compared to the syn-

thetic antioxidants BHT at 200 ppm. Therefore, some agro-industrial waste can be recommended as a viable source of antioxidants for the stabilization of food types, especially unsaturated edible oils such as sunflower oil (El-Shourbagy *et al.*, 2013).

Conjugated dienes (CD) and trienes (CT):-

The results presented in Figs. (3 & 4) indicate

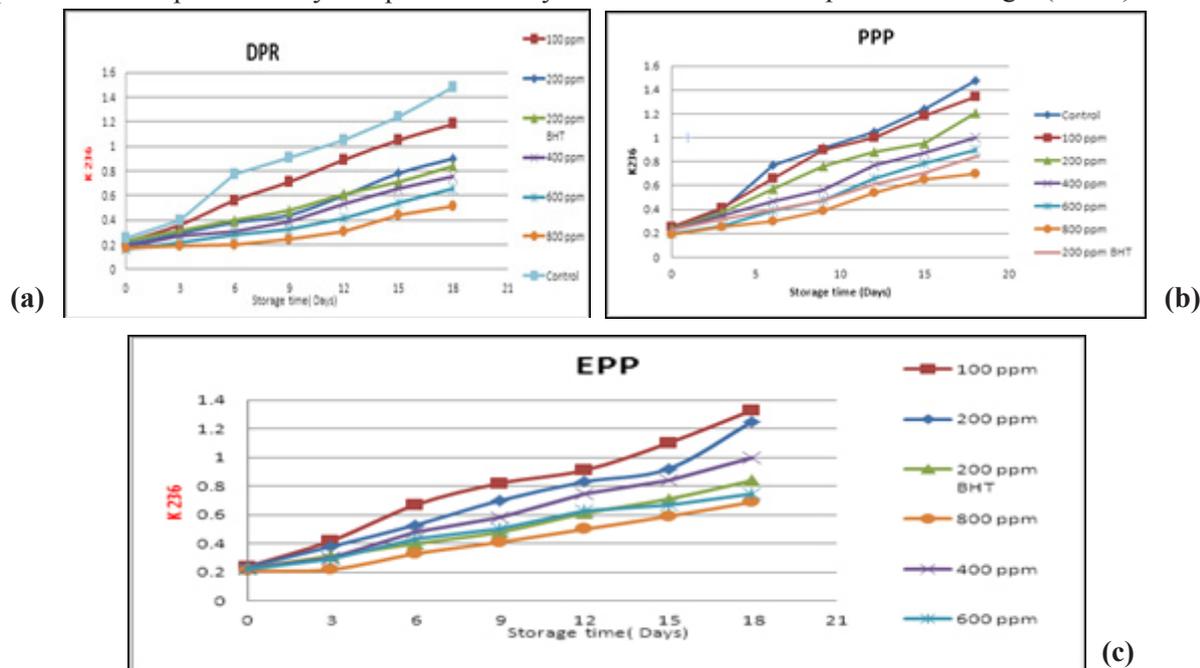


Fig. 3: Conjugated Diene(CD)of treated sunflower oil samples: (a) DPR, (b) PPP and (c) EPP methanolic extracts under accelerated storage conditions ($60 \pm 2^\circ \text{C}$).

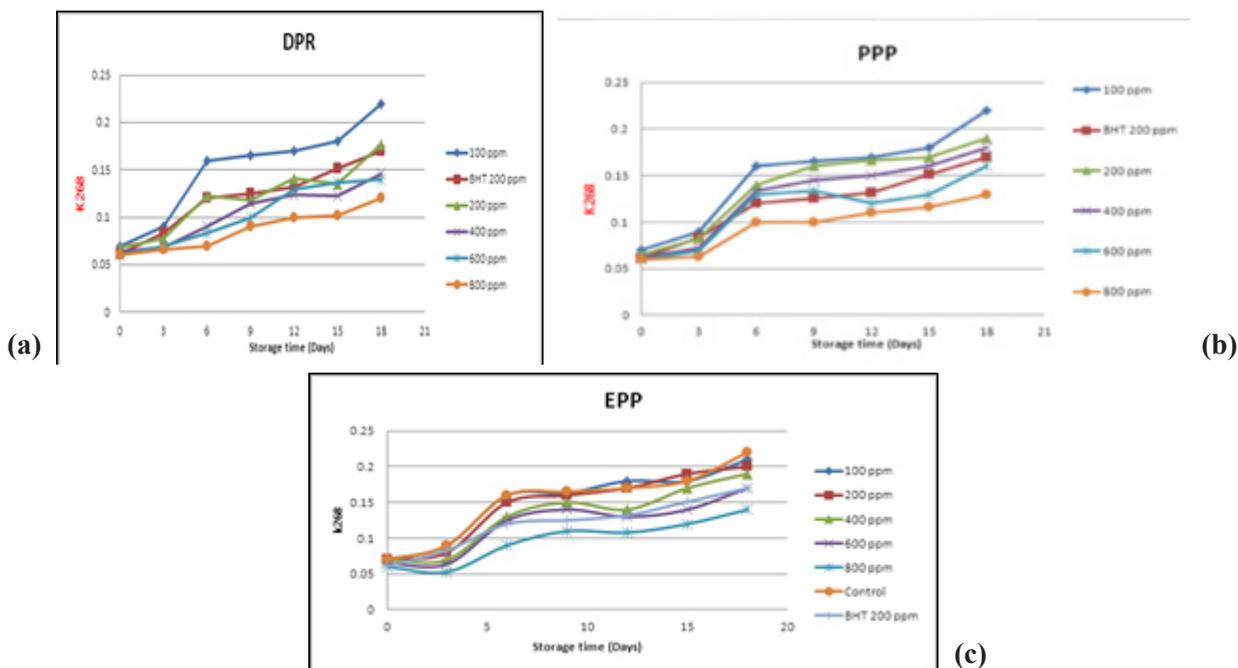


Fig. 4: Conjugated Triene (CT)of treated sunflower oil samples: (a) DPR, (b) PS, (c) EPP methanolic extract under accelerated storage conditions ($60 \pm 2^\circ \text{C}$).

that as rearrange of the double bonds sites in SFO and, therefore, non-conjugated system was transformed to conjugated diene and triene system under accelerated storage conditions. These indicate the presence of oxidation by-products for instance carbonyl compounds (Ramadan & Moersel 2003). A significant decrease was perceived in the values of CD and CT in SFO samples containing different concentrations of the methanolic extracts of DPR, PPP and EPP with a dose response manner and synthetic antioxidant BHT. DPR at the concentration of 200 ppm exhibited similar efficiency in decreasing the amount of carbonyl compounds comparing with BHT at the same concentration. The concentration of 800 ppm DPR revealed the superiority efficacy in decreasing CD and CT. The data obtained here are harmonious with those reported by El Anany (2007). The natural phenolics extracted from AIW induced antioxidant activity and at the concentration of 800 ppm shown superiority to BHT in inhibiting the oxidation deterioration of sunflower oil (Ali, 2010). The data of the present study are in agreement with the results of Kiralan *et al.* (2015) and Reza *et al.* (2018).

As a conclusion, agro-industrial wastes (AIW) such as date palm racemes, prickly pear peels and empty pea pods contained several phenolic compounds in their methanolic extracts. These extracts showed antioxidant activity in a concentration – dependent manner. In addition, these AIW can be recommended as a potent source of antioxidants for stabilization unsaturated vegetable oils such as sunflower oil.

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تركيب المواد الفينولية والنشاط المضاد للأكسدة لبعض مخلفات التصنيع الزراعي

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هدفت هذه الدراسة إلى تقدير المحتوى الكلي للمواد الفينولية و تقدير النشاط المضاد للأكسدة و التعرف على المركبات الفينولية لثلاثة من المخلفات الزراعية (شماريخ البلح ، قشور التين الشوكى ، قرون البسلة الفارغة) ودراسة نشاط المستخلصات الميثانولية لهذه المخلفات على منع التدهور التأكسدى لزيت دوار الشمس و لقد أوضحت الدراسة أن أكثر المركبات الفينولية تواجداً هي الريبوتين فى التين الشوكى ، الميرسيتين فى قرون البسلة الفارغة ، الكيورستين فى شماريخ البلح وأوضحت الدراسة أن شماريخ البلح أعطت أعلى نشاط كاسح وبقية IC_{50} (21.6 μ g/ml) لاحتوائه على مركبات فينولية وغير فينولية لها نشاط مضاد للأكسدة.

فى حين أن قرون البسلة الفارغة أعطت أقل نشاط كاسح وبقية IC_{50} (94.5 μ g/ml). كما أظهرت النتائج أن هناك اختلافاً جوهرياً فى قيمة أكسدة الدهون مابين العينة الكنترول وعينات أعطت أقل نشاط كاسح وبقية IC_{50} (21.6 μ g/ml). زيت دوار الشمس المحتوية على المستخلصات الميثانولية مما يوصى باستخدام هذه المستخلصات لإطالة فترة صلاحية الزيت بدلاً من استخدام مضادات الأكسدة المخلفة صناعياً.