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Antioxidant Activity of Purslane (*Portulaca oleracea L.*) Seed Hydro-alcoholic Extract on the Stability of Soybean Oil

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ABSTRACT

In this study, the antioxidant activity and protective effects of Purslane Seed Water-Methanolic Extract (PSWME) in stabilizing SoyBean Oil (SBO) were tested. DPPH radical scavenging activity of PSWME at different concentrations varied significantly ($P< 0.05$), ranging from 15.41% to 79.06%. Total phenolic content in PSWME was 121.09 mg Gallic Acid Equivalent (GAE) kg^{-1} dw. The protective effects of PSWME at the level of 100 mg L^{-1} in stabilizing SBO were tested and compared to BHT in a concentration of 100 mg L^{-1} by measuring Peroxide Value (PV) and ThioBarbituric Acid (TBA) during accelerated storage (at 60°C). The results showed that PSWME, similar to BHT, reduced the formation of primary and secondary oxidation products in SBO. Therefore, the use of this extract as a natural antioxidant is recommended to prevent the oxidation of vegetable oils.

Keywords: DPPH, Oven test, Oxidation, Phenolic compounds**.**

INTRODUCTION

Lipid oxidation has been reported to be the cause of off-flavors, formation of toxic compounds, and many diseases due to reactive oxygen species (Shaker, 2006; Ahn *et al*., 2012). Adding antioxidants is a method of increasing shelf life, especially that of lipids and lipid-containing foods (Jayaprakasha *et al*., 2003). Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidizing chain reactions (Inan *et al*., 2012). Since the use of synthetic antioxidants such as BHA and BHT in foods is restricted due to their toxicological effects on various species and suspected carcinogenic potential, the search for natural and safe antioxidants, especially of plant origin, has greatly increased in recent years (Spigno and Faveri, 2007). Aromatic and medicinal plants are sources of natural antioxidants thanks to their main secondary metabolites such as polyphenols and essential oils. Phenolic compounds are biochemically synthesized via the shikimate pathway, which produces a group of phenolics called phenylpropanoids. They can act as antioxidants by donating hydrogen to highly reactive radicals, thereby preventing further radical formation (Wannes *et al*., 2010). Their antioxidant potential depends on the number and arrangement of the hydroxyl groups and the extent of structure conjugation (Salmanian *et al*., 2014). Common purslane (*Portulaca oleracea L.*) is a member of Portulacaceae, which is comprised of more than 120 species of succulent herbs and shrubs (Rinaldi *et al*., 2010). Purslane is found growing in the wild or cultivated in many parts of the world (Besong *et al*., 2011). This plant is native to Iran, and the history of its cultivation there goes back more than 2000

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years. It is an important vegetable crop in southern regions of the country and is known as Perpyn (Heidarzadeh *et al*., 2013). It also grows in other areas, such as Europe, Africa, the United States, China, India, and Australia (Oliveira *et al*., 2009). Recent research has demonstrated that purslane is a good source of several compounds that have a positive impact in human health. Those compounds include omega-3 fatty acids and *β*-carotene (Oliveira *et al*., 2009), kaempferol, quercetin, and apigenin (Lim and Quah, 2007), ascorbic acid (Erkan, 2012), *α*-tocopherol, and glutathione through which their ability to neutralize free radicals prevents associated disorders (Rinaldi *et al*., 2010). Purslane is listed by the World Health Organization as one of the most used medicinal plants and has been given the term 'Global Panacea' (Demirhan and Ozbek, 2010). This plant has a variety of pharmacological activities, including analgesic, anti-inflammatory, antifungal, wound healing, and hypoglycemic (Ahmadi Moghadam *et al*., 2014). The majority of recent studies on purslane have focused on identifying fatty acid (Oliveira *et al*., 2009; Teixeira *et al*., 2009; Simopoulos, 2004; Liu *et al*., 2000), phenolic compounds (Oliveira *et al*., 2009; Erkan, 2012), and antioxidant activity (Erkan, 2012; Lim and Quah, 2007; YouGuo *et al*., 2009), but no information is currently available on the application of purslane in the food industry. Therefore, this study aimed to determine the total phenols and the radical scavenging capacity of the Purslane Seed Water-Methanolic Extract (PSWME) and to evaluate the antioxidant effectiveness of PSWME as a source of natural antioxidant, for the prevention of SoyBean Oil (SBO) oxidation in comparison with the commercially available antioxidant BHT.

MATERIALS AND METHODS

Materials

Purslane seeds were obtained from the Fars province of Iran. Refined soybean oil without additives was obtained from Alya Santa Co. (Kordkoy, Iran). All chemicals and reagents used were of analytical reagent rade and were purchased from Merck (Germany) and Sigma Alhdrich Co. (USA).

Extraction

Ground purslane seeds were blended at a ratio of 1:4 (w/v) with methanol:water (80:20 v/v ratio) at room temperature for 24 hours in the dark. Then, the solvent was evaporated in a vacuum below 40°C on a rotary evaporator (Heidolph, 60 rpm Laborta 4000) until dryness. The extract was kept frozen (−18°C) until usage.

Determination of Antiradical Activity

Antiradical activity was determined following the method of Kukic *et al*. (2008). Briefly, a 4 mL solution of the extract at different concentrations (100-600 mg mL^{-1}) was mixed with 1 mL of methanolic solution of DPPH (500 µM). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured in a spectrophotometer (Shimadzu series UV1700) at 517 nm gainst methanol as the blank. Similarly, different concentrations of Butylated HydroxyToluene (BHT) (5-25 mg mL^{-1}) were used instead of an extract sample as the reference standard during the experiment. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The antioxidant activity (%) was determined by the following formula:

DPPH scavenging capacity (%)=

$$100\times\left(A_{blank}-\frac{A_{Sample}}{A_{blank}}\right) \quad (1)$$

where, A_{blank} is the absorbance of the control, and A_{sample} is the absorbance of the extract sample. IC_{50} values, which represented the concentration of the extract that caused 50% neutralization of DPPH radicals, were calculated by plotting

inhibition percentage against concentration. All determinations were done in triplicate.

Determination of Total Phenolic Content

Total phenolic contents of the extract were determined spectrophotometrically according to the Folin-Ciocalteu (FC) colorimetric method of Shahidi and Naczk (2004) using gallic acid as the standard phenolic compound. A 0.5 mL of extract was mixed with 2.5 mL of FC which was diluted 10-fold with distilled water. After standing at room temperature for 8 minutes, 2.5 mL of Na_2CO_3 (7.5%) was added. The mixture was kept at room temperature for 30 minutes, and then absorbance was measured at 765 nm. Total phenolic content expressed as Gallic Acid Equivalent (GAE) was calculated using the following linear equation based on the calibration curve:

$Y= 0.009x+0.035$ ($R^2= 0.993$) (2)

where, Y is the absorbance and X is the concentration. The results were expressed in milligrams of gallic acid per kilogram of extract (mg GAE kg^{-1} dw).

Oven Test

Oxidation rates were investigated through daily measurements of peroxide and thiobarbituric acid values of the samples. PSWME and BHT were added into the SBO at levels of 100 ppm, and the mixtures were stirred well and stored at 60^0C for 14 days in oven (Memmert UNB 500, Germany). SBO was also prepared as a control sample under the same conditions without any additions.

Peroxide Value (PV)

PV was measured according to the method of Shantha and Decker (1994). Briefly, the sample (0.01-0.30 g, depending on the extent of peroxidation) was mixed in a disposable glass tube with 9.8 mL chloroform-methanol (7:3, v/v), then, a 50 µL ammonium thiocyanate solution and 50 µL iron (II) solution were added and the sample was mixed on a vortex mixer for 2-4 seconds. After 5 minutes of incubation at room temperature, the absorbance of the sample was determined spectrophotometrically at 500 nm against a blank that contained all the reagents except the sample. The entire procedure was conducted in subdued light and completed within 10 minutes. The iron (III) chloride solution was used as the standard curve. The *PV*, expressed as milli equivalents of peroxide per kilogram (meq kg^{-1}) of sample, was calculated using the following formula:

$$\text{Peroxide value} = \frac{(As - Ab) \times m}{55.84 \times W \times 2} \quad (3)$$

Where, A_s= Absorbance of the sample; A_b= Absorbance of the blank; m= Slope obtained by standard curve with iron (III) chloride standard solution (in this experiment, m was 36.37); w= Mass in grams of the sample, and 55.84= Atomic weight of iron.

ThioBarbituric Acid (TBA)

Three grams of oil sample was dissolved in 10 mL chloroform, and 10 mL of solution 0.07% thiobarbituric acid (that in water was mixed with the same volume of acetic acid) was added to it. The mixture was then centrifuged at 1,000 rpm for 5 minutes. The aqueous portion was removed and placed in a boiling water bath for 30 minutes; then, absorbance was measured at 532 at room temperature (Mehran, 1976). TBA was expressed as mg of MalonDiAldehyde (MDA) kg^{-1} oil.

Statistical Analyses

All determinations were carried out in triplicate and data is reported as mean±standard deviation. Significant

differences (P< 0.05) were calculated using Duncan's multiple range test.

RESULTS AND DISCUSSION

DPPH Radical Scavenging Activity

DPPH is a very stable organic free radical with a deep violet color which gives absorption maxima within the 515–528 nm range. Upon receiving a proton from any hydrogen donor, mainly from phenolics, it loses its chromophore and becomes yellow (Sultana *et al.,* 2007). As the concentration of phenolic compounds or degree of hydroxylation of the phenolic compounds increases, their DPPH radical cavenging activity also increases and can be defined as antioxidant activity (Mohdaly *et al*., 2011).

The radical scavenging activity of BHT and PSWME at different concentrations are shown in Figure 1. As the concentration increased, a regular increase was observed in the scavenging activities of PSWME. The IC_{50} value, defined as the concentration of extract causing 50% inhibition of absorbance, was calculated. Since IC_{50} is a measure of inhibitory concentration, a lower IC_{50} value reflects greater antioxidant activity of the sample (Khomdram and Devi, 2010). The inhibition concentration IC_{50} values for PWME and BHT were 346.47 and 8.5 mg mL^{-1}, respectively. This result indicates BHT had a higher antioxidant activity at very low concentration compared with PSWME.

Total Phenolic Content (TPC)

The TPC of the water-metanolic extract of purslane seed was 121.09 mg kg^{-1} in this research. Oliveira *et al*. (2009) showed that total phenolic content of purslane stems and leaves was very different between samples, ranging from 78.3 to 633.9 mg kg^{-1} dry weight. Lim and Quah (2007) determined that the TPC of purslane cultivars ranged from 127 to 478 mg GAE 100 g^{-1} of fresh weight of the plant. Such different results could be attributed to different extraction procedures, time of harvest, and environmental conditions (Oliveira *et al*., 2009).

Analysis of Primary and Secondary Lipid Oxidation Products

PV

(PV) is a widely used measure of primary lipid oxidation indicating the amount of peroxides formed in fats and oils during oxidation (Baydar *et al*., 2007). The antioxidant activity of PSWME was compared with that of BHT and the control samples. The results given in Table 1 show that (PV) increased linearly with storage time and increased in acceleration after the 4th day. This increase in the peroxide value can be attributed to the formation of hydroperoxides, which are the initial products of oxidation. Initially, the difference in values of (PV) for the control

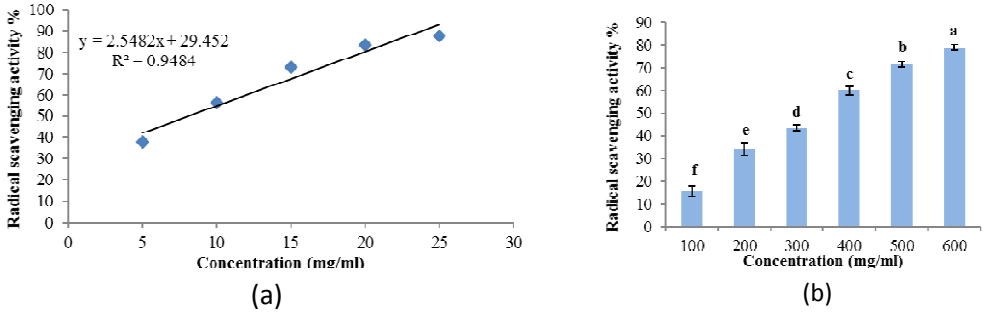

Figure 1. The radical scavenging activity of BHT (a) and PSWME (b) at different concentrations. Data are mean ±SD (n= 3). Values with different letters are significantly different (P< 0.05).

Table 1. Storage stability of SBO (control), SBO+PSWME and SBO+BHT as measured by peroxide value (meq kg^{-1}) during 14 days of incubation at 60°C.

Time (Day)	SBO [a]	SBO+PWME [b]	SBO+BHT [c]
0	1.57±0.08^{fA}	1.12±0.09^{hB}	1.55±0.23^{gA}
1	2.10±0.16^{efA}	1.72±0.18^{gB}	1.85±0.17^{gAB}
2	2.22±0.40^{eA}	1.77±0.02^{gB}	1.89±0.01^{gAB}
3	2.66±0.01^{deA}	1.66±0.02^{gC}	2.36±0.09^{efB}
4	2.65±0.02^{deA}	2.26±0.06^{fC}	2.6±0.09^{cdA}
5	3.05±0.01^{dA}	2.80±0.25^{dA}	3.14±0.48^{cA}
6	4.40±0.18^{cA}	2.69±0.01^{deC}	3.17±0.20^{cB}
7	4.28±0.08^{cA}	3.74±0.02^{bC}	3.95±0.30^{bB}
8	5.99±0.11^{aA}	5.92±0.11^{aA}	5.46±0.01^{aB}
9	6.12±0.11^{aA}	5.83±0.47^{aA}	5.90±0.20^{aA}
10	2.36±0.31^{eB}	1.89±0.01^{gC}	4.07±0.09^{bA}
11	2.70±0.14^{deA}	2.46±0.18^{efA}	3.12±0.31^{cdA}
12	5.56±0.85^{abA}	2.82±0.07^{dB}	2.84±0.06^{cdB}
13	5.76±0.04^{abA}	3.35±0.01^{cC}	4.00±0.005^{bB}
14	5.23±0.01^{bA}	3.96±0.09^{bB}	2.65±0.47^{cdC}

Means±SD: Similar capitalized and lowercase letters indicate no significant difference in each row and column, respectively (P< 0.05). [a] SoyBean oil, [b] Purslane seed water-methanolic extract. [c] Butylated hydroxytoluene

and the other samples was significant (P< 0.05); SBO+PSWME had a lower peroxide value. The peroxide content for all samples increased, but on the 9th day a sharp decrease was seen in all samples. This may be related to the observation of Iqbal and Bhanger (2007), who suggested that a decrease in (PV) after long heating times may be due to the volatilization of some breakdown products of lipid hydroperoxides formed in the primary stages of oxidation. The peroxide value of the control sample increased from 1.57 to 5.23 meq kg^{-1} during this time. On all days, the highest (PV) was observed for the control sample. Maximum (PV) contents for SBO+PSWME was 5.92 meq kg^{-1}, which is far less than those of linseed oil and grape seed oil stabilized with essential oils of mint (Inan *et al*., 2012), soybean oil stabilized by orange peel extract (Abd El-aal and Halaweish, 2010), and flaxseed oil stabilized by peanut skin extract (Taha *et al*., 2012).

TBA

Thiobarbituric acid reactive substances measure the formation of secondary oxidation products, mainly aldehydes (or carbonyls), which may contribute to the off-flavors of oxidized oils (Chang *et al*., 2002).

The TBA values of all SBO samples gradually increased with an increase in storage period (Table 2). The TBA values were in the range of 0.01-0.28 mg MDA kg^{-1} oil for the SBO treated with antioxidants after 14 days, while the maximum value of TBA for the control sample was 0.48 mg MDA kg^{-1} oil. At all stages, the highest TBA was observed in the control sample followed by SBO+PSWME and SBO+BHT, respectively. The TBA of SBO+PSWME was significantly lower (P< 0.05) than the control and comparable to SBO+BHT at all storage times initially, which predicted its high antioxidant potential. These results indicated that PSWME can effectively inhibit the oxidation of SBO. Soybean oil stabilized in the presence of mung bean hulls extract (100 ppm); but with the same concentration of BHA after 10 days of storage at 60 °C, it had lower TBA (Duh *et al*., 1997). Abd El-aal and Halaweish (2010) reported soybean oil stabilized by orange

Table 2. Storage stability of SBO, SBO+PSWME and SBO+BHT as measured by thiobarbituric acid (mg MDA kg^{-1} oil) during 14 days of incubation at 60°C.

Time (day)	SBO [a]	SBO+PWME [b]	SBO+BHT [c]
0	0.06±0.04^{dA}	0.01±0.01^{jB}	0.01±0.01^{bB}
1	0.27±0.11^{bcA}	0.05±0.01^{hB}	0.03±0.03^{bB}
2	0.38±0.07^{abA}	0.06±0.01^{ghB}	0.04±0.04^{bB}
3	0.18±0.05^{cdA}	0.02±0.01^{iB}	0.06±0.02^{bB}
4	0.46±0.05^{aA}	0.05±0.01^{hC}	0.20±0.06^{aB}
5	0.16±0.11^{cdA}	0.05±0.01^{hAB}	0.01±0.01^{bB}
6	0.25±0.11^{bcA}	0.08±0.01^{efB}	0.03±0.02^{bB}
7	0.37±0.09^{abA}	0.07±0.01^{fgB}	0.04±0.04^{bB}
8	0.20±0.07^{cdA}	0.08±0.01^{fgB}	0.07±0.03^{bB}
9	0.50±0.08^{aA}	0.07±0.01^{fgC}	0.22±0.03^{aB}
10	0.24±0.08^{bcA}	0.10±0.01^{dB}	0.01±0.01^{bC}
11	0.26±0.11^{bcA}	0.09±0.01^{edB}	0.03±0.02^{bB}
12	0.37±0.07^{abA}	0.20±0.01^{cB}	0.04±0.04^{bC}
13	0.19±0.06^{cdA}	0.25±0.01^{bA}	0.07±0.03^{bB}
14	0.48±0.06^{aA}	0.28±0.01^{aB}	0.21±0.05^{aB}

[a] Means±SD: Similar capitalized and lowercase letters indicate no significant difference in each row and column, respectively (P< 0.05).). [a] SoyBean Oil, [b] Purslane seed water-methanolic extract. [c] Butylated hydroxytoluene

peel extract (400 ppm) had a lower TBA compared with BHT (200 ppm).

CONCLUSION

The results of the present study indicate that despite significantly lower radical scavenging capacity of PSWME, the effects of purslane extract in stabilizing SBO are comparable to those of BHT. Purslane extract had a strong antioxidative effect during initial and final steps of oxidation in the dark in an oven at 60°C for 14 days. Therefore, this extract can be used as an easily accessible source of natural antioxidants. It can also be used safely in the edible oil industry to delay its oxidation, and it can be applied in other food industries as a natural antioxidant instead of synthetic antioxidants. Further studies should be done in the future to identify the phenolic compounds of purslane extract and to determine the correlation between their antioxidant effect and chemical compositions.

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بررسی فعالیت آنتی اکسیدانی عصاره الکلی-آبی دانه خرفه (*Portulaca oleracea L.*) در پایداری روغن سویا

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چکیده

در این بررسی، فعالیت آنتی اکسیدانی و اثر محافظتی عصاره متانولی-آبی دانه خرفه در پایداری روغن سویا مورد آزمون قرار گرفت. فعالیت مهارکنندگی رادیکال DPPH عصاره متانولی-آبی دانه خرفه در غلظت های مختلف به طور معنی داری متفاوت بود ($P< 0.05$) و در محدوده ۱۵/۴۱ درصد تا ۷۹/۰۶ درصد قرار داشت. محتوی کل ترکیبات فنولی در عصاره متانولی-آبی دانه خرفه ۱۲۱/۰۹ میلی گرم اسید گالیک در کیلوگرم وزن خشک بود. اثر محافظت کنندگی عصاره متانولی-آبی دانه خرفه در غلظت ۱۰۰ میلی‌گرم در لیتر در پایداری روغن سویا مورد آزمون قرار گرفت و با آنتی اکسیدان سنتزی BHT در غلظت ۱۰۰ میلی‌گرم در لیتر برای اندازه گیری عدد پراکسید و عدد اسید تیوباربیتوریک در شرایط تسریع یافته در دمای ۶۰ درجه سانتی گراد مورد مقایسه قرار گرفت. نتایج نشان داد که عصاره متانولی-آبی دانه خرفه همانند آنتی اکسیدان سنتزیBHT ، تشکیل فراورده های اولیه و ثانویه اکسایش را کاهش داد. بنابراین استفاده از این عصاره به عنوان آنتی اکسیدان طبیعی برای جلوگیری از اکسایش روغن های گیاهی توصیه می شود.