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Antioxidant activity of 100% and 80% methanol extracts from barley seeds (*Hordeum vulgare* L.): stabilization of sunflower oil

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RESUMEN

Actividad antioxidante de extractos de metanol al 80% y al 100% de semillas de cebada (*Hordeum vulgare* L.): estabilización del aceite de girasol.

El potencial antioxidante de extractos de metanol al 100% y el 80% de semillas de tres variedades de cebada (Jou 83, Jou 87 y Haider 93) fue evaluada. El rendimiento de los extractos de las semillas de cebada vario desde un 3.23% (Haider, 100% methanol) a un 5.31% (Jou 83, 80% metanol). El contenido total de fenoles, la actividad atrapadora del radical DPPH (valores IC_{50}) y la inhibición de la oxidación del ácido linoleico de los extractos de semilla de cebada (BSE) fueron 88.1-145.7 mg/100g, 90.8-168.6 µg/mL y 62.6-74.6%, respectivamente. La efectividad antioxidante de BSE fue también evaluada mediante su capacidad para estabilizar aceite de girasol con concentraciones de BSE de 600 ppm (respecto al peso del aceite). La muestras estabilizadas (tratadas con extractos) y el control (sin adición de extractos) SFO fueron tratadas bajo condiciones de almacenamiento acelerado (calentamiento en un horno a 60°C durante 30 días y ciclos de calentamiento de 8 h/día). Estas fueron analizadas a intervalos regulares para evaluar la extensión de los cambios oxidativos mediante la medida del valor de peróxidos, valor de *para*-anisidina y los contenidos de dienos conjugados y trienos congujados. Generalmente, los extractos de semilla de cebada al 80% demostraron una mejor acción antioxidante que los extracto al 100% de metanol. La actividad antioxidante de BSE varió también considerablemente entre las distintas variedades ensayadas. Los presentes resultados sugieren que los extractos antioxidantes de semillas de cebada podrían ser usadas para proteger aceites vegetales de la oxidación.

PALABRAS CLAVE: Aceite de girasol – Almacenamiento acelerado – Antioxidantes – Atrapadores de DPPH – Estabilización – Fenoles – Hordeum vulgare *L.– Productos de oxidación.*

SUMMARY

Antioxidant activity of 100% and 80% methanol extracts from barley seeds (*Hordeum vulgare* L.): stabilization of sunflower oil.

The antioxidant potential of 100% and 80% methanol extracts from the seeds of three barley varieties (Jou 83, Jou 87 and Haider 93) was assessed. The extract yields from barley seeds ranged from 3.23% (Haider 93,100% methanol) to 5.31% (Jou 83, 80% methanol). The total phenolic contents, DPPH radical scavenging activity (IC_{50} values) and inhibition of linoleic acid oxidation of barley seed extracts (BSE) were determined to be 88.1-145.7 mg/100g, 90.8-168.6 µg/mL and 62.6-74.6%, respectively. The antioxidant effectiveness of BSE was also assessed by stabilizing sunflower oil (SFO) with BSE at a concentration of 600 ppm (oil weight basis). The stabilized (treated with extract) and the control (without extract addition) SFO samples were subjected to accelerated (oven heating at 60°C for 30 days, 8 h heating cycle/day) storage. These were analyzed at regular intervals for the extent of oxidative changes according to the measurements of their contents of peroxide value, *para*-anisidine value, conjugated dienes and conjugated trienes. Generally, the 80% methanol extract of barely seeds demonstrated better antioxidant action than the 100% methanol extract. The antioxidant activity of BSE was also found to be considerably varied among the varieties tested. The present results suggest that antioxidant extracts from barely seeds might be used to protect vegetable oils from oxidation.

KEY-WORDS: *Accelerated storage – Antioxidants – DPPH scavenging –* Hordeum vulgare *L. – Oxidation products – Phenolics – Stabilization – Sunflower oil.*

1. INTRODUCTION

Nature has blessed aerobic organisms with an inner defense system that resists against oxidative damage due to reactive oxygen species (ROS). However, supplementing the natural defense mechanism with dietary antioxidants might offer better protection against the risk of certain cancers, inflammation and other degenerative diseases (Madhujit and Shahidi, 2008). The addition of synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) in lipid-containing foods is often discouraged due to their safety and perceived carcinogenicity. On the other hand, the use of plant-based antioxidant compounds in foods and preventive medicine are gaining a great deal of interest because of their potential health benefits (Ghiselli *et al.,* 2000; Hussain *et al.,* 2008).

It is well accepted that plants are the richest source of antioxidants. Among plants, cereals and legumes are prominent because they contain a

wide array of phenolics (Shahdi, 1997). Phenolic acids occurring in the grain of cereals, primarily, in bound form as conjugates with sugars, fatty acids, or proteins act as effective natural antioxidants (Shahdi, 1997; Wyen *et al.,* 2000). Plant phenolics have multiple biological functions such as antioxidant, anti-inflammatory, anti-cancer and anti-microbial activities (John and Grohmann, 2001). In view of the beneficial effects and vital role that natural antioxidants can play in human health, a greater demand currently exists for their isolation from more and more plants and agro-waste materials using some effective extracting techniques.

Barley (*Hordeum vulgare* L.), a member of the grass family, *Poaceae,* is one of the main cereal crops grown around the world (Madhujit *et al.,* 2006). Barley grains have been used for several food purposes as whole grains or in the form of value-added products. They contain a wide array of phytochemicals, primarily phenolic compounds including flavonols, phenolic acids, and procyanidins. Some studies on the antioxidant activity and phenolic contents of barley have been reported in the literature (Juntunen *et al.,* 2000; Karppinen *et al.,* 2003; Bonolie *et al.,* 2000). Madhujith *et al.* (2004) reported ferulic-, caffeic-, and vanillic- acids as the major phenolic components in barley seeds. In another study, Madhujith and Shahidi (2009) optimized the extraction process for the recovery of antioxidant components from six cultivars of barely using response surface methodology (RSM). They also evaluated the antioxidant properties of the extracted components using different antioxidant assays. The antioxidant attributes of barely as affected by alkaline hydrolysis and the release of bound phenolics have also been studied more recently (Madhujith and Shahidi, 2009).

The extract yields and the antioxidant activity of the extracts produced are significantly affected by the nature of the plant matrix and extracting solvent, owing to the presence of different antioxidant compounds of varying chemical nature (Sultana *et al.,* 2008). Polar solvents such as methanol and ethanol in their pure state or as aqueous mixtures are often recommended for the extraction of phenolic antioxidant components from a plant material (Peschel *et al.,* 2006; Arabshahi-Delouee and Urooj, 2007). However, in most cases, a single solvent is not useful to extract maximum amounts of antioxidant components from different types of plant materials (Li *et al.,* 2006).

The present research work was aimed appraising the efficacy of 100% and 80% methanol in the extraction of potent antioxidants from the seeds of three barley varieties (Jou 83, Jou 87 and Haider 93), commonly grown in Pakistan. The antioxidant effectiveness of the produced barley seed extracts was assessed using different in-vitro antioxidant assays and sunflower oil as oxidation substrate.

2. MATERIALS AND METHODS

2.1. Materials

The seeds of three local varieties (Jou 83, Jou 87 and Haider 93) of barley (*Hordeum vulgare* L.) grown under similar environmental (same place and same time) conditions were procured from Ayub Agricultural Research Institute, Faisalabad, Pakistan. The specimens of seeds were further identified and authenticated from the Department of Botany, University of Agriculture, Faisalabad, Pakistan. Refined, bleached and deodorized (RBD) sunflower oil (SFO), without additives was obtained from the deodorization section of United Industries Pvt. Ltd., Kashmir Road, Faisalabad, Pakistan. Folin-Ciocalteu's phenol regent, 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical, linoleic acid, butylated hydroxytoluene (BHT), β-carotene, Gallic acid and Tween 40 were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All the other chemicals/reagents and solvents used in this study were purchased from Merck (Darmstadt, Germany), unless stated otherwise.

2.2. Preparation of Antioxidant Extracts of Barely Seeds

Twenty grams of ground (80-mesh size) barley seeds were placed in a 500 mL flask and mixed separately with 200 mL of 100% methanol (pure methanol) and 80% methanol (methanol:water, 80:20 v/v). Extraction was performed in an electric shaker (Pamico Technologies, Faisalabad, Pakistan) for 48 hours in ambient conditions (shaking intensity 120 rpm). After extraction, the residues were separated from the extracts by filtering through a filter paper (Whatman No. 1). The residues were re-extracted twice with the fresh solvent and the extracts pooled. The extracts were then concentrated to dryness under reduced pressure at 45°C, using a rotary evaporator (EYELA, N-N Series, Rikakikai Co. Ltd. Tokyo, Japan). The crude extracts were weighed to calculate the yield and stored in a refrigerator (– 4°C), until used for further work (Anwar *et al.*, 2006; Sultana *et al.*, 2008).

2.3. Evaluation of Antioxidant Activity of Barley Seed Extracts

Estimation of total phenolics contents

Total phenolic contents (TPC) of barley seed extracts (BSE) were assessed spectrophotometrically using the Folin-Ciocalteu reagent method as reported by Chaovanalikit and Wrolstad (2004). Briefly, 50 mg of extract was mixed with 0.5 mL of Folin-Ciocalteu reagent and 7.5 mL deionized water. The mixture was left to stand at room temperature for 10 min, and then 1.5 mL of a 20% aqueous sodium carbonate (w/v) solution was added. After this, the mixture was heated in a water bath (40°C) for 20 min and

then cooled in an ice bath; finally absorbance was recorded at 755 nm using a spectrophotometer (U-2001, Hitachi Instruments Inc. Tokyo, Japan). The amounts of total phenolics were calculated from a calibration curve, constructed by running a series of standard solutions of gallic acid (R^2 = 0.9986). The results are expressed as gallic acid equivalents (GAE) milligrams per 100 grams of extract.

DPPH• scavenging assay

The DPPH (2, 2'– diphenyl–1–picrylhydrazyl) free radical scavenging activity of BSE was determined following the method of Bozin *et al.* (2006). The extracts (1.0 mL), containing 1 to 200 µg of dry matter per 1 mL of methanol) were mixed with 1 mL of 90 µM DPPH• solution. These were further diluted with 95% methyl alcohol, to a final volume of 4 mL. The resulting extract solutions and a blank were kept at room temperature for 1 h and then absorbance was measured at 515 nm using a spectrophotometer. The synthetic antioxidant, butylated hydroxytoluene (BHT) was used as a positive control. The percentage of free radical DPPH scavenging was calculated according to the following relationship:

(% DPPH• scavenging) = 100 X (A_{blank} – A_{sample} / A_{blank})

Where A_{blank} is the absorbance of the control reaction mixture excluding the test compounds, and A_{sample} is the absorbance of the test compounds. IC_{50} values, which correspond to the concentration of barley extracts that caused a 50% neutralization of DPPH•, were calculated from the plot of percent DPPH• scavenging versus concentration.

Determination of antioxidant activity in a linoleic acid system

The antioxidant activity of BSE was also determined by measuring percent inhibition of peroxidation in a linoleic acid system (Iqbal *et al.,* 2005). Extracts (5 mg) were added to a solution mixture 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 diluted to 25 mL volume with distilled water. The resulting solution was incubated at 40°C for 360 h (15 days). The degree of oxidation was measured by the peroxide values following the thiocyanate method. Briefly, 10 mL of ethanol (75% v/v), 0.2 mL of an aqueous solution of ammonium thiocynate (30%), 0.2 mL of extract solution and 0.2 mL of ferrous chloride ($FeCl_2$) solution (20 mM in 3.5% HCl v/v) were added in sequence. After 3 minute of stirring, the absorbance of resulting mixture solution and control was read at 500 nm using a spectrophotometer. The synthetic antioxidant, butylated hydroxytoluene (BHT) was used as a positive control. The percent inhibition of linoleic acid peroxidation was calculated using the following formula:

100 - [(Abs. increase of sample at 360 h/Abs. increase of control at 360 h) x 100]

2.4. Antioxidant Activity of BSE Using Sunflower Oil (SFO) as Oxidation Substrate

Stabilization of SFO

The crude BSE were separately added to preheated (50°C), refined, bleached and deodorized SFO at a concentration of 600 ppm (oil weight basis). Duplicate samples were prepared. The oil samples were stirred for 30 min at 50°C for uniform dispersion. In order to compare the antioxidant efficacy of BSE, an SFO sample was separately stabilized with a synthetic antioxidant (BHT) at a concentration of 200 ppm. Sunflower oil, without the addition of BSE, was used as a control. The stabilized and the control SFO samples (100 mL), packed in dark brown glass bottles with a narrow neck, were subjected to accelerated storage in an electric hot air-oven (IM-30, Irmeco, Germany), at 60°C for 30 days (8 h heating cycle per day).

During storage, the oil samples were taken out for analysis after every 10 days. The oxidative deterioration levels of the oils were assessed by the determinations of peroxide value (PV), conjugated dienes (CD), conjugated trienes (CT) contents and *para*-anisidine values (Sultana *et al.,* 2008).

Determination of Oxidation Parameters

Peroxide value (PV). The peroxide value (PV) of the stabilized and control SFO samples was measured following the AOCS official method Cd 8-53 (AOCS, 1997).

Conjugated dienes (CD) and conjugated trienes (CT). The secondary oxidation products CD & CT were determined as specific extinctions following the IUPAC method II. D. 23 (IUPAC, 1987). Briefly, the samples of oil were diluted with *iso*-octane. The absorbance values, recorded at 232 and 268 nm, using a spectrophotometer (U-2001, Hitachi Instruments Inc., Tokyo, Japan) were used to calculate the contents of CD and CT, respectively.

Para-anisidine value. *Para*-anisidine value was determined following the IUPAC method II. D. 26 (IUPAC, 1987). The sunflower oil samples, dissolved in *iso*-octane, were allowed to react with *para*-anisidine solution in acetic acid (0.25% w/v) for 10 min at room temperature. The absorbance of the resulting colored complex was measured at 350 nm, using a spectrophotometer.

2.5. Statistical Analysis

All determinations were made in triplicate and data is reported as mean ± SD. Data were analyzed by analysis of variance ANOVA using Minitab 2000 Version 13.2 statistical software (Minitab Inc., Pennysalvania, USA). A probability value at $p \leq 0.05$ was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1. Extract yields

The extract yields of antioxidant components from the tested varieties of barely seeds ranged from 3.23 to 4.10% and 5.02 to 5.31% with 100% methanol and 80% methanol, respectively (Table 1). The highest yield (5.31%) was obtained from var. Jou 83 with 80% methanol while the minimum (3.23%) was from Haider 93 using 100% methanol. Significant ($p < 0.05$) differences of extract yields among barely varieties and extracting solvents might be attributed to the varied polarity of 80% and 100% methanol as well as the availability of extractable components from seeds depending on varieties. In the present study, the highest yield of extract was established with 80% methanol revealing the greater efficacy of this solvent to extract maximum amounts of antioxidant components from barley seeds. The extract yields from barley seeds as determined in the present study were found to be in close agreement with that reported by Liu and Yao (2007).

3.2. Antioxidant activity of barely seed extracts (BSE)

Phenolic compounds are considered as a major group of chemicals that contribute to the antioxidant potential of cereals (Liu and Yao, 2007). The amounts of total phenolics determined in BSE are presented in Table 1. The TPC of extracts, ranging from 88.1 to 145.7 mg/100g gallic acid equivalents (GAE), varied significantly ($p < 0.05$) among barley varieties tested. The highest TPC were extracted with 80% methanol from Jou 83 (145.7 mg/100g) and the lowest with absolute methanol (100% methanol) from Haider 93 (88.1 mg/100g). Considerably, higher amounts of total phenolics were determined in 80% methanol extracts than those observed for absolute methanol extracts.

Aqueous methanol (80% methanol) is known to be an efficient and widely used solvent system to extract natural antioxidative components, especially the phenolics, from plant materials. This is due to the fact that the methanol-water mixture has high polarity and thus greater efficacy towards the extraction of polar phytochemicals such as phenolics and flavonoids (Siddiq *et al.,* 2005). The results of the present study were in agreement with the findings of Liu and Yao (2007), who also reported that higher amounts of phenolics from barely seeds were recovered using 70% ethanol and 70% methanol. The literature revealed that barely grains are a potential source of phenolics, the amounts even higher than wheat and rye (Zeilinski and Hozlowoka, 2000).

In the present study, we also investigated the free radical scavenging capacity of BSE. The DPPH radical scavenging activity of BSE increased in a concentration dependent manner (Table 1). The tested seed extracts exhibited good DPPH radical scavenging activity, IC_{50} values ranging from 90.7 to 168.6 µg/mL. Eighty percent methanol (80%) extract of Jou 83, having higher TPC (145.7 mg/100g) also showed greater DPPH radical scavenging capacity (IC_{50} value 90.7 µg/ mL), indicating a strong correlation between the results of TPC and DPPH scavenging assays. The DPPH radical scavenging capacity of BSE in the present analysis varied considerably with respect to the extracting solvent and varieties tested. The DPPH radical scavenging capacity of BSE was found to be in agreement with the studies of Zhao *et al*. (2008) and Choi *et al*. (2007).

The extracts from seeds of the tested varieties of barely (Jou 83, Jou 87 & Haider 93) exhibited inhibition of peroxidation ranging from 62.6-74.6% (Table 1). However, these levels were considerably lower than BHT (89.7% inhibition). Eighty percent methanol extract of Jou 83 offered the highest inhibition of peroxidation (74.6%), reflecting higher antioxidant activity among the extracts tested. The inhibition of peroxidation of 100% methanol extract of Jou 87 (62.6%) was found to be the lowest. The inhibition of peroxidation of BSE within the varieties tested followed the order: Jou 83 > Haider 93 > Jou 87.

The antioxidant activity of BSE as revealed by the extent of inhibition of linoleic acid peroxidation might

Table 1
Yield, TPC, DPPH radical scavenging activity and % inhibition of linoleic acid oxidation of extracts from seeds of different varieties of barley

Parameter	BHT	Jou 83		Jou 87		Haider 93	
		80% Methanol	100% Methanol	80% Methanol	100% Methanol	80% Methanol	100% Methanol
Extract yield (g/100g)	------	5.31 ± 0.35^{a}	4.01 ± 0.23^{b}	5.02 ± 0.41^{a}	4.10 ± 0.34^{b}	5.16 ± 0.40^{a}	3.23 ± 0.27^{c}
TPC (mg /100g of extract)	-------	145.7 ± 2.9^{a}	113.4 ± 3.4^{c}	131.1 ± 2.6^{b}	118.5 ± 3.5^{c}	98.0 ± 1.9^{d}	88.1 ± 1.7^{e}
DPPH radical scavenging activity (IC_{50} ug/mL)	19.2 ± 0.4^{d}	90.7 ± 2.7^{c}	144.1 ± 2.9^{b}	146.9 ± 5.1^{b}	168.6 ± 3.4^{a}	94.7 ± 2.8^{c}	166.9 ± 5.0^{a}
% Inhibition of linoleic acid oxidation	89.7 ± 1.8^{a}	74.6 ± 2.2^{b}	63.9 ± 1.3^{c}	71.7 ± 2.1^{b}	62.6 ± 1.2^{c}	72.5 ± 2.2^{b}	63.8 ± 1.3^{c}

Values are mean ± SD for triplicate determinations.
Means followed by different superscript letters in the same row present significant difference ($p \leq 0.05$)

be ascribed to the presence of considerable amounts of total phenolics. Over all, in the present analysis, a strong correlation was established between the contents of total phenolics and the levels of percent inhibition of linoleic acid peroxidation of BSE. The extracts showing greater amounts of total phenolics also exhibited higher levels of percent inhibition of linoleic acid peroxidation. These results are in line with the findings of Velioglu *et al*. (1998) who reported that extracts with higher TPC also showed strong activity against linoleic acid peroxidation.

3.3. Antioxidant potential of barley seed extracts (BSE) for stabilization of sunflower oil (SFO)

Peroxide value (PV)

Peroxide value is commonly used to assess the magnitude of primary oxidation products (mainly the peroxides) in oils (Shahidi and Wanasundara, 1997). The relative increases in the peroxide values (PV) of the stabilized and control SFO, stored under accelerated conditions, are presented in Table 2. The stabilized and control oils showed characteristic increases in PV. At the end of the storage period of 30 days, the control had the highest level of PV (relative to the initial value), indicating a higher extent of primary oxidation. The samples of sunflower oil stabilized with 80% methanol extract of Jou 83 showed the minimum increase in PV, while the samples treated with 100% methanol extract of Haider 93 depicted the maximum level.

A slow raise in PV of the stabilized SFO as compared with those of the control clearly indicated the antioxidant activity of the BSE. The order of antioxidant efficacy among tested barley varieties was found to be: Jou 83 > Jou 87 > Haider 93. Of the two extracting solvents used, 80% methanol offered the most effective barley antioxidant extracts towards protection of SFO.

Conjugated dienes and trienes (CD & CT)

The determination of CD and CT in terms of specific extinctions at 232 nm and 268 nm, respectively is considered to be useful for the assessment of the oxidative state of auto and thermo oxidized oils (Shahdi and Wanasundara, 1997). The conjugated dienes and trienes contents determined for the control and stabilized SFO are shown in Tables 3 and 4, respectively. As the

Table 2
Increase in peroxide value (meq/kg) of sunflower oil stabilized with extracts from seeds of different varieties of barley

Incubation period in days	Control	Jou 83		Jou 87		Haider 93	
		80% Methanol	100% Methanol	80% Methanol	100% Methanol	80% Methanol	100% Methanol
0	4.85 ± 0.29^{a}	4.85 ± 0.29^{a}	4.85 ± 0.29^{a}	4.85 ± 0.29^{a}	4.85 ± 0.29^{a}	4.85 ± 0.29^{a}	4.85 ± 0.29^{a}
10	19.68 ± 0.22^{a}	6.50 ± 0.36^{d}	6.68 ± 0.25^{d}	7.83 ± 0.17^{c}	9.61± 0.32^{b}	8.61 ± 0.40^{c}	9.47 ± 0.33^{b}
20	25.43 ± 0.19^{a}	9.43 ± 0.20^{c}	9.82 ± 0.51^{c}	9.92 ± 0.34^{c}	11.46 ± 0.32^{b}	9.56 ± 0.31^{c}	10.66 ± 0.32^{b}
30	28.67 ± 0.26^{a}	10.30 ± 0.41^{c}	11.46 ± 0.60^{bc}	10.86 ± 0.51^{c}	12.89± 0.71^{b}	10.45 ± 0.51^{c}	12.24 ± 0.70^{b}
Increase in PV from initial	23.82	4.45	6.61	6.01	8.04	5.60	7.39

Values are mean ± SD for triplicate determinations.
Means followed by different superscript letters in the same row present significant difference ($p \leq 0.05$)
PV peroxide value

Table 3
Increase in conjugated dienes ($\varepsilon_{1cm\ (\lambda\ 232\ nm)}$) of sunflower oil stabilized with extracts from seeds of different varieties of barley

Incubation period in days	Control	Jou 83		Jou 87		Haider 93	
		80% Methanol	100% Methanol	80% Methanol	100% Methanol	80% Methanol	100% Methanol
0	7.19 ± 0.20^{a}	7.19 ± 0.20^{a}	7.19 ± 0.20^{a}	7.19 ± 0.20^{a}	7.19 ± 0.20^{a}	7.19 ± 0.20^{a}	7.19 ± 0.20^{a}
10	11.57 ± 0.59^{a}	9.43 ± 0.18b^{c}	9.86 ± 0.50^{bc}	9.02 ± 0.32^{bc}	9.24 ± 0.33^{b}	8.38 ± 0.37^{c}	9.97 ± 0.83^{bc}
20	14.08 ± 0.69^{a}	11.65 ± 0.39^{c}	12.61 ± 0.37^{b}	11.23 ± 0.28^{bc}	11.64 ± 0.32^{b}	11.31 ± 0.63^{c}	12.01 ± 0.59^{b}
30	23.67 ± 0.98^{a}	13.64 ± 0.79^{bc}	14.78 ± 0.78^{bc}	13.75 ± 0.52^{bc}	14.87 ± 0.52^{b}	13.07 ± 0.63^{c}	14.67 ± 0.45^{bc}
Increase in CD from initial	16.48	6.45	7.59	6.56	7.68	5.88	7.48

Values are mean ± SD for triplicate determinations.
Means followed by different superscript letters in the same row present significant difference ($p \leq 0.05$)
CD conjugated dienes

storage period prolonged, the contents of CD and CT in oils increased, however these levels were considerably lower for stabilized samples. The control exhibited higher levels of these oxidation products predicting that it had undergone extensive oxidative deterioration.

Among the tested varieties of barley, Haider 93 seed extract, prepared with 80% methanol, showed the least increase in CD and CT reflecting their higher antioxidant activity for protection of SFO oil against oxidation. On the other hand, 80% and 100% methanol extracts from barely seeds Jou 87 exhibited the highest levels of CD and CT, showing the least antioxidant efficacy towards preventing the oxidation of SFO. Overall, the results of these two parameters indicated that 80% methanol extracts from barley seeds offered better protection to the sunflower oil than those of 100% methanol extracts.

Para-anisidine value

The data for *para*-anisidine value, which generally reflects the amount of aldehydic secondary oxidation products in oils (Mcginely, 1991), of stabilized and control SFO is given in Table 5. The control oil exhibited the highest increase in *para*-anisidine value indicating a higher rate of secondary oxidation. The smallest increase in *par*a-anisidine value of the oils was observed with 80% methanolic extract of Haider 93, while the maximum with 100% methanol extract of Jou 87. The assessment of antioxidant activity of plant extracts using the measurement of *para*-anisidine value of oils is generally recognized (Anwar *et al.,* 2006; Chatha *et al.,* 2006).

4. CONCLUSION

It could be concluded from the results of the present study that the antioxidant activity of barley seed extracts varied considerably depending on the nature of variety and the extracting solvents. The use of 80% methanol can be recommended for the extraction of effective antioxidant components from barley seeds. The extracts derived from barely seeds were found to be quite effective towards suppressing the primary and secondary oxidation products in the tested oil. This suggests that barley seeds might be explored as a viable source of potent antioxidants for the protection of vegetable oils from oxidation.

Table 4
Increase in conjugated trienes ($\varepsilon_{1cm\ (\lambda\ 268\ nm)}$) of sunflower oil stabilized with extracts from seeds of different varieties of barley

Incubation period in days	Control	Jou 83		Jou 87		Haider 93	
		80% Methanol	100% Methanol	80% Methanol	100% Methanol	80% Methanol	100% Methanol
0	6.10 ± 0.21^{a}	6.10 ± 0.21^{a}	6.10 ± 0.21^{a}	6.10 ± 0.21^{a}	6.10 ± 0.21^{a}	6.10 ± 0.21^{a}	6.10 ± 0.21^{a}
10	14.21 ± 0.61^{a}	7.31 ± 0.33^{b}	8.45 ± 0.42^{b}	6.78 ± 0.37^{b}	7.10 ± 0.19^{b}	6.83 ± 0.26^{c}	8.51 ± 0.33^{bc}
20	18.97 ± 0.70^{a}	8.94 ± 0.44^{bc}	10.12 ± 0.54^{bc}	8.68 ± 0.46^{bc}	9.56± 0.40^{b}	7.92 ± 0.62^{c}	10.18 ± 0.42^{bc}
30	21.64 ± 0.62^{a}	9.56 ± 0.57^{c}	11.38± 0.62^{b}	9.43 ± 0.56^{bc}	11.75 ± 0.61^{b}	9.03 ± 0.64^{c}	11.87 ± 0.60^{bc}
Increase in CT from initial	15.54	3.46	5.28	3.33	5.65	2.93	5.77

Values are mean ± SD for triplicate determinations.
Means followed by different superscript letters in the same row present significant difference ($p \leq 0.05$)
CT conjugated trienes.

Table 5
Increase in *para*-anisidine of sunflower oil stabilized with extracts from seeds extracts of different varieties of barley

Incubation period in days	Control	Jou 83		Jou 87		Haider 93	
		80% Methanol	100% Methanol	80% Methanol	100% Methanol	80% Methanol	100% Methanol
0	2.30 ± 0.32^{a}	2.30 ± 0.32^{a}	2.30 ± 0.32^{a}	2.30 ± 0.32^{a}	2.30 ± 0.32^{a}	2.30 ± 0.32^{a}	2.30 ± 0.32^{a}
10	9.30 ± 0.46^{a}	3.22 ± 0.22^{d}	5.31 ± 0.32^{c}	3.42 ± 0.39^{d}	7.90 ± 0.54^{b}	3.35 ± 0.19^{d}	3.58 ± 0.74^{d}
20	13.51 ± 0.54^{a}	4.16 ± 0.33^{d}	6.57 ± 0.44^{c}	4.37 ± 0.53^{d}	8.89 ± 0.72^{b}	4.69 ± 0.84^{d}	4.89 ± 0.95^{d}
30	19.81 ± 0.56^{a}	6.27 ± 0.66^{bc}	7.02 ± 0.59^{c}	5.68 ± 0.63^{c}	10.45 ± 0.79^{b}	6.22 ± 1.82^{bc}	6.58 ± 1.39^{bc}
Increase in *para*-anisidine value from initial	17.51	3.97	4.72	3.38	8.15	3.92	4.28

Values are mean ± SD for triplicate determinations.
Means followed by different superscript letters in the same row present significant difference ($p \leq 0.05$)

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