EXTRACTS OF SUNFLOWER HULLS: THEIR ANTIOXIDANT ACTIVITY ON LIPIDS OF COOKED MACKEREL FISH

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ABSTRACT

Sunflower (*Helianthus annus L)* hulls that have been investigated in the present work include the hulls from variety Giza 1. and hulls from hybrid Vedoc and white seeds. Methanol (M), ethanol (E), acetone (A) and ethyl acetate (EA) have been investigated as extracting solvents for the phenolic compounds present in the hulls. Solvent(S): hull (H) ratio (v/w) examined were 5:1, 7:1, and 10:1. Methanol, ethanol, acetone and ethyl acetate at a S: H ratio 7:1 extracted the highest quantities of phenolic compounds from Vedoc hulls, extracting 337.9, 367.6, 312.3 and 272.9 mg /l. extract, respectively. The highest extraction for Giza 1 and white hulls was achieved at S:H ratio 7:1 with ethanol. Vedoc and Giza 1 hull extracts were examined for their antioxidant activity using the β-carotene / linoleate system. All extracts exhibited antioxidant activity. Antioxidative power for both hulls were in the following descending order ME ≥ EE >AE > EAE. Compared to BHT, ethanolic extract of Vedoc hulls possessed 88.5% and 90.5% of the antioxidant power of BHT-200 and BHT-50, respectively. For further confirmation, the ethanolic extract of Vedoc hulls (EEV) was investigated on a fish meat model system. Mackerel fish was chosen for this purpose. Different concentrations of EEV and BHT were added to minced mackerel meat along with a control and cooked at 75±2°C for 30 min. After cooling to room temperature, the cooked samples were stored under refrigerated conditions at 4°C for 7 days. The thiobarbituric acid (TBA) values proved that ethanol extract of Vedoc sunflower hulls (EEV)-100 had a power of inhibiting lipid oxidation comparable to BHT-200, while EEV-500 and EEV-1000 proved to exhibit antioxidant activity superior to BHT-200 in the mackerel meat model system. The highest % inhibition of TBA formation was achieved with EEV-1000 at day 5 (75.05%). Surface colour was measured by Hunter Lab. Values of L^* and b^* increased while a^* values decreased during the storage period, but addition of EEV resulted in meat samples with less L^* and b^* values and higher a^* values than the control.

Keywords : sunflower hulls, phenolic compounds, antioxidants, BHT, mackerel, TBA, Hunter colour.

INTRODUCTION

Oilseed hulls are believed to shelter the oil against oxidation, thus must contain antioxidant compounds. The major use of seed hulls include, livestock bedding, composting land application, filler material, and in animal feed mixtures. Research have proved that activated carbon can be prepared from seed hulls to be utilized as adsorbents in wastewater treatment (John & Marshall (1994), and in bleaching of edible oils (Aly & Girgis 2000; Omar *et al.* 2003). Recent investigations indicate that seed hull exhibited antioxidant activity including: rice hulls (Asamarai *et al.* 1996, Wu *et al.* 1994), navy bean hulls (Onyenecho & Hettiarachchy 1991), buckwheat hulls (Watanabe *et al.* 1997), oat hulls (Xing & White 1997), rapeseed hulls (Amarowicz *et al.* 2000), peanut hulls (Duh & Yen 1995; Yen & Duh 1995), and sesame coat (Chang *et al.* 2002).

Sunflower seeds are covered with highly fibrous hulls which comprise about 15-25% of the seed. These hulls are mostly used for livestock bedding. A small amount of sunflower hulls can be added to animal feed as a source of fiber because of its needle like nature which can damage the gastrointestinal tract. It was experimented on the use of sunflower hulls as supplementary fuel to coal-fired power plants (Crum *et al.* 1992).

Lipid and lipid-soluble substances that may be susceptible to oxidation are present in almost all foods. Lipid peroxidation can result in rancidity in the finished products and make them unacceptable to consumers. Furthermore, oxidation can cause other degrading effects

such as discoloration, vitamin destruction, nutritional losses and polymerization (Yen & Duh 1995). In addition, toxic substances formed by lipid peroxidation may lead to other adverse effects such as carcinogenesis, mutagenesis, and aging (Yagi 1990). The addition of antioxidants to foods is one of the most effective means in retarding lipid oxidation. There is currently an interest in replacing synthetic antioxidants with natural ones (Ito *et al.* 1982). On the other hand, Velioglu *et al.* (1998) and Pedrosa *et al.* (2000), reported the presence of phenolic compounds in sunflower hulls.

The present work was designed with the aim of examining the antioxidant activity of three different sunflower hulls as extracted with different organic solvents at different solvent: hull ratio. The total phenolic content of these extracts were determined as well as their antioxidant activity *in vitro*. The best extract with optimum antioxidant activity was chosen to be tested in a fish (mackerel) meat model system.

MATERIALS AND METHODS

Materials

Sunflower seeds (*Helianthus annus L*) varieties namely: Giza 1 (striated), hybrid Vedoc (black) were supplied by the Agricultural Seed Research Station, Ministry of Agriculture, Dokki, Giza, Egypt, and a (white) hybrid was bought from a local market. Sunflower seeds were partially crushed and sieved to separate hulls by aspiration, the hulls were then ground using a Wiley Mill (Teactor Cemoetc 1090, Hoganas, Sweden).

Frozen mackerel (*Scomber scombrus*) was bought from a local market. The white muscle was separated and homogenized using a Waring blender (Model 33BL73, Waring Products, New Hartford, CT). The meat was then packaged in polyethylene pouches and kept in a freezer to be used later.

Methods

Extraction of total phenolic compounds

Ground sunflower hulls of the three types of sunflower were extracted with several organic solvents namely, methanol (M), ethanol (E), acetone (A) and ethyl acetate (EA) at solvent to hull ratios of 5:1, 7:1 and 10:1.

Extraction was carried out using shaking incubator at room temperature overnight, filtered through Whatman No. 4 filter paper. The residue was re-extracted in the same manner. Two filtrates were combined and evaporated on a rotary evaporator (Rotavapor R-124-BUCHI-Switzerland) at 40°C to dryness.

Determination of total phenolic compounds

Total phenolic compounds were quantitatively determined according to Hung *et al.* (2002) by measuring the absorbance at 765 nm using Folin Ciocalteu's phenol reagent. one ml of Folin Ciocalteu's phenol reagent of (1:10) dilution was added to 0.2 ml of the different solvent extracts of sunflower hulls, 0.8 ml of the 7.5% (w/v) sodium carbonate was added and mixed. The mixture was left for 30 min. for colour development. The absorbance (A_{765}) was read against milli-Q water. The concentration of phenol groups was calculated from a standard curve obtained by subjecting various amounts of gallic acid to the same treatment as the test samples. The equation for the standard curve was as follows:

$$PG = 95.1 . A_{765} - 2.6$$

Where PG is the concentration of phenol groups (mg/l.).

Determination of antioxidant activity (AOA)

Antioxidant activity of hull extracts and standard BHT (50 and 200ppm) was determined according to the β-carotene bleaching method following a modification of the procedure described by Marco (1968). For a typical assay, one ml of β-carotene solution (0.2 mg β-carotene /ml in chloroform) was added to round bottom flasks containing 0.2 ml linoleic acid and 0.2 ml of Tween 20. After evaporation to dryness under vacuum at room temperature, oxygenated distilled water (50ml) was added and the mixture was shaken to form a liposome solution (emulsion). A 5ml aliquot of emulsion was placed in test tubes contained 0.2 ml of 80% methanol (as a control) or corresponding hull extract or standard and the mixture was thoroughly mixed. The samples were then subjected to thermal autoxidation at 50°C for 2hrs. (Velioglu *et al.* 1998). The absorption of the solution at 470 nm was measured using a spectrophotometer (Shimadzu UV-Visible Recording Spectrophotometer, Model UV240 Graphtcord) by taking measurements at 15-20 min. intervals. The rate of bleaching of β-carotene was calculated by fitting linear regression to data over time. All samples were assayed in triplicate. Antioxidant activity (AOA) was calculated as % inhibition relative to control using the following equation (Al-Shaikhan *et al.* 1995)

$$AOA\,\% = \frac{R_{control} - R_{sample}}{R_{control}} \times 100$$

Where:

AOA : Antioxidant activity

R : $R_{control}$ and R_{sample} were the bleaching rates of β-carotene in reactant mix without antioxidant and with hull extracts or BHT, respectively.

Preparation of cooked mackerel as a model system

Ethanol extract of Vedoc sunflower hulls (EEV) was chosen and its antioxidant activity was examined on a fish (mackerel) model system according to Shahidi & Pegg (1990). The hull extract was dissolved in 2 ml of 99% ethanol (absolute) and added to the minced mackerel fish (20% by weight of water) at the following concentrations (100, 500 and 1000 ppm). These concentrations were used to test their antioxidant effectiveness (Onyeneho & Hettiarachchy 1991). A sample with BHT (200 ppm) and a control sample containing only 2 ml of 99% ethanol without antioxidant were also prepared. Meat systems were thoroughly homogenized and cooked at 75±2°C (He & Shahidi, 1997) in a thermostated water bath (Julabo Labortetechnik GMBH Sw 20 seelbach / Germany) for 30 min., samples were cooled to room temperature, transferred into plastic bags and then stored for 7 days at 4±1°C. Samples were analysed for lipid oxidation (TBA) and colour changes on days 0, 1, 3, 5 and 7.

Thiobarbituric acid (TBA)

The TBA was determined spectrophotometrically according to the procedure described by Siu & Draper (1978). Cooked fish samples (10g) were homogenized in 25 ml of 10% trichloroacetic acetic. The mixture was mixed and filtered. One milliliter of 0.06 M thiobarbituric acid was added to 4 ml aliquots of the filtrate and heated in a boiling water bath for 10 min for colour development. The absorbance was read at 532 nm using a spectrophotometer (Shimadzu UV-Visible Recording Spectrophotometer, Model UV 240 Graphtcord). The TBA values were expressed as milligrams of malonaldehyde / kilogram of dry sample. Triplicate analyses were performed on all mackerel samples.

Measurement of colour

Changes in the surface colour of the mackerel samples was measured in duplicate for each sample using a spectrophotometer with colour scale (Hunter, Lab. Scan XE, Reston, VA). This instrument was standardized against a white tile of Hunter Lab. Colour standard (LX No. 16379: X= 77.26, Y= 81.94 and Z= 88.14). Colour was expressed in terms of lightness (L^* –value), redness (a^* –value), and yellowness (b^* –value).

Proximate analysis

Moisture, protein, lipid, ash and crude fiber contents of sunflower hulls and muscle of mackerel were determined according to AOCS (1998). All analysis was carried out in triplicate.

RESULTS AND DISCUSSION

Current usage of sunflower seed or its by-products as human food is so low that it might be considered as underutilized. Its use, however as an animal feed is considerable and growing. For humans, sunflower seeds are used mainly as a snack, the seeds being roasted and eaten as peanuts and chestnuts. Decorticated sunflower seeds feature much more in vegetarian diets and are sold primarily in health food stores as an effective alternative protein source (Nwokolo, 1996). To prepare refined protein products from sunflower such as flour, concentrates, isolates, textured vegetable protein etc., thus sunflower seeds have to be decorticated. Hence, effective utilization of the hulls seems to be essential.

Proximate composition of sunflower hulls

Table (1) gives the proximate composition of the three sunflower hulls. Data revealed that Giza 1, Vedoc and White hulls contained 6.14, 5.69 and 4.33 % protein; 1.65, 2.20 and 1.89% oil; 1.35, 1.68 and 1.89% ash; 20.05, 18.28 and 19.26% crude fiber, and 62.58, 62.42 and 64.22% nitrogen free extract (NFE), respectively. Earle *et al.* (1968) reported that sunflower hulls contained between 1.7- 6.1% protein. Bau *et al.* (1983), reported 2.8% protein, 1.2% lipid, 2.5% ash and 0.2 ethanol soluble for sunflower seed hull.

Extraction of phenolic compounds from sunflower seed hulls

Table (2) shows the total extracted phenolic compounds from the three seed hulls using four extracting solvents namely, methanol (M), ethanol (E), acetone (A) and ethyl acetate (EA). Results generally, revealed that M and E extracted more phenolic

Table 1: Proximate composition of different sunflower seed hulls

Analysis %	Sunflower hulls		
	Giza 1	Vedock	White
Moisture	8.53 ±0.090	9.80±0.05	8.81±0.11
Protein	6.14±0.025	5.69±0.08	4.33±0.06
Oil	1.65 ±0.180	2.20±0.15	1.89±0.08
Ash	1.35 ±0.140	1.68±0.21	1.49±0.02
Crude Fiber	20.05 ±0.31	18.28±0.27	19.26±0.21
NFE*	62.58±0.06	62.42±0.11	64.22±0.09

Data reported are the mean values with standard deviation (SD)
* NFE = Nitrogen free extract

Table 2: Total phenolic compounds extracted from the three sunflower seed hulls using different solvents

Sunflower variety	S:H	Total phenolic compounds (mg/l.)			
		ME	EE	AE	EAE
	5:1	316.3 ± 0.11	311.6 ± 0.03	256.3 ± 0.07	255.9 ± 0.06
Giza 1	7:1	337.8 ± 0.03	337.8 ± 0.06	239.9 ± 0.02	246.7 ± 0.05
	10:1	309.5 ± 0.09	298.9 ± 0.21	216.2 ± 0.21	250.3 ± 0.16
	5:1	330.3 ± 0.09	303.4 ± 0.05	306.6 ± 0.02	256.9 ± 0.18
Vedoc	7:1	337.9 ± 0.02	367.6 ± 0.09	312.3 ± 0.01	272.9 ± 0.21
	10:1	321.6 ± 0.06	333.5 ± 0.11	309.9 ± 0.13	228.8 ± 0.03
	5:1	266.6 ± 0.03	226.8 ± 0.07	209.9 ± 0.09	199.9 ± 0.08
White	7:1	283.3 ± 0.06	299.6 ± 0.21	253.9 ± 0.12	203.3 ± 0.05
	10:1	236.3 ± 0.21	263.3 ± 0.06	222.2 ± 0.03	165.6±0.07

Data reported are the mean values with standard deviation (SD).
ME : Methanol extract **AE : Acetone extract** **S:H : Solvent : Hull ratio**
EE : Ethanol extract **EAE : Ethylacetate extract**

compounds than A and EA from the three types of hulls. The highest amount of total phenolic compounds was extracted from Giza 1 hulls using methanol (ME) and ethanol (EE) at a 7:1 solvent: hull ratio. The methanolic (ME) and ethanolic (EE) extracts from Vedoc and white hulls by using the previously mentioned ratio gave the following total phenolic compounds corresponding to 337.9, 283.3, 367.6 and 299.6 mg/l, respectively. On the other hand, A and EA extracted less phenolic compounds as compared to the other two solvents. In most cases 7:1 S:H ratio extracted more phenolic compounds except in the case of AE and EAE of Giza 1, where 5:1 was the optimum. Results obviously revealed that E was the most effective extracting solvent of phenolic compounds from sunflower seed hulls.

Bau *et al.* (1983) reported phenolic content of sunflower hulls to be 4.55% of sample of which 3.08% were in free state and 96.92% were bound. Velioglu *et al.* (1998), studied the antioxidant activity and total phenolics in selected fruits, vegetables and grain products. They found that sunflower hulls (purple) contained 9747 mg total phenolics/100g, while sunflower seed contained 1601 mg/100g. Pedrosa *et al.* (2000) used the HPLC for the determination of caffeic and chlorogenic acids and their derivatives in different sunflower seeds (kernels and hulls). They found that the total phenolic compounds (g/kg) of the hull in different genotypes of sunflower were 0.0780 for Tesoro, 0.376 for Marks, 0.2350 for Clip, 0.732 for Vyp and 0.1810 for Nanta. They added that the total phenolic compounds of kernels of different genotypes ranged between 1.6867 and 1.2867 g/kg kernel.

Phenolic contents that were reported for other seed hulls included sesame coat which

contained phenolic compounds and tetranortriterpenoids that contribute to its antioxidant activity (Chang *et al.* 2002). Hulls of ten legumes namely mung bean, field pea, lentil, fababean, pigeon pea, cowpea, navy bean, lima bean, chick pea and lupine were found to contain phenolic acids including p-hydroxybenzoic, protocatechuic, synergic, gallic, trans-p-coumaric and trans – ferulic acids that were present as both soluble esters and residues on alkaline hydrolysis of the hulls (Sosulski & Dabrowski 1984). On the other hand, Yen & Duh (1995), reported that the total phenolics of the hulls of four cultivars of peanut being in a range between 4.2 – 10.2 mg/g of hulls. The ethanolic extract of buckwheat hulls were reported to contain protocatechuic acid 4.3 mg/100g, rutin 4.3 mg/100g, and quercitin 2.5 mg/100g hulls (Watanabe *et al.* 1997). Phenolic compounds present in oat hulls as determined by GC-MS were ferulic acid, p-coumaric acid, vanillic acid, p-hydroxybenzoic acid, catechol, 4-hydroxyl phenyl acetate, o-coumaric, catechol, sinapic acid, and salycilic acid with a total of 3508 mg/kg hull (Xing & White 1997). Furthermore, Amarowicz *et al.* (2000), found that the total phenolic content of canola and rapeseed hulls ranged between 128 and 129 mg of sinapic acid equivalents/ 1g of extract. Ramarathnam *et al.* (1988), reported on the presence of phenolic compounds in rice hull methanolic extract.

Antioxidant activity of sunflower hull extracts

Table (3) gives the antioxidant activity (AOA%) of different extracts of sunflower hull (Vedoc) along with different concentrations of BHT (50 and 200 ppm) as determined by the β-carotene/linoleate system at different time intervals. Figure (1) is a plot of the absorbance of different extracts of Vedoc hulls against time, the difference in absorbance between extracts and control is taken as an indicator for the AOA. It was clear from Table (3) and Fig. (1) that all Vedoc hull extracts exhibited AOA. Comparing the obtained results with synthetic antioxidants (BHT), the AOA of the examined extracts were in the following descending order: BHT-200> BHT-50> ME> EE> AE> EAE. It is also worth to note that the extracts which showed relatively higher antioxidant activity (ME and EE) contained higher amount of total phenolic compounds (Table 2). The ME and EE possessed 92.88% and 90.47% of the antioxidant power of BHT-50, respectively, and 90.88% and 88.5% of the antioxidant power of BHT-200, respectively.

The obtained results also revealed the same pattern in antioxidative effects when the different extracts of sunflower variety (Giza 1) were investigated. The results of this variety are shown in Table (4) and Fig. (2).

From the aforementioned results, the antioxidants recovered from both ME and EE of sunflower hull (Giza 1 and Vedoc) proved their efficiency compared to BHT. So, this natural antioxidant can be applied successfully in food industries instead of using the synthetic one.

Velioglu *et al.* (1998) reported that the antioxidant activity (AOA%) of methanolic extract of sunflower hull (purple) was 88.9%, and

Table 3: Antioxidant activity of Vedoc sunflower hull extracted by different solvents at S:H (7:1)

Time (min)	Antioxidant activity %					
	ME	EE	AE	EAE	BHT-50	BHT-200
15	74.63±0.08	66.79±0.34	19.16±0.08	86.60±0.16	86.60±0.09	94.75±0.35
30	86.51±0.13	75.05±0.31	69.12±0.36	59.45±0.29	96.64±0.04	97.36±0.28
50	84.49±0.31	84.06±0.19	74.16±0.40	54.56±0.15	96.47±0.09	97.58±0.06
70	91.60±0.26	90.14±0.08	80.64±0.09	72.57±0.16	97.30±0.16	98.42±0.34
90	91.13±0.07	90.71±0.23	85.86±0.08	83.85±0.22	98.23±0.13	98.38±0.18
110	91.08±0.17	90.06±0.28	85.35±0.34	72.94±0.32	96.63±0.28	97.93±0.11
130	90.97±0.33	86.40±0.31	78.14±0.25	70.64±0.28	95.52±0.19	97.41±0.09
150	88.50±0.09	86.20±0.11	72.67±0.22	69.79±0.06	95.28±0.17	97.38±0.13

Data reported are the mean values with standard deviation (SD).
ME : Methanol extract **AE : Acetone extract** **S:H : Solvent : Hull ratio**
EE : Ethanol extract **EAE : Ethylacetate extract**

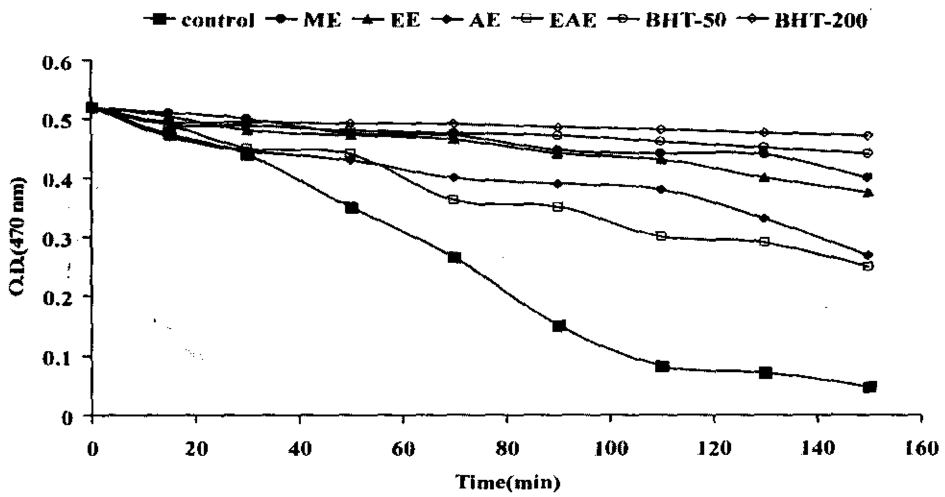

Fig. 1: Antioxidant activity of Vedoc sunflower hull extracted by different solvents at S:H (7:1) in a β-carotene / linoleate system as measured by changes in absorbance

Table 4: Antioxidant activity of sunflower hull (Giza 1) extracted by different solvents at S:H (7:1)

Time (min)	Antioxidant activity %					
	ME	EE	AE	EAE	BHT-50	BHT-200
15	32.45±0.09	29.16±0.23	18.23±0.07	7.18±0.01	86.60±0.16	94.75±0.31
30	79.84±0.09	79.93±0.06	68.07±0.16	53.21±0.09	96.64±0.13	97.36±0.28
50	88.69±0.05	88.77±0.13	72.28±0.09	54.63±0.23	96.47±0.25	97.58±0.08
70	90.80±0.12	93.75±0.28	74.81±0.22	69.31±0.08	97.30±0.06	98.42±0.25
90	91.92±0.21	94.29±0.08	80.52±0.23	79.85±0.09	98.23±0.19	98.38±0.22
110	89.79±0.06	92.01±0.07	83.84±0.05	82.79±0.13	96.63±0.05	97.93±0.18
130	90.41±0.08	90.12±0.31	80.79±0.19	77.18±0.32	95.52±0.09	97.41±0.07
150	88.62±0.16	87.96±0.09	71.73±0.30	69.86±0.27	95.28±0.08	97.38±0.27

Data reported are the mean values with standard deviation (SD).
ME : Methanol extract **AE : Acetone extract** **S:H : Solvent : Hull ratio**
EE : Ethanol extract **EAE : Ethylacetate extract**

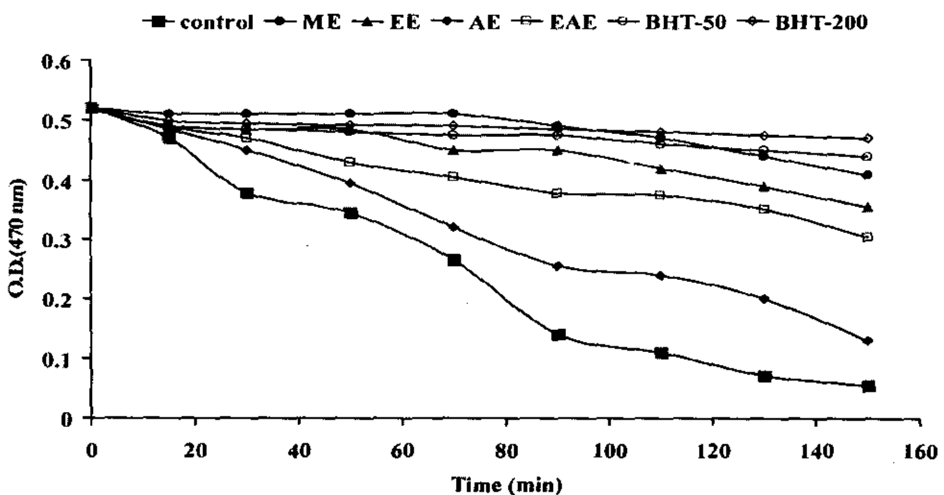

Fig. 2: Antioxidant activity of sunflower hull (Giza 1) extracted by different solvents at S:H (7:1) in a β-carotene / linoleate system as measured by changes in absorbance

its oxidation rate ratio (ORR) was 0.111 and its antioxidant activity coefficient (AAC) was 714.5. They reported BHT-200 and BHT-50 (mg/l) exhibited 97.2 and 84.3 AOA%, respectively. Other investigators reported the AOA of other seed hulls. Ramarathnam *et al.* (1988), studied the hulls of two rice varieties namely Katakutara (Kat) and Kusabue (Kus). They found a strong antioxidant activity for crude methanolic extract of (Kat) rice hull, inhibited lipid peroxidation to the extent of 88% in comparison with rice hull (Kus) which inhibited lipid peroxidation to the extent of 81%. However, both varieties showed AOA stronger than BHA and α-tocopherol which showed inhibition of lipid peroxidation to the extent of 83% and 68%, respectively. Chang *et al.* (2002), working on the seed coat of sesame proved that the AOA of 1.0 mg ethanolic extract of sesame coat (EESC) was 91.4% and being rather equal to 1.0 mg tocopherol 90.4% but was weaker than 1.0mg BHA 98.6%. Amarowicz *et al.* (2000) found that crude tannin extract of canola hulls exhibited stronger AOA than rapeseed hulls. Duh *et al.* (1997) reported that the methanolic extract of mung bean hulls at a concentration of 100 ppm exhibited stronger AOA than 100 ppm BHA and 100 ppm dl-α-tocopherol on the peroxidation of linoleic acid. The methanol hull extract also showed good inhibitory activity in soybean oil oxidation which was examined by peroxide value , thiobarbituric acid and gas chromatography of the fatty acid methyl esters. Xing & White (1997), reported different concentrations of oat groats and hull extracts when added to soybean oil at 60°C and compared to TBHQ and a control (no additives) to act as an antioxidant as measured by peroxide value. The AOA of extracts of groat and hulls increased with concentration. Onyencho & Hettiarachchy (1991), studied the effectiveness of freeze dried navy bean hull extract (NBHE) as an antioxidant, this was evaluated in storage studies with soy and sunflower oils. Monthly peroxide values determination indicated NBHE to be a stronger antioxidant than BHT-BHA mixture and rosemary (AR) but was less effective than TBHQ at all levels and storage conditions. Watanabe *et al.* (1997) separated ethanolic extract of buckwheat hulls by Sephadex LH-20 column chromatography into eight fractions. Five of the fractions exhibited peroxyl radical scavenging activity by inhibiting the oxidation of methyl linoleate in solution. Yen & Duh (1994, 1995) reported that methanolic extract of peanut hulls (MEPH) showed marked activity as a radical scavenger in the experiments using DPPH* radical. The MEPH also possessed AOA towards H_2O_2 and suuperoxide (O_2^-), it also exhibited a marked scavenging activity on hydroxyl radicals.

Antioxidant activity of sunflower hull in fish meat as a model system

Mackerel, a fatty fish, because of its high content of unsaturated fatty acids (He & Shahidi, 1997) was chosen in the present study. The authors stated that the extension of shelf life of foods and especially fish may be achieved by addition of antioxidants. The antioxidant chosen in the present study to inhibit lipid oxidation was the ethanol extract of sunflower hull "Vedoc" (EEV). This choice was based on the fact that ethanol is more safer than methanol, acetone or ethyl acetate and therefore more suitable for the food industry. Vedoc is the sunflower hybrid used industrially for its high oil content, thus the availability of hulls is assured. The EEV was investigated in the fish model system at three levels (100, 500 and 1000 ppm) along with BHT-200 ppm. and a control (minced mackerel meat with no addition), for comparison. Table (5) gives the proximate composition of the minced mackerel meat. Data revealed that mackerel meat contained moisture (67.75%), protein (18.63%), fat (11.24%), and ash (1.34%). These values are in agreement with those reported by He & Shahidi (1997).

Table 5: Proximate composition of frozen mackerel

Components	Percentage
Moisture	67.75±0.30
Protein	18.63±0.35
Fat	11.24±0.04
Ash	1.34±0.07

Data reported are mean values with standard deviation (SD)

Changes in TBA values of cooked mackerel fish: The TBA values of mackerel meat treated with different levels of EEV along with a sample treated with BHT and a control are presented in Table (6). It was clear that the addition of EEV at 100, 500 and 1000 ppm, delayed lipid oxidation for cooked mackerel compared to the control sample at all storage times. The EEV-500 and EEV-1000 competed well with BHT-

Table 6: Effect of ethanol extract of sunflower hulls (Vedoc) on the TBA values of cooked mackerel stored at 4°C (mg malonaldehyde / kg sample)

Cooked mackerel	ppm	Storage time (days)				
		0	1	3	5	7
Control		9.00±0.10	11.54±0.40	14.54±0.03	16.01±0.03	17.38±0.20
+BHT	200	5.20±0.31	5.36±0.20	6.31±0.10	6.90±0.15	7.42±0.40
+EEV*	100	5.70±0.04	6.40±0.01	7.10±0.28	8.06±0.09	8.90±0.42
	500	4.00±0.23	4.40±0.03	6.00±0.04	6.31±0.03	7.35±0.01
	1000	3.40±0.02	3.60±0.01	4.50±0.03	4.03±0.15	5.72±0.05

Data reported are the mean values with standard deviation (SD).

*** Ethanolic extract of Vedoc hulls.**

200 in inhibiting the formation of thiobarbituric acid reactive substances (TBARS), but EEV-100 was less effective than BHT-200 in the fish model system. It could be also noticed that TBARS formation either with the addition of EEV or BHT or without any addition was time dependent at 4°C.

Figure (3) shows the % inhibition of lipid oxidation as % of control. Results of Figure 3 confirmed results in Table (6). The highest % inhibition of lipid oxidation was achieved with EEV-1000 followed by EEV-500. EEV at these two levels of addition surpassed BHT-200 in their power of inhibiting lipid oxidation in mackerel meat system. At day 7, the % inhibition of lipid oxidation (compared to control) reached 57.3%, 48.8%, 57.7% and 67.1% for BHT-200, EEV-100, EEV-500, and EEV-1000, respectively.

He & Shahidi (1997) examined the AOA of green tea catechins in fish meat model system (mackerel). They monitored progression of oxidation by measuring changes in TBA and total volatiles of samples. They reported that samples treated with ground green tea leaves, tea extracts and pure catechins showed excellent oxidative stability as compared with samples that contained α-tocopherol, BHT, BHA and TBHQ. Yu *et al.* (2002), reported that rosemary extracts (water-soluble) exhibited

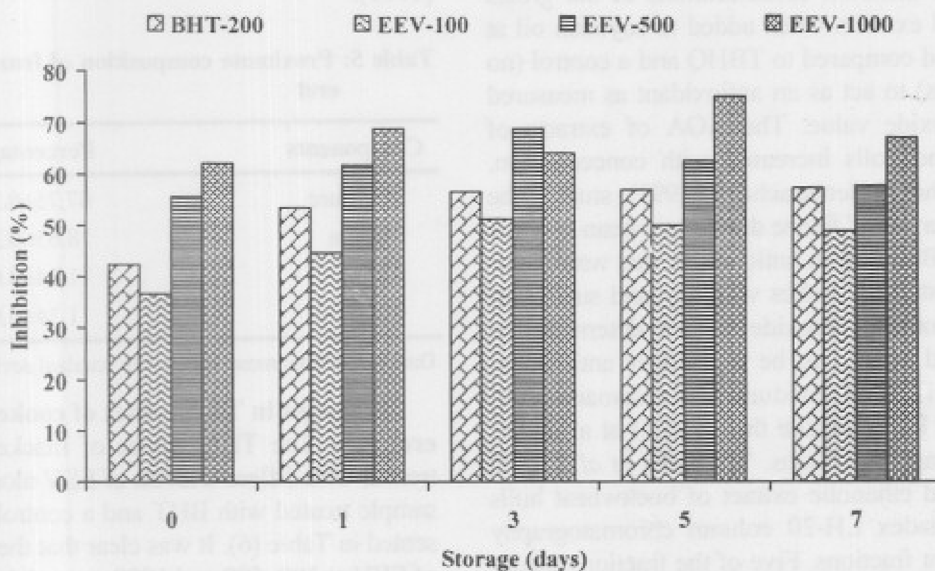

Fig. 3: Inhibition percentage of oxidation as measured by TBA in a mackerel meat model system

significant protection of cooked turkey meat from lipid oxidation. Sanchez-Escalente *et al.* (2003a), prepared beef patties containing natural antioxidants- cayenne hot pepper, red sweet pepper, lycopene-rich tomato pulp, and extracts of tomato rich in lycopene, and packaged in modified atmosphere were evaluated for storage stability at 2±1°C, by measuring colour surface metmyoglobin, TBA, psychrotropic bacteria counts and sensory off-odour and discolouration. Results revealed that lipid oxidation was found to be dramatically reduced (P<0.05) in beef patties containing the peppers, either hot or sweet. The TBA values were below 1 in both types of patties. Inhibition was even greater by the effect of cayenne hot peppers, which did not result in TBA elevation.

Sanchez-Escalaste *et al.* (2003b), also examined the antioxidant action of borage, rosemary, oregano and ascorbic acid in beef patties packaged in modified atmosphere. They reported that all the antioxidants except ascorbic acid reduced (P<0.01) TBA formation. Tang *et al.* (2001), studied the effect of tea catechins (TC) on improving oxidative stability of cooked chicken meat and beef compared with α-tocopherol (VE). Oxidative stability (TBA) was measured on days 1, 3, 6 and 10. The susceptibility of cooked meat to lipid oxidation was species dependent. Chicken meat was more susceptible to oxidative deterioration than beef. There were no significant changes in TBA numbers during the course of the test for cooked beef containing either TC or VE at the levels of 300 mg/kg^{-1} meat.

Colour of cooked mackerel fish: The effect of addition EEV at different levels along with BHT on the surface colour characteristics of cooked mackerel meat is presented in Table (7) and Figs. (4 and 5). Results of b^* values are not shown in figures because they follow the same trend as L^*. The EEV clearly decreased the L^* (lightness) and b^* (yellowness) values of cooked mackerel meat when compared to the control. On the other hand, values of L^* and b^* for control and treated samples increased throughout the storage period. On day 0, L^* values for control, BHT-200, EEV-100, EEV 500 and EEV-1000 were 47.2, 46.24, 46.15, 45.54 and 45.2, respectively, and b^* values were 13.34, 12.13, 12.11, 11.1 and 10.15, respectively.

At the end of the storage period, L^* values were 59.93, 48.91, 49.53, 47.31 and 46.15 for control, BHT-200, EEV-100, EEV-500, EEV-1000, respectively, and b^* values were 17.54, 14.95, 13.11, 12.92 and 11.6, respectively. The difference in colour between control and treated samples as demonstrated by L^* and b^* values increased with storage. On the other hand, values of a^* decreased as storage time was elongated. On day 0, a^* value was 5.37 for control which decreased to 2.54 on day 7. The a^* values for BHT-200 and EEV-100 were 6.76, 4 and 6.12 at the beginning of the experiment and decreased to 4.31 and 4.73 at the end of storage time. The addition of all antioxidants under study protected the mackerel meat from redness fading to varying degrees.

Sanchez- Esclante *et al.* (2003a) worked on the stabilization of colour and odour of beef patties by lycopene-rich tomato and peppers as a source of antioxidants. The antioxidant sources included cayenne hot pepper, red sweet pepper, lycopene rich tomato pulp (LRTP) and extract of tomato rich in lycopene (Lyc-o-Mato). Results demonstrated that the addition of ground peppers (both sweet and hot) to beef patties significantly inhibited (P<0.05) the oxidation of myoglobin and lipid. Regarding values of L^* and b^* no trend or significant difference among treatments were traced while both peppers gave a dramatic (P<0.05) inhibition of metmyoglbin formation. Yet, both peppers gave the highest a^* values. All treatments resulted in decrease of a^* colour of beef patties during the storage period. Yu *et al.* (2002) also worked on rosemary extract to inhibit oxidation of cooked turkey meat. They found that Hunter L^* values of cooked turkey meat at all tested storage periods significantly decreased when water-soluble rosemary extract (WSRE) was added. Turkey samples containing WSRE had higher a^* values than that of the control. Rosemary extracts delayed the decrease of the Hunter a* values of cooked turkey significantly at all storage periods tested.

Sanchez- Esclante *et al.* (2003b) examined the antioxidant activity of oregano, borage rosemary and ascorbic acid in beef patties packaged in modified atmosphere. They found that a^* values decreased progressively during storage in all the beef patties (treated with antioxidants) and that values of a^* were significantly higher (P<0.01) than those of control between days 8-20 in patties treated with oregano. On day 4 of storage, the a^* values were similar for all samples (P<0.05) except those with rosemary (alone or with ascorbic acid), but after 8 days storage they varied significantly (P<0.01).

Table 7: Changes in colour of cooked mackerel with sunflower hull (Vedoc) or BHT during storage

Treatment		Storage time (days)														
Sample	ppm	0			1			3			5			7		
		L*	a*	b*	L*	a*	b*	L*	a*	b*	L*	a*	b*	L*	a*	b*
Mackerel (Control)		47.20± 0.21	5.73± 0.43	13.34± 0.11	48.01± 0.39	4.11± 0.44	14.13± 0.38	50.44± 0.34	3.32± 0.09	15.97± 0.12	51.36± 0.45	2.98± 0.26	16.5± 0.11	52.93± 0.19	2.54± 0.16	17.54± 0.21
Mackerel + BHT	200	46.24± 0.31	6.76± 0.16	12.13± 0.11	47.58± 0.25	5.95± 0.36	13.7± 0.41	48.07± 0.32	5.04± 0.33	14.13± 0.26	48.5± 0.43	4.90± 0.25	14.56± 0.41	48.91± 0.36	4.31± 0.29	14.95± 0.13
	100	46.15± 0.36	6.12± 0.33	12.11± 0.51	47.23± 0.43	5.85± 0.16	13.02± 0.25	48.66± 0.33	4.02± 0.46	14.38± 0.49	49.00± 0.13	4.30± 0.29	13.6± 0.46	49.53± 0.24	4.73± 0.17	13.11± 0.28
Makerel + EEV	500	45.54± 0.45	7.27± 0.21	11.1± 0.11	46.26± 0.32	6.27± 0.51	12.48± 0.45	47.90± 0.39	5.29± 0.36	13.32± 0.1	47.6± 0.41	5.42± 0.4	13.11± 0.11	47.31± 0.46	5.85± 0.50	12.92± 0.33
	1000	45.20± 0.39	8.82± 0.50	10.15± 0.21	45.91± 0.45	7.79± 0.46	11.46± 0.21	47.23± 0.38	6.13± 0.29	12.24± 0.41	47.01± 0.55	6.56± 0.46	11.32± 0.31	46.15± 0.29	6.76± 0.46	11.60± 0.52

Data reported are the mean values with standard deviation (SD)
L* : Lightness a* : redness b* : yellowness

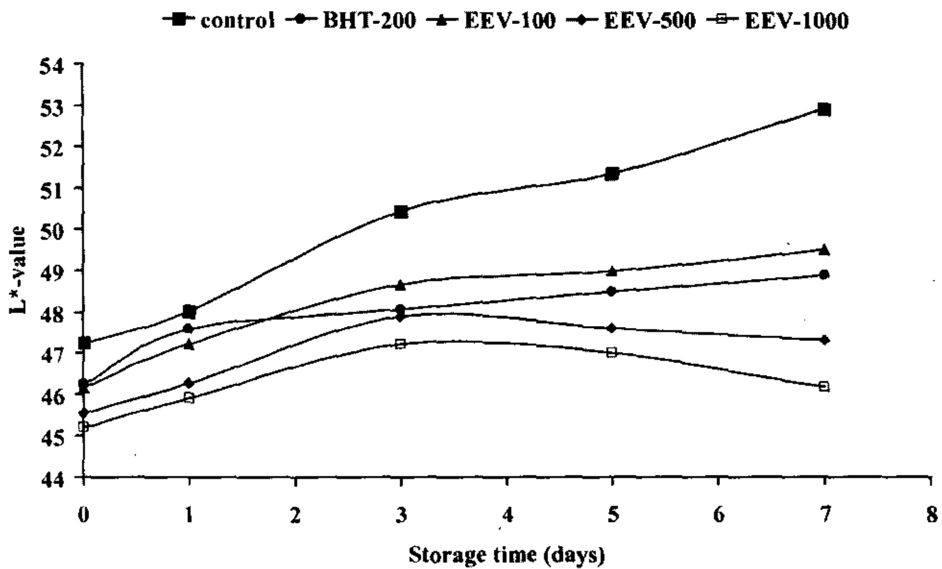

Fig. 4: Hunter L*-value of cooked mackerel with sunflower hull (Vedoc) or BHT during storage
EEV : Ethanolic extract of Vedoc hulls

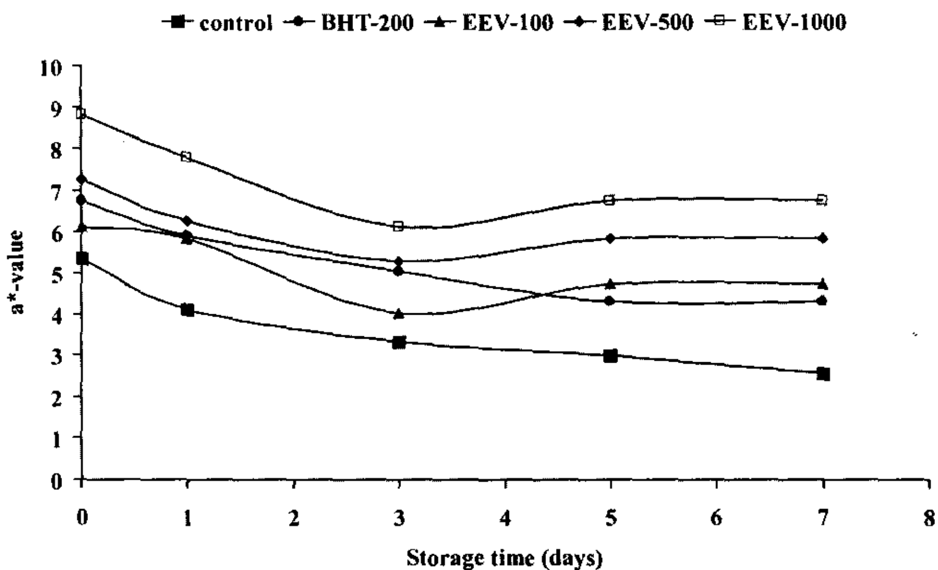

Fig. 5: Hunter a*-value of cooked mackerel with sunflower hull (Vedoc) or BHT during storage
EEV : Ethanolic extract of Vedoc hulls

Previous results indicate that the potential use of the ethanolic extract of sunflower hulls (Vedoc) as an antioxidant has been confirmed by the β-carotene- linoleate method. The ethanolic hull extract has been also tested in a fish (Mackerel) meat model system and has been proved to delay oxidation of fish fat (TBA) as well as preserving the surface colour of fish meat as measured by Hunter Lab. In conclusion, ethanol extract of sunflower hull (Vedoc) presented a potential cheap natural source of antioxidant.

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مستخلصات قصرة بذور دوار الشمس: نشاطها المضاد للأكسدة على ليبيدات سمك الماكريل المطهي

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استخلصت المركبات الفينولية الموجودة فى قصرة ثلاثة أصناف من بذور دوار الشمس (*Helianthus annus L*) وهى جيزة ١ ، وهجين Vedoc ودوار الشمس الأبيض بواسطة مذيبات (الميثانول، الإيثانول، الأسيتون، خلات الإيثيل) بنسب استخلاص مختلفة [قصرة (وزن) : مذيب (حجم)]. أوضحت النتائج أن استخدام النسبة ١:٧ هى الاكثر كفاءة فى استخلاص المركبات الفينولية من قصرة هجين دوار الشمس (Vedoc) بواسطة الميثانول، الإيثانول، الأسيتون، وخلات الإيثيل حيث تم استخلاص ٣٣٧,٩، ٣٦٧,٦، ٣١٢,٣ و ٢٧٢,٩ ملجم/ لتر على التوالى. كما تبين أن الايثانول بنسبة ١:٧ هو الأكثر كفاءة على الاستخلاص للصنفين جيزة ١ ، وقصرة البذورالبيضاء ثم تم تقدير النشاط المضاد للأكسدة antioxidant activity بنظام (β-carotene-linoleate) لمستخلصات قصرة كل من جيزة ١، والهجين Vedoc. أثبتت النتائج أن جميع المستخلصات كان لها نشاط مضاد للأكسدة والتى تدرجت فى فعاليتها كالآتى (الميثانول≥، الايثانول> الاسيتون> خلات الإيثيل). بالمقارنة مع مضاد الأكسدة الصناعى BHT وجد أن مستخلص الإيثانول لقصرة هجين دوار الشمس (EEV) Vedoc يمثل ٩٠,٥% و ٨٨,٥% من كفاءة قوة تضاد الأكسدة للـــ BHT عند استخدامه بتركيزات ٥٠، ٢٠٠ جزء فى المليون على الترتيب. لتأكيد هذه النتائج أضيفت تركيزات مختلفة (١٠٠، ٥٠٠، ١٠٠٠ جزء فى المليون) من هذا المستخلص (EEV) بالاضافة إلى استخدام مضاد الأكسدة الصناعى (BHT – ٢٠٠ جزء فى المليون) وذلك للمقارنة إلى مفروم سمك الماكريل ثم طهيه على درجة حرارة ٧٥±٢°م لمدة ٣٠ دقيقة ثم التبريد والتخزين على ٤°م لمدة سبعة أيام وأجريت إختبارات الأكسدة بتقدير رقم حمض الثيوباربيتيوريك (TBA) وقياس التغير فى اللون باستخدام جهاز (Hunter Lab.). أثبتت النتائج أن استخدام (EEV) بتركيز ١٠٠ جزء فى المليون يماثل تقريبا تأثير مضاد الأكسدة الصناعى فى تثبيط عملية الأكسدة ، بينما تبين أن استخدام تركيزات عالية (٥٠٠، ١٠٠٠ جزء فى المليون) أدت إلى زيادة كفاءة عملية التثبيط والتى تفوق استخدام مضاد الأكسدة الصناعى حيث وصلت أعلى نسبة لتثبيط تكون حمض الثيوباربيتيوريك لمفروم سمك الماكريل المطهى (٧٥,٠٥%) والمخزن بالتبريد عند استخدام (EEV) بتركيز ١٠٠٠ جزء فى المليون فى اليوم الخامس عند ٤°م.