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Antioxidant Effect of Microwave-assisted Extracts of Olive Leaves on Sunflower Oil

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ABSTRACT

The antioxidant properties and total phenols' content of 80% methanol, acetone and water extracts of olive leaves procured through Microwave Assisted Extraction (MAE) were examined. Two varieties of olive namely: Cronaiky and Roghani were the ones studied. Various experimental models were employed for antioxidant activity evaluation of the extracts. The results revealed the highest phenolic content (230.50 mg tannic acid equivalent g^{-1} extract) and the lowest IC_{50} in DPPH (86.81 µg ml^{-1} of extract), reducing power (166.45 µg ml^{-1} extract) and total antioxidant capacity (128.32 µg ml^{-1} of extract) indices were attributed to the methanol extract of Cronaiky variety. The efficacy of the extracts at three experimental levels (200, 500, 1,000 ppm) in blocking the oxidation process was investigated using peroxide value and thiobarbituric acid index for sunflower oil during its heating at 70°C (oven test method). The lowest peroxide value and thiobarbituric acid index was observed for the methanol extract of Cronaiky at its 1,000 ppm concentration. This extract was competitively comparable with BHA and BHT at either of the tested levels (100, 200 ppm). Thus, methanol extract of Cronaiky olive variety can be employed in place of synthetic antioxidants to establish oxidation stability in edible oils.

Keywords: Antioxidant activity, Microwave, Olive leaf, Sunflower oil.

INTRODUCTION

Vegetable oils and fats constitute an important part of human diet. They are the providers of essential fatty acids, which in turn are the precursors of important hormones controlling many physiological factors (Iqbal and Bhanger, 2007). It is generally accepted that a major cause of deterioration of foods containing fats is due to oxidation of unsaturated fatty acids. Free radicals exert undesirable effects on the nutritional value and on many other such important food parameters as aroma, taste, texture, consistency and appearance. Due to such changes, oxidized products become unacceptable to consumers and along with that, the related industries suffer from financial losses (Valenzuela *et al*., 2003). Such synthetic antioxidants as BHA, BHT, and TBHQ have been utilized, as food additives, to overcome the instability problems of foods due to their oil and fat contents, but the point in question is that some synthetic antioxidants are very volatile and thermosensitive. On the other hand, these compounds may be contributors to many such health problems as cancer causing and formation of mutagens.

Due to these concerns, an alternative strategy of industries is established as based on the natural sources of antioxidants that could replace the synthetic ones or at least diminish their uses as food additives. Phenols are recognized as one of the most important groups of natural antioxidants that have high demonstrated capability for scavenging free radicals. Furthermore, such trait enrichment

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also highly benefits human health (Antolovich *et al*., 2004).

Extraction is an essential step to obtain valuable natural compounds such as phenolics from plants. Traditional methods have been employed for many decades, but they are often much time-consuming and require large volumes of organic solvents. Also, extra energy is needed to accelerate the process of extraction, to reduce the organic solvent use up, and to diminish environmental pollution. Microwave Assisted Extraction (MAE) is based on heating the solvent by an application of microwave energy, disruption of hydrogen bonds and migration enforcement of the ions (Mandal *et al*., 2007). These are some of the ways by which MAE promotes the efficiency of extraction. The accurate choice of solvent is very critical in achieving an optimal extraction. Solvent choice for MAE depends on solubility of the target analyte, the interaction between solvent and plant matrix, microwave absorbing properties of solvent [(dielectric constant (ε'), dielectric loss (ε'') and dissipation factor (ε''/ε')]. Dielectric constant (ε') indicates the absorption capability of microwave energy. Dielectric loss (ε'') describes the efficiency of converting microwave energy into heat. Dissipation factor is the capability of the solvent for absorption of microwave energy and its transformation into heat to be transferred to the surrounding molecules (Jain *et al*., 2009). Some applications of MAE for biologically active compounds have been presented in literature, examples of which are: extraction of polyphenols and caffeine from green tea leaves (Pan *et al*., 2003) and phenolic compounds from pistachio green hull (Rajaei *et al*., 2010).

Phenolic compounds act as antioxidants *via* such different mechanisms as radical scavenging, metal chelation, reducing power, and quenching of singlet oxygen, among others. There are several analytical methods including 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, reducing power, and phosphomolybdenum assay for evaluation of antioxidant activity of biological samples. Numerous studies have been carried out on antioxidant activity of different plants' organs. Oliveira *et al* (2009) reported that strawberry leaves disply high reducing power as well as scavenging capacity on DPPH radical. Also, a methanol extract of *Cinnamomum verum* leaf exhibited free radical scavenging activity, as well as reducing power especially against DPPH radical (Mathew *et al*., 2006). Furthermore, the efficiency of different natural antioxidants in stabilizing vegetable oils has already been put to study. Effect of rice bran (Chatha *et al*., 2006) as well as garlic extracts (Iqbal and Bhanger, 2007) for an inhibition of oxidation in sunflower oil has been reported.

The leaves of olive tree *Olea europaea*, contain secoiridoids (oleuropein, ligstroside, dimethyloleuropein, and oleoside), flavonoids (apigenin, kaempferol, luteolin), as well as phenolic compounds (caffeic acid, tyrosol, hydroxytyrosol). The leaves carry the highest content of these compounds among other different plant organs of the tree. For instance, percentage of oleuropein (as an olive biophenol model) in olive oil, alperujo and olive leaves ranges between 0.005-0.12%; 0.87 and 1-14% respectively (Lujan *et al*., 2006). Bouaziz and Sayadi (2005) demonstrated that olive leaf extracts exercise a considerable scavenging activity on DPPH. In addition, on enrichment of some oils (olive oil, sunflower oil, palm oil, as well as a vegetable shortening) with polyphenols of olive leaf extract secured protective effect against oxidation (Salta *et al*., 2009). In another study (Bouaziz *et al*., 2008) it was shown that hydrolysate and Chemlali olive leaf extracts are excellent antioxidants and can serve as substitutes for synthetic antioxidants in refined olive and in husk oils. This is while antioxidant effect of MAE olive leaf extracts have not been put to investigation yet.

In this work, olive leaf extracts obtained through Microwave Assisted Extraction (MAE) while applying different solvents were made use of, as a source of natural polyphenolic antioxidants, to have sunflower oil prevented from oxidation.

MATERIALS AND METHODS

Materials

Refined, Bleached, Deodorized (RBD) sunflower oil was obtained from Parto Danekhazar Company in Behshahr city (Mazandaran Province, North of Iran)**.** Olive leaves of Cronaiky and of Roghani varieties were collected from Kordkoy (Golestan Province, North of Iran). All the chemicals and reagents utilized were of analytical grade purchased from either Sigma or Merck Company.

Extraction

The leaves were dried by being exposed to open air, shady conditions until completely dry. They were then ground to a powder before being passed through a 60-mesh sieve. A household microwave oven (Samsung CF3110N-5, Korea) was modified in the laboratory by adding a magnetic stirrer, water condenser, temperature sensor and time controlling device as depicted in Figure 1 (Gharekhani *et al*., 2009). Olive leaf powder was mixed with different solvents (80% methanol, acetone, and water), irradiated under microwave in pre-setting procedures of: three times to the desired temperature and then 3 seconds power on for heating up and 15 seconds power off for cooling, but not allowed to super-boil. Irradiation was continued for 15 minutes. The extracts were filtered through Wattman No.1 filter paper to remove the fine particles. The filtrates obtained from methanol and acetone were evaporated to dryness at 40°C in a rotary evaporator (IKA RV 05 basic, Germany). The water extract was freeze-dried (Epron FDV5503, South Korea). The dried sample of each extract was stored at 4°C until further later use.

Determination of Total Phenolic Content

Total phenolic content in each extract was found out through Folin–Ciocalteu micro-method (Slinkard and Singleton, 1977). Briefly, 20 µl of each extract solution was mixed with 1.16 ml of distilled water plus 100 µl of Folin–Ciocalteu reagent, followed by addition of 300 µl of Na_2CO3 solution (20%) after 1 minutes and before 8 minutes. Subsequently, the mixture was incubated in a shaking incubator (Memmert WB14, Germany) at 40°C for 30 minutes and its absorbance determined at 760 nm (PG Instruments T80, UK). The phenolic content was determined using the following linear equation and based on the calibration curve:

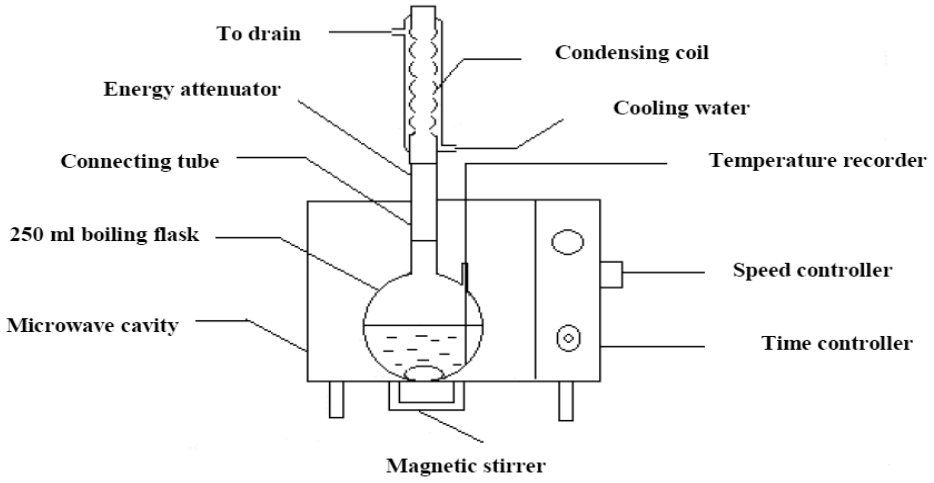

Figure 1. Modifications done on a household microwave oven.

$Y= 0.00114X+0.01062\ R^2 = 0.9964$ (1)

Where, *Y* stands for the absorbance and *X* is the concentration (µg ml^{-1}) as tannic acid equivalents.

DPPH Radical Scavenging Activity

DPPH radical scavenging activities of all the samples were evaluated by the method of Arabshahi and Urooj (2007). In brief, 1 ml of a 1 mM methanol solution of DPPH was mixed with 3 ml of extract solution in methanol (50–1,000 µg ml^{-1}). Then, the reaction mixture was incubated for 30 minutes at room temperature in the dark. The blank sample contained all the reagents excluding the olive leaf extract. The absorbance was measured at 517 nm and DPPH radical scavenging activity (%) of the sample calculated as:

$(A-B/A)\times100$ (2)

Where, *A* stands for the absorbance of control while *B* represents the absorbance of the sample.

The DPPH radical scavenging activities for BHA and BHT were also assayed for comparison.

Reducing Power Assay

The capacity of extracts to reduce iron (III) was determined according to the method of Yildirim *et al*. (2001). The dried extract (50–1,000 µg) in 1 ml of the pertinent solvent was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) plus 2.5 ml of potassium ferricyanide ($K_3Fe(CN)_6$, 10 g l^{-1}), then the mixture incubated at 50°C for 30 minutes. Following incubation, 2.5 ml of trichloroacetic acid (100 g l^{-1}) was added and the mixture was centrifuged (Centurion k2042, USA) at 1650g for 10 minutes. Finally, 2.5 ml of the supernatant solution was mixed with 2.5 ml of distilled water plus 0.5 ml of $FeCl_3$ (1 g l^{-1}) and the absorbance measured at 700 nm. High absorbance indicates high reducing power. The reducing powers of BHA and BHT were also determined for comparison.

Total Antioxidant Capacity

This assay is based on the reduction of Mo (VI) to Mo (V) by the sample and the subsequent formation of a green phosphate/Mo (V) complex at acidic pH (Prieto *et al*., 1999). An aliquot of 0.1 ml of sample solution (containing 50–1,000 µg of dried extract in 1 ml of the corresponding solvent) was mixed in an Eppendorf tube with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM of ammonium molybdate). The tubes were capped and incubated at 95°C for 90 minutes. Following cooling, the absorbance was measured at 695 nm against the blank. A typical blank contained 1 ml of the reagent solution and an appropriate volume of each solvent, and incubated under the same conditions as the rest of samples. The antioxidant activity of extracts was expressed as the absorbance of the sample. The higher absorbance value indicates a higher antioxidant activity. BHA and BHT were also assayed for making comparisons.

Oxidative Stability

Olive leaf extracts (200, 500 and 1,000 ppm concentration) and synthetic antioxidants (BHA, BHT and TBHQ at 100 and 200 ppm levels) were added to RBD sunflower oil with all the samples stored at 70°C in an oven for 12 days to evaluate their capability in retarding the oxidation processes. Control samples bearing no antioxidants were also placed under the same storage conditions. Analyses were made through the measurement of the Peroxide Value (PV) according to the method of AOAC (1990), with the antioxidant activity calculated while using the following equation:

$100-(PV_{sample}/PV_{control}\times100)$ (3)

Also, thiobarbituric acid value was estimated (Goli *et al*., 2005) to analyze the secondary metabolites of oxidation.

Statistical Analyses

The experiments were all carried out in triplicate. The data were analyzed using Analysis of Variance (ANOVA) while significant differences among means being determined through Duncan's Multiple Range Test at $P< 0.05$ and by use of SAS software.

RESULTS AND DISCUSSION

Total Phenolic Content

Legend keys have been appropriated for identification of different extracts. First letters are variety indicators (C= Cronaiky, R=Roghani) and second ones show the solvent type (W= water; M= methanol, A= acetone), respectively.

Table 1 presents total phenolic contents and Inhibition Concentration (IC_{50}) for different extracts. The concentration of phenolics, expressed as mg Tannic Acid Equivalent (TAE) g^{-1} extract, was dependent on the solvent type and variety. As seen in Table 1, the highest (230.5 mg TAE g^{-1}) and the lowest (131.67 mg TAE g^{-1}) phenolic compounds were obtained with methanol and actone extracts from Cronaiky variety. In terms of the solvent, methanol gave the highest extraction yield while acetone the lowest, respectively. Excluding actone extracts, Cronaiky carried higher yields of phenolic content than Roghani.

Polar solvents (methanol, ethanol, and water) benefit from a good ability for absorption of microwave energy due to their high dielectric constants (Zhang *et al*., 2008). Water presents the highest dielectric constant among such common solvents as methanol and ethanol but the dissipation factor for this solvent is significantly lower than those for the other solvents. Hence, the rate of microwave energy absorption by water is higher than the dispersion of this energy into heat. Finally, heating efficiency for ethanol and for methanol will be higher than that for water due to a high dissipation factor. Thus, solvents with a high dielectric constant and a high dissipation factor facilitate distribution of heat throughout the matrix and increase the extraction yield of target analytes (Jain *et al*., 2009).

Non-polar solvents remain transparent to microwaves due to their lower dielectric constant and dissipation factor, producing no heat under microwave and thus are not efficient in MAE (Mandal *et al*., 2007). That is why with acetone (non- polar solvent), total phenolic contents of extracts were low and the methanol extracts carried higher phenolic contents than water extracts. Mohsen and Ammar (2008) also confirmed for other plant species, polar solvents produce a higher yield of phenolic concentration are compared with the non-polar ones.

DPPH Scavenging Activity

In this work, antioxidant activity was evaluated with IC_{50} values and was expressed as µg of dry extract per 1 ml. The sample with the lowest IC_{50} presents a higher antioxidant activity. IC_{50} indicates the concentration of extract at which radical scavenging activity is 50% in DPPH assay. Extracts with high radical scavenging activities present lower IC_{50} values. IC_{50} values of the extracts stood between 86.81 and 168.73 µg ml^{-1}. The highest IC_{50} (86.81 µg ml^{-1}) of DPPH scavenging effect was related to CM. CA extract with an IC_{50} value of 168.73±0.11 µg ml^{-1}had the lowest antioxidant activity among all the extracts (Table 1).

Variety type and solvents used for polyphenol extraction exerted significant effects on DPPH scavenging. Cronaiky variety showed a higher antioxidant activity than Roghani except for its acetone extracts. Regardless of variety, methanol and acetone

Table 1. Total phenolic contents and IC_{50}* (µg ml^{-1}) of different solvent extracts from olive leaves.

Antioxidant	Total phenols	DPPH	Reducing power	Total antioxidant capacity
CM	230.5±0.13[a**]	86.81 ±0.43[f]	166.45 ±0.14[g]	128.37 ±0.07[h]
RM	226.84±0.39[b]	90.05 ±0.28[e]	215.91 ±0.79[e]	139.88 ±0.3[g]
CW	203.9±0.26[c]	101.66 ±0.6[d]	270.48 ±0.43[d]	310.07 ±0.35[e]
RW	190.68±0.3[d]	121.05 ±0.57[c]	280.05 ±0.29[c]	330 ±0.64[d]
CA	131.67±0.8[f]	168.73 ±0.11[a]	506.02 ±0.1[a]	453.45 ±0.32[a]
RA	144.41±0.45[e]	165.65 ±0.5[b]	381.78 ±0.2[b]	424.87 ±0.11[b]
BHA	-	89.05 ±0.38[e]	205.54 ±0.65[f]	422.3 ±0.5[c]
BHT	-	41.60 ±0.42[g]	59.15 ±0.19[h]	179.43 ±0.1[f]

* IC_{50} values: The effective concentration at which the absorbance was 0.5 for reducing power and total antioxidant capacity; DPPH radicals were scavenged by 50%.

** Values denoted by different letters within each column are significantly different (P< 0.05).

extracts exhibited the highest and lowest antioxidant activities in DPPH assay, respectively.

As BHT had the lowest IC_{50} (41.6 µg ml^{-1} none of the extracts could compete with this compound except CM which showed higher scavenging activity than BHA (IC_{50}= 89.05 µg ml^{-1}). Thus, CM extract can be used as a natural antioxidant replacement for BHA.

Reducing Power

In the case of reducing power, the IC_{50} value presents the concentration at which the absorbance is 0.5. IC_{50} values of the extracts were in the range of 59.154 to 506.02 µg ml^{-1}. Significant differences (P< 0.05) were observed between varieties and among solvents. According to the obtained results, Cronaiky presented a higher reducing power than Roghani (with an exclusion of acetone extract). Moreover, in terms of the type of solvent applied, methanol was shown to be the most effective solvent, producing highest reducing power while acetone resulting in the lowest antioxidant activity (Table 1).

CM with an IC_{50} value of 166.45 bore the highest reducing power, while CA presenting the lowest antioxidant activity (IC_{50}= 506.02) among all the extracts. Reducing power' of the extracts were of the following order:

BHT> CM> BHA> RM> CW> RW> RA> CA

Furthermore, as shown in Table 1, all the olive leaf extracts were of a lower reducing power than BHT, but the antioxidant activity of CM surpassed that of BHA.

Total Antioxidant Capacity

Like the reducing power, IC_{50} is the effective concentration at which the absorbance is 0.5. As seen in Table 1, among all samples, the highest IC_{50} (128.37) and lowest (453.45) for total antioxidant capacity belonged to CM and CA, respectively. IC_{50} values obtained for olive leaf extracts varied between 128.37 and 453.45 µg ml^{-1} with the following order observed:

CA> RA> BHA> RW> CW> BHT> RM> CM

Also, total antioxidant capacities of CM and RM were higher than that for BHT and with an exclusion of acetone extracts, all other extracts possessed stronger antioxidant activity than BHA. Thus, BHA can be replaced with methanol and water extracts obtained from olive leaf.

A significantly negative linear correlation was observed between the polyphenol content and IC_{50} values (y= -0.8785x+287.49, R^2= 0.98), reducing power (y= -2.8486x+839, R^2= 0.93), ans total antioxidant capacity (y= -

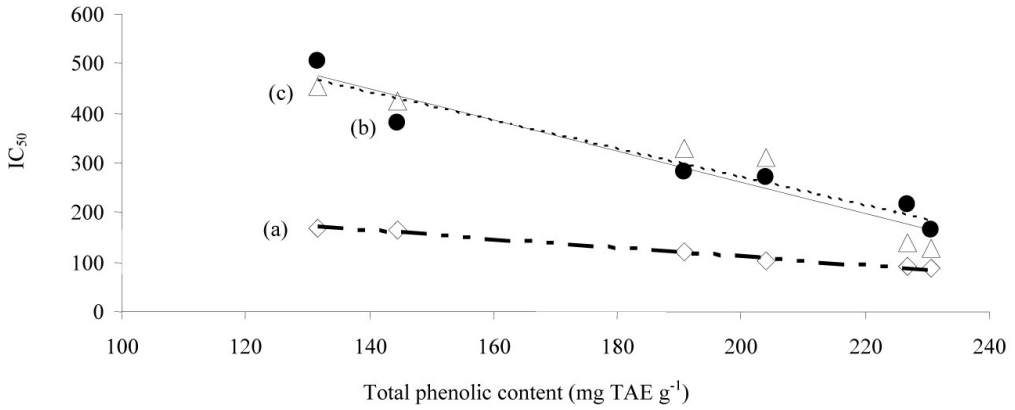

Figure 2. Correlation between total phenolic content and antioxidant activity: (a) DPPH scavenging activity; (b) Reducing power, (c) Total antioxidant activity. IC_{50} values: The effective concentration at which the absorbance was 0.5 for reducing power and total antioxidant capacity, DPPH radicals were scavenged by 50%.

3.1637x+892.56, R^2= 0.90) assays (Figure 2). This negative linear correlation means the samples with lower IC_{50} values were of higher polyphenol contents.

The results reveal that phenolics are likely to contribute to 98% of scavenging activity, 93% of reducing power, and 90% of the antioxidant capacity of the olive leaves. Also, it implies that antioxidant activity of plant extracts is not restricted to phenolics. Other such secondary antioxidant metabolites volatile oils, carotenoids, and vitamins may contribute to 2% scavenging activity, 7% reducing power and 10% of total antioxidant activity. The present study's results are similar to those of several other works done for the case of other plant species (Cai *et al*., 2004; Pan *et al*., 2007).

Furthermore, antioxidant activity depends on the nature and concentration of antioxidants, interaction between antioxidants inside the extracts, specific plant materials, reaction conditions and quantification method. So, acomparison of the data from different analytical approaches is difficult and making it necessary to use at least two methods for a determination of antioxidant activity (Contini *et al*., 2008).

As shown in Table 1, for each solvent, antioxidant activities of the two studied varieties were statistically different. Genotype and growth conditions (water availability, light quality and temperature) by affecting the quantity and/or types of phenolics can contribute to the differences among varieties. The data obtained for tea (Turkmen *et al*., 2006), leaf of Cinnamonum species (Prasad *et al*., 2009) and Iranian pomegranate seed extracts (Sadeghi *et al*., 2009) were similar to the present study's ones results.

Evaluation of the Antioxidant Activity of Olive Leaf Extracts on Sunflower Oil

Peroxide Value (PV)

Peroxide value measures hydroperoxide products formed in the initial stages of lipid oxidation and is a good indicator of oxidation state for oils. In the present study, oxidation degree of sunflower oil samples was determined by measuring the *PV* and Thiobarbituric Acid (TBA) value either in the absence or presence of antioxidants for a duration of 12 days.

The results indicated the influence of antioxidant type and storage time on *PV* as being statistically significant. Total mean *PV* and *TBA* value as well as the influence of antioxidants, during storage, on oxidation are shown in Tables 2 and 3, respectively.

Table 2. Total mean Peroxide value (meq kg^{-1} oil) and TBA (mg malonaldehyde/kg oil) for different antioxidants.

Treatment*	*PV*	*TBA*
Control	171.81[a]	0.203[a]
CM-200**	106[h]	0.117[m]
CM-500	76.39[m]	0.102[r]
CM-1000	50.39[p]	0.095[s]
RM-200	128.65[d]	0.127[h]
RM-500	82.61[l]	0.11[o]
RM-1000	63.10[o]	0.107[p]
CW-200	143.29[b]	0.163[b]
CW-500	123.08[f]	0.139[e]
CW-1000	107.52[h]	0.126[j]
RW-200	134.32[c]	0.151[d]
RW-500	112.61[g]	0.135[f]
RW-1000	101.77[i]	0.122[k]
CA-200	124.83[ef]	0.139[e]
CA-500	89.01[k]	0.126[i]
CA-1000	66.94[n]	0.115[n]
RA-200	105.27[h]	0.135[f]
RA-500	80.48[l]	0.121[l]
RA-1000	62.54[o]	0.106[q]
BHA-100	136.71[c]	0.155[c]
BHA-200	127.5[de]	0.134[g]
BHT-100	108.34[h]	0.136[f]
BHT-200	96.14[j]	0.122[k]
TBHQ-100	34.84[q]	0.082[t]
TBHQ-200	22.69[r]	0.061[u]

* Values denoted by different letters are significantly different (P< 0.05).

** The values following legend keys indicate concentration of antioxidants in oil.

For all the samples, an increase in *PV* was observed for longer durations of storage times. The *PV* of control sample which contained no antioxidant was at the highest level for all the days as compared with other treatments. Peroxide Value (PV) raised from 45.34 meq kg^{-1} of oil on the second day to 282.31 meq kg^{-1} on the 12th for control (As for control sample, the mean PV during 12 days was 171.81 meq kg^{-1} of oil). The lowest PV (22.69 meq kg^{-1} of oil) was observed for TBHQ-200 while the highest antioxidant activity achieved through CM-1000 with *PV* equal to 50.39 among all the extracts, followed by RA-1000 (62.53 meq kg^{-1} of oil) and RM-1000 (63.10 meq kg^{-1} of oil). As a general trend, antioxidant activity increased in higher extract concentrations. Roghani benefited from a higher antioxidant activity than Cronaiky expect for methanol extracts. At a concentration of 200 ppm, the highest level of *PV* were allocated to CW (143.29 meq kg^{-1} of oil), while CM (106 meq kg^{-1} of oil) accounted for the least. At a concentration of 500 ppm, the lowest and highest *PVs* went to CM (76.39 meq kg^{-1} oil) and CW (123.08 meq kg^{-1} of oil), respectively.

As regards synthetic antioxidants, it should be noted that CM-500, CM-1000, RM-500, RM-1000, RW-1000, CA-500, CA-1000, RA-500 and RA-1000 could compete with BHA and BHT at 100 and 200 ppm. However, no extract could retard the oxidation process as compared with TBHQ. Finally, CM-1000 can be recommended as a more suitable antioxidant for controlling the PV in sunflower oil.

As shown in Tables 3 and 4, the antioxidant efficacy of all the antioxidants decreased with storage. This reduction was more pronounced at lower concentrations. For example, the antioxidant activity of CM-200 on the second day was 72.66 which reached 25.49 meq kg^{-1} of oil on the 12th day, while in the case of CM-1000, this value decreased from 76.60 on the second day to 68.83 meq kg^{-1} of oil on the 12th day. This can be attributed to the oxidation phenomenon of antioxidants during their storage period. However, further reductions at lower concentrations of the extracts could be due to lower levels of hydroperoxides at their initial stage, so the lower concentrations of the extract can control the formation of these compounds. However on subsequent days, because of increasing and accumulation of these compounds, lower concentrations were not able to modulate the *PV* making it necessary to use a higher concentration of the extract. The most potent antioxidant activity was observed for TBHQ-200. During all the storage time, except for the second day, the greatest antioxidant activity belonged to CM-1000 among all the extracts and test materials.

Thiobarbituric Acid Value (TBA)

The *TBA* value is an indication of the formation of secondary oxidation products, reported as malonaldehyde equivalents. The

Table 3. Influence of antioxidants on *PV* (meq kg oil^{-1}) of sunflower oil during storage.

Treatment *	Day					
	2	4	6	8	10	12
Control	45.34 ^{a}	108.87 ^{a}	134.13 ^{a}	208.29 ^{a}	251.93 ^{a}	282.31 ^{a}
CM-200**	12.39 ^{ghi}	42.71 ^{ijk}	70.61 ^{ij}	123.73 ^{fg}	176.22 ^{f}	210.33 ^{g}
CM-500	14.5 ^{ghi}	35.48 ^{kl}	62.38 ^{k}	98.8 ^{i}	116.5 ^{j}	130.68 ^{n}
CM-1000	10.61 ^{ghij}	21.37 ^{m}	39.49 ^{m}	60.68 ^{k}	82.06 ^{m}	88.12 ^{q}
RM-200	14.05 ^{ghi}	49.59 ^{ghi}	102.66 ^{cd}	143.97 ^{cd}	208.63 ^{cd}	253.0 1 ^{c}
RM-500	10.36 ^{hi}	36.38 ^{kl}	69.23 ^{jk}	^{h} 107.68	126.79 ^{i}	145.23 ^{m}
RM-1000	9.37 ^{ij}	36.25 ^{l}	55.19 ^{l}	79.23 ^{j}	95.45 ^{kl}	106.1 ^{p}
CW-200	17.45 ^{fgh}	59.36 ^{de}	104.04 ^{c}	169.5 ^{b}	246.73 ^{a}	262.64 ^{b}
CW-500	11.24 ^{ghij}	45.69 ^{hij}	95.83 ^{de}	146.68 ^{c}	191.39 ^{e}	247.65 ^{cd}
CW-1000	9.62 ^{ij}	42.48 ^{ijk}	84.53 ^{g}	135.49 ^{e}	171.6 ^{f}	201.37 ^{h}
RW-200	14.78 ^{ghi}	52.82 ^{efgh}	95.78 ^{de}	151.45 ^{c}	223.56 ^{b}	267.5 ^{b}
RW-500	13.71 ^{ghi}	51.21 ^{fgh}	92.36 ^{ef}	147.4 3 ^{c}	174.49 ^{f}	196.47 ^{h}
RW-1000	11.44 ^{ghij}	47.69 ^{ghi}	86.63 ^{fg}	136.63 ^{de}	153.31 ^{gh}	174.9 ^{jk}
CA-200	25.62 ^{cde}	^{d} 66.28	86.53 ^{fg}	144.32 ^{cd}	191.07 ^{e}	235.17 ^{e}
CA-500	18.26 ^{efg}	54.3 ^{efg}	70.83 ^{ij}	108.45 ^{h}	125.63 ^{i}	156.61 ^{l}
CA-1000	15.72 ^{ghi}	37.1 ^{kl}	54.90 ^{l}	79.86 ^{j}	99.35 ^{k}	114.71 ^{o}
RA-200	23.53 ^{def}	55.39 ^{efg}	82.90 ^{gh}	126.7 6 ^{f}	160.85 ^{g}	182. 17 ^{ij}
RA-500	17.51 ^{fgh}	39.31 ^{jkl}	66.88 ^{jk}	101.47 ^{hi}	116.66 ^{j}	141.08 ^{m}
RA-1000	15.64 ^{ghi}	35.30 ^{kl}	52.80 ^{l}	77.593 ^{j}	88.78 ^{lm}	105.09 ^{p}
BHA-100	32.49 ^{bc}	90.85 ^{b}	114.61 ^{b}	135.68 ^{e}	201.47 ^{d}	245.12 ^{d}
BHA-200	38.44 ^{ab}	75.24 ^{c}	106.66 ^{c}	130.2 ^{ef}	193.71 ^{e}	220.75 ^{f}
BHT-100	36.46 ^{b}	65.85 ^{d}	84.68 ^{g}	117.28 ^{g}	157.31 ^{g}	188.45 ^{i}
BHT-200	26.73 ^{cd}	57.34 ^{ef}	77.34 ^{hi}	97.39 ^{i}	148.67 ^{h}	169.4 ^{k}
TBHQ-100	9.55 ^{ij}	15.64 ^{mn}	25.72 ^{n}	43.65 ^{l}	51.71 ^{n}	62.78 ^{r}
TBHQ-200	4.08 ^{j}	8.38 ^{n}	15.63 ^{o}	25.44 ^{m}	33.23 ^{o}	49.36 ^{s}
SD	1.2					

* Values denoted by different letters are significantly different (P< 0.05).
** The values following legend keys indicate concentration of antioxidants in oil.

effect of treatment and time on *TBA* was statistically significant (P< 0.05). Table 5 presents treatment effects on this index during the storage time.

A maximum *TBA* of 0.203 mg malonaldehyde equivalent kg^{-1} oil was observed for the control. This value was 0.079 malonaldehyde equivalent kg^{-1} oil after the second day while on the 12th day, it reached around 0.420 meq kg^{-1} of oil. TBHQ treated oil contained the lowest level of *TBA* (0.061 mg malonaldehyde equivalents kg^{-1} of oil) but CM-1000 (0.095 mg malonaldehyde equivalent kg^{-1} oil) was the most suitable extract for monitoring *TBA* among all the extracts.

The higher the extract content, the higher was the antioxidant activity. At 500 ppm concentraction, the highest antioxidant activity belonged to CM (0.102 mg malonaldehyde kg^{-1} oil), while CW (0.139 mg malonaldehyde equivalent kg^{-1} oil) presented the lowest. As for 200 ppm, methanol *vs.* water extracts of Cronayki contained the highest and the lowest *TBA*, respectively.

Excluding methanol extracts, Roghani extracts affected the formation of *TBA* reactive substances more strongly than the Cronaiky ones.

The *TBA* content of CM at 200, 500 and 1,000 ppm levels, RM at 500 and 1,000 ppm, CA and RW of 1,000 ppm were lower than those for BHA and BHT but all treatments were less effective than TBHQ. Hence, these treatments can be replaced for BHA and BHT in foods. Nevertheless, none of the treatments could compete with TBHQ.

Table 4. Antioxidant activity for different samples during storage.

Treatment	Day					
	2	4	6	8	10	12
CM-200	72.66±0.01^{e}	60.76±0.65^{g}	47.34±0.46^{h}	40.59±0.61^{j}	30.05±0.31^{n}	25.49±0.33^{jk}
CM-500	68±0.07^{fg}	67.4±0.57^{e}	53.49±0.28^{f}	52.56±0.28^{f}	53.75±0.18^{g}	53.71±0.14^{de}
CM-1000	76.6±0.24^{cd}	80.37±0.64^{c}	70.55±0.34^{c}	70.86±0.24^{c}	67.42±0.45^{c}	68.83±0.09^{b}
RM-200	68.99±0.52^{f}	54.44±0.26^{j}	23.46±0.48^{o}	30.88±0.033^{n}	17.18±0.21^{r}	10.37±0.35^{mno}
RM-500	77.14±0.54^{bcd}	66.57±0.59^{e}	48.37±0.16^{h}	48.3±0.08^{h}	49.67±0.04^{h}	48.55±0.04^{ef}
RM-1000	79.32±0.24^{b}	69.45±053^{d}	58.85±0.45^{e}	61.95±0.19^{de}	62.11±0.2^{e}	62.41±0.11^{bc}
CW-200	61.51±0.27^{i}	45.47±0.23^{o}	22.43±0.23^{o}	18.62±0.37^{q}	2.06±0.29^{t}	6.96±0.06^{no}
CW-500	75.2±0.54^{de}	58.02±0.42^{h}	28.54±0.04^{n}	29.57±0.29^{o}	24.03±0.22^{o}	12.27±0.18^{mno}
CW-1000	78.78±0.24^{bc}	60.97±0.72	36.97±0.53^{jk}	34.95±0.24^{m}	31.88±0.2^{m}	28.67±0.27^{ijk}
RW-200	67.4±0.27^{fgh}	51.48±0.39^{kl}	28.58±0.11^{n}	27.28±0.65^{p}	11.26±0.43^{s}	5.24±0.05^{o}
RW-500	69.75±0.11^{f}	52.96±0.37^{jk}	31.13±0.24^{m}	29.21±0.12^{o}	30.73±0.32^{n}	30.4±0.2^{ij}
RW-1000	74.75±0.06^{de}	56.19±0.15^{i}	35.4±0.55^{l}	34.4±0.19^{m}	39.14±0.29^{j}	38.04±0.12^{gh}
CA-200	43.48±0.5^{k}	39.12±0.5^{p}	35.48±0.47^{l}	30.71±0.09^{n}	24.15±0.15^{o}	16.69±0.12^{lm}
CA-500	59.71±0.71^{i}	50.12±0.55^{lm}	47.19±0.44^{h}	47.93±0.17^{h}	50.13±0.45^{h}	44.52±0.1^{fg}
CA-1000	65.31±0.27^{h}	65.91±0.24^{e}	59.06±0.43^{e}	61.65±0.04^{e}	60.56±0.11^{f}	59.36±0.06^{cd}
RA-200	48.1±0.19^{j}	49.12±0.56^{m}	38.19±0.42^{j}	39.14±0.34^{k}	36.15±0.23^{l}	35.47±0.17^{hi}
RA-500	61.376±0.77^{i}	63.88±0.46^{f}	50.13±0.43^{g}	51.28±0.36^{g}	53.69±0.16^{g}	50.02±0.03^{ef}
RA-1000	65.5±0.414^{gh}	67.56±0.64^{e}	60.63±0.44^{d}	62.74±0.25^{d}	64.75±0.2^{d}	62.77±0.11^{bc}
BHA-100	28.33±0.24^{m}	16.54±0.52^{r}	14.55±0.86^{q}	34.86±0.26^{m}	20.02±0.35^{q}	13.17±0.42^{mn}
BHA-200	15.22±0.52^{o}	30.88±0.44^{q}	20.48±0.47^{p}	37.49±0.34^{l}	23.1±0.43^{p}	21.8±0.24^{kl}
BHT-100	19.59±0.53^{n}	39.5±0.54^{p}	36.86±0.47^{k}	43.69±0.39^{i}	37.55±0.23^{k}	33.24±0.25^{hi}
BHT-200	41.03±0.36^{l}	47.32±0.55^{n}	42.33±0.43^{i}	53.24±0.39^{f}	40.98±0.42^{i}	39.99±0.27^{gh}
TBHQ-100	78.93±0.13^{bc}	85.63±0.57^{b}	80.82±0.51^{b}	79.04±0.24^{b}	79.47±0.07^{b}	77.76±0.24^{a}
TBHQ-200	90.99±0.01^{a}	92.29±0.27^{a}	88.34±0.27^{a}	87.78±0.15^{a}	86.8±0.19^{a}	82.51±0.24^{a}

* The values after Legend keys indicate concentration of antioxidants in oil.
** Values followed by different letters are significantly different ($p < 0.05$).

Table5. Influence of antioxidants on *TBA* (mg malonaldehyde kg^{-1} oil) of sunflower oil during storage time.

Treatment*	Day					
	2	4	6	8	10	12
Control	0.079^{a}	0.146^{a}	0.186^{a}	0.24^{a}	0.317^{a}	0.42^{a}
CM-200**	0.056^{no}	0.107^{k}	0.111^{n}	0.148^{l}	0.156^{k}	0.205^{l}
CM-500	0.053^{p}	0.105^{l}	0.108^{o}	0.124^{s}	0.128^{p}	0.163^{r}
CM-1000	0.052^{p}	0.104^{l}	0.106^{p}	0.116^{u}	0.123^{q}	0.138^{s}
RM-200	0.068^{ef}	0.111^{hi}	0.128^{h}	0.161^{h}	0.169^{j}	0.221^{k}
RM-500	0.066^{g}	0.109^{ij}	0.118^{k}	0.126^{r}	0.138^{n}	0.183^{o}
RM-1000	0.063^{ij}	0.105^{l}	0.112^{mn}	0.133^{p}	0.132^{o}	0.169^{q}
CW-200	0.062^{klm}	0.109^{j}	0.125^{i}	0.168^{e}	0.276^{b}	0.366^{b}
CW-500	0.062^{jklm}	0.106^{k}	0.121^{j}	0.157^{i}	0.201^{e}	0.296^{d}
CW-1000	0.057^{n}	0.104^{l}	0.115^{l}	0.133^{p}	0.185^{f}	0.253^{g}
RW-200	0.063^{ijk}	0.133^{d}	0.144^{de}	0.174^{c}	0.209^{c}	0.298^{c}
RW-500	0.061^{m}	0.125^{f}	0.133^{g}	0.14^{o}	0.186^{f}	0.271^{f}
RW-1000	0.057^{n}	0.12^{g}	0.127^{h}	0.128^{q}	0.168^{j}	0.221^{k}
CA-200	0.069^{d}	0.137^{c}	0.143^{e}	0.172^{d}	0.183^{g}	0.238^{h}
CA-500	0.067^{fg}	0.134^{d}	0.137^{f}	0.145^{m}	0.175^{h}	0.194^{n}
CA-1000	0.064^{hi}	0.121^{g}	0.128^{h}	0.132^{p}	0.154^{l}	0.176^{p}
RA-200	0.065^{h}	0.136^{c}	0.145^{d}	0.165^{f}	0.176^{h}	0.228^{j}
RA-500	0.062^{ijkl}	0.129^{e}	0.133^{g}	0.142^{n}	0.15^{m}	0.136^{t}
RA-1000	0.061^{lm}	0.111^{h}	0.113^{m}	0121^{t}	0.138^{n}	0.164^{r}
BHA-100	0.074^{b}	0.145^{a}	0.153^{b}	0.19^{b}	0.207^{d}	0.285^{e}
BHA-200	0.072^{c}	0.128^{e}	0.15^{c}	0.154^{j}	0.171^{i}	0.23^{i}
BHT-100	0.069^{de}	0.14^{b}	0.153^{b}	0.163^{g}	0.171^{i}	0.222^{k}
BHT-200	0.055^{o}	0.122^{g}	0.144^{de}	0.15^{k}	0.152^{l}	0.196^{m}
TBHQ-100	0.058^{n}	0.077^{m}	0.084^{q}	0.096^{v}	0.108^{r}	0.12^{u}
TBHQ-200	0.042^{q}	0.054^{n}	0.061^{r}	0.068^{w}	0.073^{s}	0.097^{v}
SD	0.0005					

* Values denoted by different letters are significantly different ($P< 0.05$).
** The values following legend keys indicate concentration of antioxidants in oil.

During the early days, *TBA* was low but on subsequent days, the rate of hydroperoxide decomposition was higher than the rate of formation for these products. Therefore, the content of TBA reactive substances increased with an increase in storage period and a higher concentration of the extract exhibited a more prononged delay of oxidation. Moreover, in early days, differences among extracts were insignificant for *TBA* and *PV*, but on subsequent days, significant differences were observed and the 1,000 ppm concentration showed a strong stabilization of sunflower oil as compared with other concentrations.

The results revealed that methanol extracts were the most effective in retardation of oxidation, a phenomenon that can be explained by their high phenolic contents. However, acetone extracts resulted in the lowest total phenolic content among the solvents, but because of their dicarboxylic phenolic contents and their high solubility in oil, these extracts presented more inhibition effects against oxidization than water extracts (Korukluoglu *et al*., 2004).

As TBA reactive substances are the secondary oxidation products obtained from decomposition of such initial oxidation products as hydroperoxides, an increasing trend of *TBA* value was lower than *PV* with the results for *TBA* and *PV* being different. This can be attributed to various mechanisms of antioxidants (prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, and radical scavenging). Hence some antioxidants can inhibit chain initiation and formation of hydroperoxides (PV), but certain antioxidants contribute to the decomposition of hydroperoxides and formation of secondary oxidation products.

Maisuthisakul and Charuchongkolwongse (2007) and Mariod *et al* (2006) reported similar results for the cases of *Cratoxylum formosum* and *Guiera senegalensis*, respectively.

CONCLUSIONS

Results finally indicated that methanol extracts possess the highest phenolic content among the extracts, leading to the highest antioxidant activity in DPPH assay, reducing power as well as total antioxidant activity. Application of MAE extracts to prevent sunflower oil oxidation showed that *PV* and *TBA* values increased with duration of storage while being decreased with extract concentration. One thousand ppm concentration led to the most antioxidant activity for retardation of oxidation and there was a significant difference observed among extracts, except for the early day durations.

CM-1000 exerted the most powerful effect on oxidation control and was stronger than BHA and BHT, still lower than TBHQ. Finally CM-1000 can be recommended as a potential source of antioxidants for maintaining stabilization in food systems, especially unsaturated vegetable oils.

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اثر آنتی اکسیدانی عصاره های مایکروویوی برگ های زیتون بر روغن آفتابگردان

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چکیده

ویژگی های آنتی اکسیدانی و میزان ترکیبات فنولی کل عصاره های برگ زیتون (دو واریته کرونایکی و روغنی) استخراجی توسط حلال های متانول ۸۰ درصد، آب و استون به کمک امواج مایکروویو مورد آزمون قرارگرفت. آزمون های مختلفی برای سنجش فعالیت آنتی اکسیدانی استفاده شد. نتایج نشان داد بالاترین میزان ترکیبات فنولی (۲۳۰/۵ میلی گرم تانیک اسید در گرم عصاره) و کمترین میزان IC ۵۰ در آزمون های DPPH (۸۶/۸۱ میکروگرم در میلی لیترعصاره) نیروی احیاکنندگی (۱۶۶/۴۵ میکروگرم در میلی لیترعصاره) و ظرفیت آنتی اکسیدانی کل (۱۲۸/۳۲ میکروگرم در میلی لیترعصاره) مربوط به عصاره متانولی واریته کرونایکی بود. در ادامه، کارایی عصاره ها در سه سطح (۲۰۰، ۵۰۰۰ و ۱۰۰۰ پی پی ام) بر پایداری اکسیداتیو روغن آفتابگردان با اندازه گیری دو فاکتور اندیس پراکسید و اسید تیوباربیتوریک در دمای ۷۰ درجه سانتیگراد بررسی شد (تست آون). کمترین میزان اندیس پراکسید و اسید تیوباربیتوریک در عصاره های متانولی واریته کرونایکی در سطح ۱۰۰۰ پی پی ام مشاهده شد. همچنین این عصاره قابل رقابت با دو آنتی اکسیدان سنتزی BHA و BHT در هر دو سطح مورد آزمون (۱۰۰ و ۲۰۰ پی پی ام) بود. بنابراین عصاره متانولی واریته کرونایکی برگ های زیتون می تواند جایگزین آنتی اکسیدان های سنتزی در روغن های خوراکی شود.