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## Scavenging Activity of Different Garlic Extracts and Garlic Powder and their Antioxidant Effect on Heated Sunflower Oil

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### ABSTRACT

Seven garlic extracts were prepared in different solvents. The extract yield ranged from 0.36 (n-hexane) to 71.73% water (H<sub>2</sub>O at 50°C), total phenolic content from 11.16 methanol/water (MeOH/H<sub>2</sub>O, 1:1) to 17.32 (H<sub>2</sub>O) mg GAE g<sup>-1</sup> extract and antioxidant activity (DPPH) from 27.25 (H<sub>2</sub>O) to 16.39% (MeOH/H<sub>2</sub>O, 1:1) at concentration of 150 µg µL<sup>-1</sup>, whereas IC<sub>50</sub> ranged from 0.27 (H<sub>2</sub>O) to 0.53 (MeOH/H<sub>2</sub>O, 1:1) mg mL<sup>-1</sup> DPPH. The efficiency of Garlic Extracts (GE) and Garlic Powder (GP) were studied as antioxidants, in comparison with BHT, to stabilize sunflower oil (SFO) heated at frying temperature. Each GE and GP was added to SFO (0.1 g mL<sup>-1</sup> of oil) and heated in a draft oven at 180°C for 15 h (3 h day<sup>-1</sup>). Similar heating test was carried out using legal limit of BHT at concentration of 200 ppm. The progress of lipid oxidation was measured in terms of Peroxide Value (PV), Acid Value (AV), Conjugated Dienes, (CD) and Conjugated Trienes (CT). The results of this study showed that GE and GP have inhibition effect on lipid oxidation. The reduction percent in peroxide values after using GE and GP as antioxidants ranged from 53.8 (SFO-H<sub>2</sub>O) to 73.2 (SFO-MeOH) approximately and the reduction percent in acid value ranged from about 6.8 (SFO-H<sub>2</sub>O) to 24.1 (SFO-MeOH) in comparison to about 7.7 and 34.2%, after 15 h, respectively, for SFO-BHT. Whereas, the reduction percent in CD and CT, after 15 h, ranged from about 21.6 (SFO-MeOH/H<sub>2</sub>O, 1:1) to 50.3 (SFO-GP) and from about 11.5 (SFO-MeOH/H<sub>2</sub>O, 1:1) to 22.2 (SFO-GP), respectively, in comparison to about 2.6 and 24.1, respectively, for SFO-BHT.

**Key words:** Sunflower oil, garlic extract, garlic powder, phenolic compounds, scavenging activity, IC<sub>50</sub>, PV, AV, CD, CT

### INTRODUCTION

Belonging to the Alliaceae family, garlic has been used throughout recorded history for both culinary and medicinal propose (McGee, 2004). Garlic is known in history as treatment for cold, cough and asthma and is reported to strengthen immune system (Lawrence and Lawrence, 2011). It has many medicinal effects such as lowering of blood cholesterol level (Yeh and Yeh, 1994), antiplatelet aggregation (Steiner *et al.*, 1996), anti-inflammatory activity (Baek *et al.*, 2001) and inhibition of cholesterol synthesis (Piscitelli *et al.*, 2002). Garlic has been long known to have antibacterial (Rees *et al.*, 1993; Sasaki *et al.*, 1999; Shobana *et al.*, 2009), antifungal (Pai and Platt, 1995), anticancer (Unnikrishnan and Kuttan, 1990; Kaschula *et al.*, 2010), antioxidant (Galano and Francisco-Marquez, 2009) and antiviral (Weber *et al.*, 1992).

It is a well-known fact that free radicals and other reactive species formed in living cells play an important role in origin of life and biological evaluation (Lawrence and Lawrence, 2011). Free

radicals can also cause lipid peroxidation in foods and lead to their deterioration (Perry *et al.*, 2000; Sundaram and Mitra, 2007; Ozsoy *et al.*, 2008).

Although, there are some synthetic antioxidants such as Butylated Hydroxyl Anisole (BHA) and Butylated Hydroxyl Toluene (BHT), however consumers are concerned about the safety of synthetic food additives. This concern has aroused a great interest in natural additives (Pokorny, 1991).

The antioxidants are now known to play an important role in protection against disorders caused by oxidant damage (Abdou, 2011). The term antioxidants refers to compounds that can inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reaction (Velioglu *et al.*, 1998) and which can thus prevent or repair damage done to the body's cells by oxygen. They act in one or more of the following ways: reducing agents, free scavengers, potential complexers of prooxidant metals and quenchers of singlet oxygen (Hudson, 1990).

The pharmaceutical properties of garlic is strictly associated with the presence of phenolic compounds (phenolic acids, flavonoids), polysaccharides and proteins (Bianchini and Vainio, 2001; Nishimura *et al.*, 2004). Phenolic acids are important adjunctive and physiological constituent of major active substances.

Garlic is considered to be one of the best disease-preventive foods, based on its potential and varied effects (Amagase, 2006). Data that characterize the antioxidant properties related to phenolic and flavonoid compounds of garlic were recorded (Miller *et al.*, 2000; Nuutila *et al.*, 2003; Lawrence and Lawrence, 2011). Chung (2006) reported that garlic and garlic extracts possess antioxidant activity and they supply protection against free radical damage in the body. Wangcharoen and Morasuk (2009) studied the antioxidant activity of dry and wet heated garlic, they found that heating affect the antioxidant activity because of the decomposition of the phenolic compounds.

Different solvents were used for the extraction of garlic, MeOH, EtOH, water, or their admixtures were the most abundant solvents used for the extraction of garlic (Leelarungrayub *et al.*, 2006; Iqbal and Bhanger, 2007; Bozin *et al.*, 2008; Temitope *et al.*, 2010; Drozd *et al.*, 2011; Abdou, 2011; Rafieian-Kopaei *et al.*, 2013). Other solvents like: ether, acetone, hexane or ethyl acetate were also used for the extraction of garlic (Iqbal and Bhanger, 2007; Bozin *et al.*, 2008).

The aim of this work was to evaluate the effect of different extracted solvents on the yield of the antioxidants of garlic and their antioxidant activities. It was also aimed to study the effect of the different garlic extracts as well as garlic powder on the stability of sunflower oil (SFO) (heated at frying temperature) in comparison with a synthetic antioxidant BHT.

## **MATERIALS AND METHODS**

**Materials and reagents:** Fresh garlic cloves were purchased from the local market in March 2014. The RBD sunflower oil (refined, bleached and deodorized SFO and free from any antioxidants) was supplied by Cairo Oil Company refineries, Cairo, Egypt. All the Reagents used were of analytical grade and were purchased from E. Merck. Folin and Ciocalteu's Phenol Reagent (FCP reagent) AR, purchased from Sisco Research Laboratories Pt. Ltd. Butylated Hydroxy Toluene (BHT) was obtained from NICE Chemicals, India. Whereas, 2,2-diphenyl-1-picrylhydrazyl (DPPH) was obtained from Sigma Chemical Co (St. Louis, MO, USA). Gallic acid 1-hydrate, purchased from ARABLAB.

## **METHODS**

**Determination of moisture content:** A fresh garlic sample (about 20 g) was minced to fine particles and dried in an oven at 40°C for three days until constant weight. The dried garlic was weighed and the moisture content was calculated.

**Preparation of dried garlic stock:** A sample of fresh garlic (4.5 kg) was minced and dried in an oven at 40°C for three days. The dried sample was ground into fine particles to give Garlic Powder (GP).

### **Extraction procedure**

**Screening of solvents used in extraction:** Samples of GP (20 g each) were accurately weighed and each one was extracted with 120 mL of the following solvents: methanol (MeOH), chloroform/methanol (CHCl<sub>3</sub>/MeOH, 2:1, v/v), methanol/water (MeOH/H<sub>2</sub>O, 1:1, v/v), methanol/chloroform (MeOH/CHCl<sub>3</sub>, 2:1, v/v), acetone, n-hexane and water. The extraction was carried out by refluxing the GP sample with 120 mL of each of the above solvents (except water) for 1 h on a water bath then left at room temperature overnight. The solution was then filtered and the extraction procedure was repeated twice on the residue. The filtrates were collected and subjected to evaporation by a rotary evaporator under reduced pressure at 40°C to remove the solvent. The extract was weighed and the yield percent was calculated before storing under nitrogen in a refrigerator for further analysis.

**Preparation of water extract:** A sample of about 20 g GP was accurately weighed and mixed with 120 mL water then warmed on water bath (50°C) with stirring for three days (4 h day<sup>-1</sup>) according to Abdou (2011). The solution was filtered and the filtrate was evaporated on a rotary evaporator under reduced pressure with alternative addition of small quantity of acetone and absolute ethanol to remove the water. The percent crude yield of water extract was calculated.

**Determination of total phenolics:** The concentration and the total phenolics content of the four garlic extracts [MeOH, MeOH/CHCl<sub>3</sub> (2:1, v/v), MeOH/H<sub>2</sub>O (1:1, v/v) and H<sub>2</sub>O] were determined colorimetrically using Folin-Ciocalteu (FC) reagent according to Singh *et al.* (2002) with slight modification. Samples containing polyphenols are reduced by the FC reagent by producing blue colored complex. The phenolic concentration of the extracts was evaluated from a gallic acid calibration curve. Thus, 0.01 g Gallic Acid (GA) was dissolved in 100 mL methanol (i.e. each 10 µL MeOH contains 1 µg gallic acid). A series of different concentrations (25, 50, 75, 100, 125, 150, 175 and 200 µg/10 µL) were prepared. Each concentration was brought up to 3 mL by distilled water. Two milliliter of 10-fold diluted FC reagent was added to the test tube. After 5 min, 1 mL of 7.5% sodium carbonate solution was added and standing for 30 min at room temperature. The blank was prepared by replacing the GA with 3 mL distilled water. The absorbance was measured at 765 nm using a UV-visible spectrophotometer (UV-160 1PC, UV-visible spectrophotometer, Shimadzu, Tokyo, Japan). The calibration curve was performed by plotting the value of absorbance versus concentration. A similar procedure was adopted for the extracts as described above in the preparation of the calibration curve. All determinations were performed in triplicate. Total phenolic content was expressed as milligrams of Gallic Acid Equivalent per gram of extract (GAE). For the extracts, briefly, 90 mg of each dried extract were dissolved in the least amount of its solvent and transferred to 100 mL measuring flask and make up with distilled water

to the mark. Suitable aliquot (150  $\mu\text{L}$ ) from the above solution, was put in a test tube and the volume was brought up to 3 mL by distilled water and similar steps were adopted for the four extracts as described above in the preparation of the calibration curve. The linear equation on the calibration curve is as follows:

$$y = 31.68 x - 0.0171, [\text{i.e. } x = (y + 0.0171) / 31.68]; (R^2 = 0.9687)$$

where, y is the absorbance (nm) and x is the concentration ( $\text{mg GAE g}^{-1}$  dried extract).

**Determination of DPPH<sup>•</sup>-Free Radical Scavenging Activity (FRSA):** The hydrogen atom or electron donating ability of garlic extracted samples and BHT was determined from bleaching of purple colored solvent solution of DPPH. This spectrometric assay uses the stable radical DPPH as a reagent (Lawrence and Lawrence, 2011). The antioxidant activity of the four garlic extracts [MeOH, MeOH/CHCl<sub>3</sub> (2:1, v/v), MeOH/H<sub>2</sub>O (1:1, v/v) and H<sub>2</sub>O] were determined. The standard was assessed on basis of the radical scavenging effect of the stable DPPH radical by the method described by Elmastas *et al.* (2007) with slight modification. Briefly, 90 mg of each dried extract were dissolved in its solvent, ( $\text{mg mL}^{-1}$ ) and transferred to 100 mL measuring flask and make up with its solvent to a final volume of 100 mL. Three different volumes containing 50, 100 and 150  $\mu\text{g mL}^{-1}$  from the above solution were put in a graduated test tube and brought up to 4 mL of freshly prepared DPPH (0.0024% in methanol) by micropipette and thoroughly mixed by vortex. The mixture was shaken for 1 min and kept for 30 min in a dark place. The absorbance (A) of the reaction mixture at 517 nm was measured with a spectrophotometer. The blank was prepared by replacing the extract with methanol or methanol/water (1:1, v/v) or water, according to the used extract. The BHT was used as positive control and the same procedure was followed. All measurements were carried out in three replicates. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The percentage of free radical scavenging activity was calculated as follows:

$$\text{FRSA (\%)} = \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \times 100$$

**Calculation of IC<sub>50</sub>:** From plotting the different concentrations (50, 100 and 150  $\mu\text{g mL}^{-1}$ ) of the four garlic extracts [MeOH, MeOH/CHCl<sub>3</sub> (2:1, v/v), MeOH/H<sub>2</sub>O (1:1, v/v) and H<sub>2</sub>O], against FRSA, IC<sub>50</sub> was calculated via linear equation for the four extracts and BHT then the IC<sub>50</sub> (X at Y = 50%) was calculated. IC<sub>50</sub> is the concentration of the inhibitor extract where the response is reduced by half and caused 50% decrease in DPPH absorbance.

### **Thermal oxidation of SFO**

**Preparation of samples:** Five beakers (100 mL each) were used for each of the three extracts [MeOH, MeOH/H<sub>2</sub>O (1:1, v/v) and H<sub>2</sub>O] as well as GP, BHT and a control sample. It is noteworthy to mention that the methanol/chloroform (2:1) extract was excluded in this experiment because chloroform is not safe in food applications. In each beaker and according to Oyewole and Olayinka (2007), a sample of 30 mL SFO was mixed separately with 3 g of GP or one of the above extracts (0.1 g antioxidant per mL of oil, dissolved in a least amount of water) and heated at  $180 \pm 2.0^\circ\text{C}$  in a draft oven for five days (3 h per day). During heating in the oven, the samples were stirred

occasionally with a glass rod to ensure well aeration and mixing. After 3 h of heating the beakers were allowed to cool down in a desiccator at room temperature overnight. In the second day, the contents of one beaker were kept in a dark glass bottle in a refrigerator for further use. The above heating procedure was repeated for another four days to get finally six samples heated at 180°C for 3, 6, 9, 12 and 15 h. Similar heating test was carried out on SFO with BHT at their legal limit of 200 ppm as well as a control SFO sample (without addition of any antioxidant).

**Evaluation of lipid oxidation:** Peroxide Value (PV) and Acid Value (AV) of SFO (control) and the heated oil samples were determined according to the recommended methods of AOCS Official Method (AOCS., 1989).

**Conjugated Dienes (CD) and Conjugated Trienes (CT):** Conjugated dienes and conjugated trienes formed in heated oil samples were assessed based on IUPAC procedure (IUPAC., 1979). An amount of the heated oil sample was accurately weighed into a 25 mL volumetric flasks and the samples were diluted with n-hexane instead of iso-octane and the absorbance (A) was measured at selective wavelengths ( $\lambda$ ) 234 and 268 nm, for CD and CT, respectively, in 10 mm quartz cell. The absorbance reading should be lies between 0.2-0.8 using n-hexane as a blank. The UV-visible spectrophotometer (UV-160 1PC, UV-visible spectrophotometer, Shimadzu, Tokyo, Japan) was used. All the measurements were carried out in triplicate and the results are expressed as the specific extinction values K 234 and K 268 following the equation:

$$\text{The specific absorbance } E_{1\text{cm}}^{1\%}(\lambda) = A_{\lambda} / c \times d$$

where, c is the concentration of the sample solution (g/100 mL), d is the cell length in cm. K 234 and K 268: specific extinction value at  $A_{\lambda 234}$  and  $A_{\lambda 268}$  nm.

**Statistical analysis:** All analyses were performed on triplicate samples and the data was expressed as Mean $\pm$ Standard Deviations (SD) (n = 3). Statistical data analysis of the various samples were analyzed using one way analysis of variance (ANOVA PC-STAT, 1985 Version IA copyright, University of Georgia) to determine the significant differences. A probability (p) value less than 0.05 were considered statistically significant, at a 5% significance level (p<0.05).

## RESULTS AND DISCUSSION

The moisture content of fresh garlic was determined and its value was 78.51%.

**Yield extract percent:** The percentage yield extract of the different used solvents was shown in the Fig. 1. The yield percent for all used solvents was as follows: H<sub>2</sub>O>MeOH/H<sub>2</sub>O>MeOH>MeOH/CHCl<sub>3</sub>>CHCl<sub>3</sub>/MeOH>acetone>n-Hexane with values of: 71.73>57.26>21.97>12.41>4.80>1.39>0.36. It was decided to exclude the CHCl<sub>3</sub>/MeOH (2:1, v/v), acetone and n-hexane extracts because of their poor yield. The yield of MeOH extract was comparable to the results of Iqbal and Bhangar (2007), who reported that the percentage yield was 23.15%.

**Total phenolics:** The antioxidant activity of any plant is attributed to its active components present in it (Abdou, 2011; Raza *et al.*, 2014). The total phenols of garlic extracts were determined using the Folin-Ciocalteu method (Table 1). The total phenols content of the four extracts

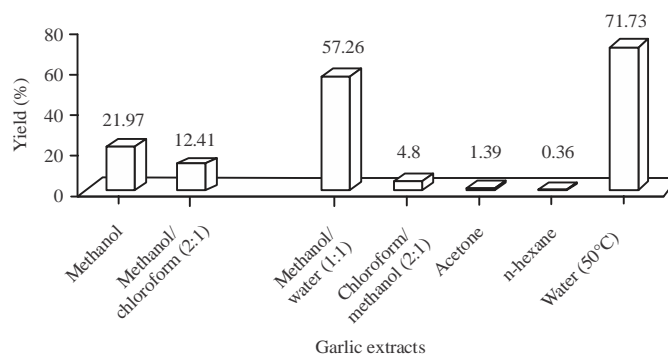


Fig. 1: Percentage yield extract of the different garlic extracts

Table 1: Total phenolics contents of garlic extracts and garlic powder (K 765 nm)

Sample extract	mg GAE g <sup>-1</sup> extract
MeOH	14.48±0.03 <sup>c</sup>
MeOH/CHCl <sub>3</sub> (2:1)	15.39±0.04 <sup>b</sup>
MeOH/H <sub>2</sub> O (1:1)	11.16±0.04 <sup>d</sup>
H <sub>2</sub> O	17.32±0.02 <sup>a</sup>

K 765: specific extinction value at 765 nm, Means which are not significantly different are followed by the same number. Significance level (p<0.05), <sup>a-d</sup>Means in each column followed by different superscripts letters are significantly different (p<0.05)

Table 2: Scavenging effect of garlic extracts and butylated hydroxyl toluene on 2,2-diphenyl-1-picryl-hydrazyl free radicals

Garlic extract	DPPH inhibition (%)			IC <sub>50</sub> (mg mL <sup>-1</sup> )
	50	100	150	
	Concentrations (µg µL <sup>-1</sup> )			
MeOH	5.97±0.00 <sup>dC</sup>	16.54±0.04 <sup>dB</sup>	18.99±0.04 <sup>dA</sup>	0.43±0.00 <sup>c</sup>
MeOH/CHCl <sub>3</sub> (2:1)	6.89±0.04 <sup>cC</sup>	17.46±0.03 <sup>cB</sup>	19.45±0.03 <sup>cA</sup>	0.44±0.00 <sup>b</sup>
MeOH/H <sub>2</sub> O (1:1)	5.82±0.03 <sup>cC</sup>	14.70±0.04 <sup>eB</sup>	16.39±0.03 <sup>eA</sup>	0.53±0.00 <sup>a</sup>
H <sub>2</sub> O	7.66±0.04 <sup>bC</sup>	25.88±0.03 <sup>bB</sup>	27.25±0.03 <sup>bA</sup>	0.27±0.00 <sup>d</sup>
BHT	77.79±0.02 <sup>aC</sup>	88.51±0.04 <sup>aB</sup>	95.25±0.03 <sup>aA</sup>	0.18±0.00 <sup>e</sup>

Means which are not significantly different are followed by the same number. Significance level (p<0.05), <sup>a-e</sup>Means in each column followed by different superscripts letters are significantly different (p<0.05), <sup>A-C</sup>Means in each row followed by different superscripts letters are significantly different (p<0.05), DPPH: 2,2-diphenyl-1-picryl-hydrazyl

decreased in the following order: H<sub>2</sub>O > MeOH/CHCl<sub>3</sub> > MeOH > MeOH/H<sub>2</sub>O with values of: 17.32 > 15.39 > 14.48 > 11.16 mg GAE g<sup>-1</sup> extract. Abdou (2011) reported that total phenolics in garlic extracts were 12.5, 27.6, 23.9 and 25.3 (mg/100 g dry weight extract) for MeOH, MeOH/H<sub>2</sub>O (1:1), water at 37°C and water at 100°C, respectively.

**Dpph-Free Radical Scavenging Activity (FRSA):** The main role of any antioxidant is its ability to trap free radicals. Antioxidant compounds like phenolic acids and polyphenols scavenge free radicals such as peroxide, hydroperoxide and thus inhibit the oxidative mechanisms. The radical scavenging activity of the garlic extracts in comparison with BHT towards DPPH free radicals was expressed as percentage inhibition. Table 2 shows that scavenging effects of garlic extracts on DPPH radical increased with increased concentration. The scavenging power of garlic extracts on the DPPH radical was in the order H<sub>2</sub>O > MeOH/CHCl<sub>3</sub> > MeOH > MeOH/H<sub>2</sub>O with values of 27.25, 19.45, 18.99, and 16.39%, respectively, at the concentration of 150 µg µL<sup>-1</sup>. However, the scavenging effect of BHT was much higher than all garlic extracts (95.25%). These results indicate that garlic extracts specially water and methanol extracts are good free radical scavenger acting



as antioxidants (Noguchi *et al.*, 1994). This means that garlic extracts can react with free radicals, which are the main initiator of the autoxidation chain of fat, thus terminating the chain reaction (Gordon, 1990; Frankel, 1991).

The IC<sub>50</sub> for garlic extracts were found to be 0.43, 0.44, 0.53 and 0.27 mg mL<sup>-1</sup> DPPH for MeOH, MeOH/CHCl<sub>3</sub>, MeOH/H<sub>2</sub>O and H<sub>2</sub>O respectively (Table 2). On the other side IC<sub>50</sub> of BHT was 0.186. The results revealed that garlic extracts have good inhibition power especially H<sub>2</sub>O extract. Sultana *et al.* (2010) reported that IC<sub>50</sub> of MeOH extract of garlic was 89.25 µg mL<sup>-1</sup>. It was also, reported by Bozin *et al.* (2008) that the IC<sub>50</sub> of immature garlic plants, dried garlic bulbs and fresh garlic bulbs (80% MeOH extracts) were: 1.03, 4.41 and 6.01 mg mL<sup>-1</sup>, respectively. Moreover, Lawrence and Lawrence (2011) reported the IC<sub>50</sub> of garlic essential oil was 0.5 mg mL<sup>-1</sup>.

### Thermal oxidation of SFO

#### Measurement of lipid oxidation

**Peroxide value:** The SFO is an unsaturated oil which is widely used in food, frying and cooking and it is considered as a good source of linoleic acid. Because of its unsaturation properties it is easily undergo rancidity when heated; for this reason it was selected to investigate the antioxidant power of garlic extracts and garlic powder in comparison with BHT.

Detection of peroxides in unsaturated fats and oils gives the initial evidence of rancidity. It gives a measure of the extent to which an oil sample has undergone primary oxidation (Ali, 2010; Zhang *et al.*, 2010). Peroxide value of an oil or fat is useful for accessing the extent to which spoilage has advanced. The double bonds found in fats and oils play a role in oxidation. Oils with a high degree of unsaturation are most susceptible to autoxidation. The best test for autoxidation is determination of peroxide value because peroxides are intermediates in the autoxidation. The PV increases only when the rate of peroxides formation exceeds that of its destruction (Poiana, 2012). The effect of the different garlic extracts, garlic powder, as well as BHT on the oxidative stability of RBD SFO heated at the frying temperature was studied. Table 3 shows the effect of heating SFO, in presence of the above mentioned compounds, on the change of PV. The peroxide value of SFO (control) was 0.682, before heating, increased to 3.013 meq kg<sup>-1</sup> after 3 h heating, reaching 5.433 at the end of heating period (15 h). The effect of the different extracts, GP and BHT on inhibiting the formation of hydroperoxides was in the order: SFO-MeOH > SFO-GP > SFO-MeOH/H<sub>2</sub>O > SFO- H<sub>2</sub>O > SFO-BHT after the heating period (15 h). PV of SFO-BHT was comparable to that of SFO- H<sub>2</sub>O in the periods up to 6 h after that it increased noticeably, this means that the effect of BHT on inhibiting peroxides formation is nearly negligible after 6 h.

Table 3: Effect of garlic extracts and garlic powder on peroxide value (meq kg<sup>-1</sup> oil) during sunflower oil heating in comparison with butylated hydroxyl toluene

Time (h)	Treated sunflower oil samples					
	SFO (control)	SFO-MeOH	SFO-MeOH/H <sub>2</sub> O (1:1)	SFO-H <sub>2</sub> O	SFO-GP	SFO-BHT
3	3.013±0.056 <sup>dA</sup>	1.086±0.008 <sup>bF</sup>	1.517±0.002 <sup>cD</sup>	2.036±0.001 <sup>cC</sup>	1.282±0.003 <sup>eE</sup>	2.302±0.067 <sup>dB</sup>
6	3.133±0.129 <sup>dA</sup>	1.146±0.003 <sup>bE</sup>	1.932±0.023 <sup>bC</sup>	2.174±0.003 <sup>cC</sup>	1.443±0.013 <sup>dD</sup>	2.529±0.145 <sup>dB</sup>
9	3.545±0.107 <sup>eA</sup>	1.164±0.154 <sup>bE</sup>	2.213±0.002 <sup>abC</sup>	2.254±0.197 <sup>bC</sup>	1.503±0.006 <sup>cD</sup>	3.254±0.131 <sup>eB</sup>
12	4.327±0.002 <sup>bA</sup>	1.183±0.156 <sup>bC</sup>	2.473±0.475 <sup>aB</sup>	2.486±0.03 <sup>abB</sup>	1.538±0.006 <sup>bC</sup>	4.015±0.15 <sup>bA</sup>
15	5.433±0.003 <sup>aA</sup>	1.457±0.001 <sup>aD</sup>	2.484±0.141 <sup>aC</sup>	2.509±0.209 <sup>aC</sup>	1.633±0.002 <sup>aD</sup>	5.014±0.227 <sup>aB</sup>
Reduction in PV after 15 h (%)	0.00	73.182	54.279	53.819	69.943	7.712

PV of control sample at zero time and at room temperature was: 0.682±0.018, Means which are not significantly different are followed by the same number. Significance level (p<0.05), <sup>a-f</sup>Means in each column followed by different superscripts letters are significantly different (p<0.05), <sup>A-F</sup>Means in each row followed by different superscripts letters are significantly different (p<0.05), SFO: Sunflower oil, BHT: Butylated hydroxyl toluene, MeOH: Methnol, GP: Garlic powder, H<sub>2</sub>O: Water



At the end of the heating period (after 15 h), PV decreased in the following order: SFO-MeOH > SFO-GP > SFO-MeOH/H<sub>2</sub>O>SFO-H<sub>2</sub>O with approximate values: 1.457, 1.633, 2.484 and 2.509 meq kg<sup>-1</sup>, respectively and with reduction percent of approximate: 73, 69, 54 and 53, respectively. Whereas, the reduction percent in PV of SFO-BHT was approximately 7 (Table 3). At any time of heating, significant differences (p<0.05) in PV were observed between the control sample and oil samples with garlic extracts, garlic powder and BHT. At the end, these results prove that garlic extracts and garlic powder are very effective inhibitors of lipid oxidation because of their long term effectiveness and stability and their inhibition power exceeds that of BHT. It is worthy to mention that MeOH/CHCl<sub>3</sub> was excluded because CHCl<sub>3</sub> is not safe in food processing.

**Acid value:** Formation of free fatty acids might be an important measure of rancidity of fats and oils. An increase in the amount of free fatty acids in an oil or fat sample indicates hydrolysis of the triglycerides (Frega *et al.*, 1999). Table 4 shows the changes in AV of SFO blended with GP, garlic extracts as well as BHT. The acid value of the control was 0.054, after 15 h heating it reached 0.456 mg KOH g<sup>-1</sup>. Whereas, the values of the AV of SFO containing GP or any of the garlic extracts were less. This indicated that GP and garlic extracts slowing the hydrolysis of the triglycerides during heating. While, the AV(s) of the SFO samples contained BHT were comparable to the values of the sample contained methanol extract. At the end of heating period, the reduction percent in AV was as follows: SFO-MeOH>SFO-GP>SFO-MeOH/H<sub>2</sub>O>SFO-H<sub>2</sub>O with approximate values of: 24, 15, 11, 6, respectively; whereas the decrease in case of SFO-BHT was approximately 34%. Based on statistical test, addition of GE(s) and GP as well as BHT, resulted in significant decrease in AV (p<0.05) relative to the control during heating process. Accordingly, it could be concluded that GP and garlic extracts have obvious antioxidant activity following the order: SFO-MeOH>SFO-GP>SFO-MeOH/H<sub>2</sub>O>SFO-H<sub>2</sub>O.

**Conjugated dienes and trienes:** The polyunsaturated fatty acids oxidation started with the formation of hydroperoxides. Immediately after peroxides have been formed, the non-conjugated double bonds present in natural unsaturated lipids (e.g., linoleic) suffer a rearrangement generated conjugated dienes which absorb at 232 nm (Gertz *et al.*, 2000). When polyunsaturated fatty acids containing three or more double bonds (e.g. linolenic acid) undergo oxidation the conjugation can be extended to include another double bond resulting in the formation of conjugated trienes which absorb at 268 nm. The changes in UV absorbance at 234 and 268 nm determined by K 234 and K 268 have been used as a relative measure of oxidation (Albi *et al.*, 1997; De Abreu *et al.*, 2010). The

Table 4: Effect of garlic extracts and garlic powder on acid value (mg KOH g<sup>-1</sup>) during heating of sunflower oil in comparison with butylated hydroxyl toluene

Time (h)	Treated sunflower oil samples					
	SFO (control)	SFO-MeOH	SFO-MeOH/H <sub>2</sub> O (1:1)	SFO-H <sub>2</sub> O	SFO-GP	SFO-BHT
3	0.076±0.003 <sup>aA</sup>	0.054±0.002 <sup>dD</sup>	0.065±0.001 <sup>eB</sup>	0.068±0.001 <sup>eB</sup>	0.058±0.001 <sup>cC</sup>	0.046±0.002 <sup>eE</sup>
6	0.096±0.002 <sup>dA</sup>	0.075±0.001 <sup>dD</sup>	0.083±0.001 <sup>dC</sup>	0.087±0.002 <sup>dB</sup>	0.077±0.001 <sup>dD</sup>	0.063±0.002 <sup>dE</sup>
9	0.226±0.003 <sup>eA</sup>	0.146±0.002 <sup>eE</sup>	0.184±0.002 <sup>cC</sup>	0.195±0.003 <sup>eB</sup>	0.166±0.002 <sup>dD</sup>	0.126±0.002 <sup>fF</sup>
12	0.383±0.002 <sup>bA</sup>	0.186±0.001 <sup>bE</sup>	0.252±0.003 <sup>bC</sup>	0.284±0.002 <sup>bB</sup>	0.212±0.002 <sup>bD</sup>	0.144±0.003 <sup>bF</sup>
15	0.456±0.003 <sup>aA</sup>	0.346±0.002 <sup>aE</sup>	0.404±0.003 <sup>aC</sup>	0.425±0.002 <sup>aB</sup>	0.384±0.002 <sup>aD</sup>	0.300±0.001 <sup>aF</sup>
Reduction in AV after 15 h (%)	0.00	24.123	11.403	6.798	15.789	34.211

AV of control sample at zero time and at room temperature was: 0.054±0.002, Means which are not significantly different are followed by the same number. Significance level (p<0.05), <sup>a-e</sup>Means in each column followed by different superscripts letters are significantly different (p<0.05), <sup>A-F</sup>Means in each row followed by different superscripts letters are significantly different (p<0.05), SFO: Sun flower oil, MeOH: Methanol, GP: Garlic powder, BHT: Butylated hydroxy toluene, H<sub>2</sub>O: Water

Table 5: Effect of garlic extracts, garlic powder on formation of conjugated dienes, during sunflower oil heating in comparison with butylated hydroxyl toluene

Time (h)	Treated sunflower oil samples					
	SFO (control)	SFO-MeOH	SFO-MeOH/H <sub>2</sub> O (1:1)	SFO-H <sub>2</sub> O	SFO-GP	SFO-BHT
3	7.522±0.011 <sup>eA</sup>	7.141±0.007 <sup>eE</sup>	7.306±0.006 <sup>eC</sup>	7.223±0.003 <sup>eD</sup>	7.065±0.004 <sup>eF</sup>	7.334±0.003 <sup>eB</sup>
6	9.580±0.005 <sup>dA</sup>	8.541±0.006 <sup>dE</sup>	8.759±0.006 <sup>dC</sup>	8.675±0.004 <sup>dD</sup>	8.277±0.003 <sup>dF</sup>	9.194±0.004 <sup>dB</sup>
9	14.533±0.012 <sup>cA</sup>	10.067±0.007 <sup>cD</sup>	10.417±0.008 <sup>cC</sup>	10.415±0.002 <sup>cC</sup>	9.624±0.004 <sup>cE</sup>	13.823±0.003 <sup>cB</sup>
12	19.523±0.007 <sup>bA</sup>	12.269±0.008 <sup>bE</sup>	12.872±0.009 <sup>bC</sup>	12.825±0.002 <sup>bD</sup>	10.736±0.003 <sup>bF</sup>	18.003±0.009 <sup>bB</sup>
15	25.025±0.017 <sup>aA</sup>	14.927±0.006 <sup>aE</sup>	19.607±0.007 <sup>aC</sup>	17.067±0.002 <sup>aD</sup>	12.436±0.007 <sup>aF</sup>	24.375±0.008 <sup>aB</sup>
Reduction in CD after 15 h (%)	0.00	40.351	21.650	31.800	50.305	2.597

CD (K 234 nm) of control sample at zero time and at room temperature was: 5.504±0.167, Means which are not significantly different are followed by the same number. Significance level (p<0.05), <sup>a-c</sup>Means in each column followed by different superscripts letters are significantly different (p<0.05), <sup>A-F</sup>Means in each row followed by different superscripts letters are significantly different (p<0.05), SFO: Sun flower oil, MeOH: Methanol, GP: Galic powder, BHT: Butylated hydroxy toluene, H<sub>2</sub>O: Water

Table 6: Effect of garlic extracts, garlic powder on formation of conjugated trienes during sunflower oil heating in comparison with butylated hydroxyl toluene

Time (h)	Treated sunflower oil samples					
	SFO (control)	SFO-MeOH	SFO-MeOH/H <sub>2</sub> O (1:1)	SFO-H <sub>2</sub> O	SFO-GP	SFO-BHT
3	1.936±0.001 <sup>eA</sup>	1.634±0.003 <sup>dD</sup>	1.805±0.003 <sup>eB</sup>	1.735±0.003 <sup>eC</sup>	1.584±0.002 <sup>eE</sup>	1.425±0.002 <sup>eF</sup>
6	2.987±0.002 <sup>dA</sup>	2.753±0.003 <sup>dD</sup>	2.893±0.002 <sup>dB</sup>	2.822±0.002 <sup>dC</sup>	2.633±0.002 <sup>dE</sup>	2.526±0.002 <sup>dF</sup>
9	3.814±0.003 <sup>cA</sup>	3.624±0.002 <sup>cD</sup>	3.773±0.002 <sup>cB</sup>	3.743±0.002 <sup>cC</sup>	3.584±0.001 <sup>cE</sup>	3.526±0.002 <sup>cF</sup>
12	4.242±0.002 <sup>bA</sup>	4.144±0.002 <sup>bD</sup>	4.195±0.002 <sup>bB</sup>	4.174±0.002 <sup>bC</sup>	4.103±0.002 <sup>bE</sup>	3.906±0.001 <sup>bF</sup>
15	5.787±0.007 <sup>aA</sup>	4.713±0.002 <sup>aD</sup>	5.124±0.003 <sup>aB</sup>	4.902±0.002 <sup>aC</sup>	4.505±0.002 <sup>aE</sup>	4.395±0.002 <sup>aF</sup>
Reduction in CT after 15 h (%)	0.00	18.559	11.457	15.293	22.153	24.054

CT (K 268 nm) of control sample at zero time and in room temperature was: 1.680±0.001, Means which are not significantly different are followed by the same number. Significance level (p<0.05), <sup>a-c</sup>Means in each column followed by different superscripts letters are significantly different (p<0.05), <sup>A-F</sup>Means in each row followed by different superscripts letters are significantly different (p<0.05), SFO: Sun flower oil, MeOH: Methanol, GP: Galic powder, BHT: Butylated hydroxy toluene, H<sub>2</sub>O: Water

presence of CD and CT is a good measurement of oxidation because they remain in the frying oil (Sulieman *et al.*, 2006). The increase in CD and CT is proportional to the uptake of oxygen and formation of peroxides during the early stages of oxidation as well as with the degradation rate of linoleic acid (Che Man *et al.*, 1999; Sulieman *et al.*, 2006).

Table 5 and 6 show the effect of blending SFO with GP and garlic extracts-GP on the values of K 234 and K 268 in comparison with that of BHT. The results revealed that the formation of CD and CT increased with increasing heating period. The SFO (control) showed the highest values of CD and CT accumulation all over the heating period. The antioxidant effect of GP and garlic extracts on inhibiting the formation of CD and CT was in the order: SFO-GP > SFO-MeOH > SFO-H<sub>2</sub>O > SFO-MeOH/H<sub>2</sub>O. On the other hand, the inhibitory effect of BHT on reducing the formation of CD was comparable to that of GP and garlic extracts in the early heating periods (3 and 6 h) after that K 234 and K 268 of SFO-BHT was higher than those of GP and garlic extracts. This means that GP and garlic extracts are effective in inhibiting progress of lipid oxidation. This may be due to their long term effectiveness and stability which make them more powerful than BHT. At the end of the heating period, GP and garlic extracts reduced CD accumulation by approximately: 50, 40, 31 and 21% for SFO-GP, SFO-MeOH, SFO-H<sub>2</sub>O and SFO-MeOH/H<sub>2</sub>O, respectively; whereas CT reduced by approximately: 22, 18, 15 and 11% for SFO-GP, SFO-MeOH, SFO-H<sub>2</sub>O and SFO-MeOH/H<sub>2</sub>O, respectively. Significant decrease (p<0.05) in CD and CT were detected by addition of GE(s), GP and BHT during heating period relative to SFO control. These results prove the efficiency of GP and garlic extracts in slowing down lipid degradation and increasing oxidative stability of oil when heated at frying temperature.

## CONCLUSION

From this study, variations in activities between garlic extracts and BHT were noticed by determination of phenolic contents, DPPH scavenging activities and IC<sub>50</sub>. It was found that water extract showed the highest phenolic content, highest DPPH scavenging activity as well as IC<sub>50</sub>. The present study also revealed that garlic extracts and garlic powder can stabilize SFO to a great extent. They inhibit thermal deterioration of oil by improving its hydrolytic stability and inhibiting double bond conjugation. Therefore, garlic extracts and GP can be considered as a potential natural antioxidant.

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