Abstract: Oil oxidation is significant for acceptability, nutritional quality, and toxicity of edible oils. Antioxidant supplementation for oil is a common and fundamental strategy for improving its oxidative stability and prolonging induction time. Ginger contains natural antioxidants such as phenolic and flavonoid compounds. Ginger extracts were prepared by extraction with different solvents (methanol, ethanol, acetone and water). Ethanolic ginger extract had the highest yield (10.52%), whereas the aqueous extract had the lowest yield (8.10%). Also, the ethanolic extract was the highest in the content of phenolic and flavonoid compounds (75.17 and 19.55 mg/g, respectively), followed by methanolic extract (67.24 and 17.46 mg/g, respectively). Thus, further elaboration focused on the ethanolic extract. The scavenging ability of ginger extract was dose-dependent; it increased with the increase in ginger extract concentration. As expected, the ginger extract had lower DPPH scavenging activity than BHT (synthetic antioxidant). Free fatty acid (FFA), peroxide value (PV), conjugated dienes (CD) and thiobarbituric acid (TBA) value were used to evaluate the effect of ginger extract as a natural antioxidant on sunflower oil oxidation. The higher the concentration of ginger extract, the lower the magnitude of FFA, PV, CD, and TBA in sunflower oil. According to our findings, the level of sunflower oil supplementation with ginger extract should be below 600 mg/kg. The result of this study suggests that ginger extract can be recommended as a natural antioxidant to retard sunflower oil oxidation.

Key words: flavonoids, phenolics, scavenging activity, solvents, storage, oxidative stability

INTRODUCTION

Sunflower (Helianthus annuus L.) is one of the main crops used for edible oil production in many countries of the world, including Egypt. It is related to the quality of its oil, which is useful for human consumption and biodiesel production. In addition, due to its large capacity for adaptation to different edaphic and climatic conditions, sunflower is an excellent option for crop rotation for several production regions (Carvalho, 2003; Taher, Abde-Twab & E-Sharhi, 2008).

Edible oils with higher contents of unsaturated fatty acids, especially polyunsaturated fatty acids (PUFA), are more susceptible to oxidation. Sunflower oil is a kind of nutritious vegetable oil that contains lots of PUFA (more
than 85%), especially represented by linoleic acid (essential fatty acid) (more than 60% of fatty acids). Over time, the reduction of overall quality takes place in sunflower oil and the linoleic acid might decrease due to oxidative deterioration of sunflower oil. The oil oxidation process not only can produce rancid odors, off flavors and discoloration, but can also decrease the nutritional quality and safety of oil due to the formation of degradation products (toxic and polymer compounds), resulting in harmful effects on human health (Elsorady & Abdl Aziz, 2011; Meng et al., 2021). Oil oxidation is affected by several factors such as oxygen, light, heat, fatty acids composition and antioxidants. Oxidative stability is an important parameter for oil quality and shelf life. It depends not only on the composition of fatty acids but also on the content of antioxidants (Lužačić, Grahovac, Hladni & Romanić, 2022).

Antioxidants are added to the oil to improve oxidative stability by scavenging free radicals, controlling transition metals, and quenching singlet oxygen. Antioxidants (synthetic and natural) are used as food additives to prolong shelf life and to retard or delay oxidative deterioration. Synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tertiary butylhydroquinone (TBHQ) and propyl gallate (PG) are widely used for high performance and their efficiencies in many countries but their safety has been questioned because recent reports revealed that these compounds may be implicated in many health risks, including cancer and carcinogenesis (Choe & Min, 2006; Elsorady & Abdl Aziz, 2011). Hence, there is a tendency towards the use of natural antioxidants of plant origin to replace these synthetic antioxidants. Therefore, it is quite meaningful to explore natural antioxidants such as essential oils from herbs, spices such as rosemary extract, thyme oil, olive oil mill wastewater, etc., which provide good antioxidant activity and improve sensory properties (Jennings & Akoh 2009; Abd-el Ghani, Ammar & Hegazy, 2010; Elsorady & Abdl Aziz, 2011; Embuscadó, 2015; Sayyari & Farahmandfar, 2016; Wang, Meng, Wang, Wang & Blasi, 2020).

Natural antioxidants such as phenolic and flavonoid compounds can protect from free radicals and retard the progress of many chronic diseases and lipid oxidative rancidity in foods (Elsorady & Abdl Aziz, 2011). Natural plant antioxidants are important because of two reasons. Firstly, the consumption of food rich in antioxidants is necessary to prevent oxidative damage of cells which is involved in the progression of many diseases. Secondly, antioxidants are used in food preservation to prevent oxidation and increase its shelf life by delaying the lipid peroxidation process. Although synthetic antioxidants are strong, they have toxic effects. Natural antioxidants could be safe for human use (Tohma et al., 2017).

Ginger is used as a traditional medicine in Asian and Arabic countries (Altman & Mar-cussen, 2001). Ginger (Zingiber officinale) rhizomes are consumed as fresh, dried powder and slices. Its unique flavor manifests from a combination of pungency (oleoresin) and aromatic essential oil (Tyler, 1993). Ginger consumption is useful in many disease treatments such as hypertension, diabetes and Alzheimer's diseases. In traditional medicine, it is used to treat headaches, colds, arthritis, rheumatic disorders and muscular discomfort. Also, ginger has anti-inflammatory, anticancer and antioxidant properties (Tohma et al., 2017). Dijeng et al. (2018) cited that ginger is rich in polyphenol compounds such as gingerol, shogaol and diarylheptanoids which exert antioxidant, anti-inflammatory, antidiabetic and antitumor effects. The essential oil and extract from ginger roots are efficient as a source of natural antioxidants for the preservation of oil stability. Womeni, Tonfack, Tiencbeu and Linder (2013) reported that the methanolic extract of ginger roots was efficient in retarding the oxidation of soybean oil. Sunflower oil is common and widely used oil in frying and culinary practices in Egypt. Therefore the objective of this investigation was to study the antioxidant activities of ginger extract and its influence on sunflower oil oxidation.

MATERIALS AND METHODS

Materials

Ginger was purchased from a local market. Sunflower oil (SO) was obtained from Arma Oils Co. 10th of Ramadan, Egypt. Chemicals and reagents were obtained from Sigma Chemical Co, (ST. Louis, US) and El-Gomhoria Co. for Pharmaceutical, Cairo, Egypt.

Proximate composition of ginger

Moisture, fat, proteins, fibers and ash content were determined according to standard AOAC.
methods (AOAC, 2000: 925.10, 963.15, 950.36, 973.18 and 942.05, respectively) and total carbohydrates were determined by difference: Total carbohydrates = 100 – (moisture + fat + proteins + fibers + ash).

**Preparation of ginger extracts**

The ginger was washed, peeled and dried at 55 °C. The dried ginger was ground in a grinder (Kenwood Ltd.) to a fine powder. The powder (10 g) was added to 100ml of different solvents (80% methanol, 80% ethanol, 80% acetone and water) overnight in a shaker at 22±2 °C. The extract was filtered, and then evaporated in a rotary evaporator below 40 °C. The solid extract was weighed to calculate the yield using Equation (1):

\[
\text{Extraction yield (\%)} = \frac{\text{Weight of solid extract}}{\text{Weight of sample taken for extraction}} \times 100
\]  

**Determination of total phenolics and flavonoid contents**

Total phenolic content (mg GAE/g) of the ginger extract was determined with the Folin-Ciocalteu reagent according to the method of Kim, Jeond and Lee (2003). The flavonoid content (mg quercetin/g) was determined according to the method of Kreft, Fabjan and Yasumoto (2006).

**Antioxidant activities of ethanolic ginger extract using (DPPH) method**

The scavenging ability of ginger extract (0 - 20.0 mg/ml) on 2, 2 diphenyl-1- picryl hydrazyl (DPPH) radicals was determined according to the method of Elsorady and Abdl Aziz (2011). The scavenging ability was calculated as follows:

\[
\text{Scavenging ability (\%)} = \frac{(\Delta A_{517} \text{ of control} - \Delta A_{517} \text{ of sample})}{\Delta A_{517} \text{ of control}} \times 100
\]

Butylated hydroxytoulene (BHT) was used for comparison.

**Sensory sensitivity (threshold) test**

This test was carried out according to Elsorady and Abdl Aziz (2011). Two sets of beakers (8 in each set) containing sunflower oil were mixed with different concentrations of ethanolic ginger extract (100, 200, 400, 600, 1000, 1200, 2000 and 3000 mg/kg). Eight panelists were chosen from personnel at the Food Technology Research Institute (Giza, Egypt), and asked to sniff and taste each sample, characterize the flavor and rate the flavor intensity on a scale from 0 (no flavor) to 5 (extremely strong flavor).

**Application of ginger extract to sunflower oil**

Sunflower oil (free of synthetic antioxidant) was used as the substrate for oxidation studies. Different concentrations of ethanolic ginger extract (200, 400 and 600 mg/kg) were added separately to the sunflower oil (100 g) which were placed in beakers (200 ml). Sunflower oil (100 g) without any addition was used as a control sample. In addition, synthetic antioxidant BHT was tested in sunflower oil for comparative purposes at its legal limit of 200 mg/kg (Elsorady & Abdl Aziz, 2011). The oxidation rates of all samples of sunflower oil were followed in an oven at 60 °C for 20 days. Samples were withdrawn (15 g approximately) periodically every 0, 5, 10, 15, and 20 days for analysis. Immediately after storage period, oil samples were analyzed.

**Measurements of sunflower oil oxidation**

Free fatty acid (FFA) (%: oleic acid), peroxide value (PV) (meq. O₂/kg oil) and conjugated dienes (CD) were determined according to standard AOAC (2000). Thiobarbituric acid (TBA) value (mg malonaldehyde/kg oil) was determined according to Elsorady and Abdl Aziz (2011).

**Statistical analysis**

One-way analysis of variance was carried out on all the data of each oil quality parameter studied using SPSS program (SPSS Statistics 16th version, IBM) with p<0.05 considered to be statistically significant. For the comparison of means, the LSD and Duncan post-hoc tests were used. The measurements were performed with three replicates, each replicate contained at least duplicate samples.

**RESULTS AND DISCUSSION**

**Proximate composition of ginger**

The proximate composition of the ginger rhizome is indicated in Table 1. Moisture content was 72.24%. It is lower than that observed by El-Ghorab, Nauman, Anjum, Hussain and Nadeem (2010), Maizura, Aminah and Wan Aida (2011). This may be due to differences in varieties and climatic conditions. It had 0.24% fat, 1.30% proteins, 1.42% fibers and 1.62% ash. These findings agreed with the data by El-Ghorab et al. (2010).
Table 1.
Proximate composition of ginger (mean ± SE)

| Parameter       | Content (g/100 g wet weight) |
|-----------------|-------------------------------|
| Moisture        | 72.24±0.32                   |
| Crude fat       | 0.24±0.04                    |
| Crude proteins  | 1.30±0.25                    |
| Fibers          | 1.42±0.21                    |
| Ash             | 1.62±0.02                    |
| Carbohydrates   | 23.18±0.85                   |

Ginger extracts characteristics

Table 2 shows the extraction yield (%) of ginger extract using different organic solvents, i.e., ethanol, methanol, acetone, and water. The maximum extraction yield was obtained with ethanol (10.52%), followed by those extracted with methanol (9.74%), acetone (8.24%) and water (8.10%). These results were in line with those of Qadir, Shahzadi, Bashir, Munir and Shahzad (2017).

The antioxidative action of phenolics and flavonoids is multiple. They are acting as...
scavengers of singlet oxygen, removing free radicals, activators of antioxidant enzymes and inhibiting oxidases (Shetty & McCue, 2003). The total amounts of phenolics and flavonoids in ginger extracts obtained by different solvents are shown in Table 2. Total phenolic and flavonoid contents of ginger extracts ranged between 57.38 and 75.17 mg/mg and 15.30 and 19.55 mg/g, respectively. Ethanol extract had the highest contents of phenols and flavonoids followed by methanol, acetone and water extracts, respectively. These findings agreed with Rehman, Saliyari and Habib (2003), Stoilova, Krustanov, Stoyanova, Denev and Gargova (2007) and Qadir et al., (2017). These findings may be related to solvent strength and polarity index which changes the solubility of bioactive compounds in the plant matrix, ethanol proved to be the best extracting solvent (Tomsone, Kruma & Galoburda, 2012; Do et al. 2014; Ngo, Scarlett, Bowyer, Ngo & Vuong, 2017). Therefore, the ethanol extract of ginger was selected for further evaluation of its antioxidant activity in sunflower oil at 60ºC for 20 days of storage. Free fatty acids, peroxide values and TBA were determined to assess the degree of sunflower oil oxidation.

Scavenging activity of ginger extract by DPPH test

Scavenging of DPPH radicals has been used to determine the antioxidant activity of natural compounds (Ozturk, Ozturk, Duru & Topcu, 2007). The antioxidant activity of ginger extracts is related to phenolics and flavonoids components such as shogaols, gingerol and gingerdrol for their capability to be donors of hydrogen atoms or electrons and to scavenge free radicals (Kikuzaki & Nakatani, 1993; Stoilova et al., 2007; Mao et al., 2019). The ginger extract showed a significant effect in inhibiting DPPH, reaching up to 85.58% at a concentration of 20 mg/ml compared with BHT 90.35% at the same concentration (Table 3). DPPH scavenging activity of BHT was higher than that of ginger extract and its activity increased with higher concentrations. This finding agreed with Ghasemzadeh, Jaafar and Rahmat (2010) who reported that plant extracts exhibited less DPPH scavenging activities than those of butylated hydroxyl toluene (BHT). On the other hand, this finding is not in line with Stoilova et al. (2007) who found that ginger extract had higher DPPH scavenging activity than BHT. Polyphenol solubility plays an important role in antioxidant activity. As a result, an increase in total phenolic content is in direct correlation with an increase in antioxidant activity (Tomsone et al., 2012). Mošovská, Nováková and Kalinák (2015) cited that the high scavenging activity of the ginger extract against ABTS and DPPH radicals was related to phenolic compounds such as gingerols, shogaols, paradols and gingerdions.

Effect of ginger extract on quality attributes of sunflower oil

Effect on sensory properties

The threshold values for ginger extract added to sunflower oil are shown in Table 4. These values were the same for ginger extract concentration in the range of 100 to 400 mg/kg and did not at all affect the flavor note of sunflower oil. Consequently, the addition ginger extract to sunflower oil is acceptable for human consumption. The addition of ginger extract to sunflower oil at 600, 1000, 1200, 2000, 3000 mg/kg caused weak, medium, medium, strong and very strong flavor, respectively. These findings suggest that ginger extract should be added below a concentration of 600 mg/kg to sunflower oil.

Effect on storage stability

a) Free fatty acid (FFA) content

In the initiation stage of oil oxidation, free fatty acids are formed which are susceptible to oxygen attack in the presence of light, resulting in the formation of many organic compounds and free fatty acids which are responsible for the rancidity and off-flavors in food. Free fatty acids and peroxide value are the primary predictors of oil oxidation (Rehman et al., 2003). Table 5 shows the effect of the storage period on the FFA content of sunflower oil (SO). A gradual increase in FFA content was observed during the storage of sunflower oil at 60 ºC for 20 days. Initially, the FFA content of sunflower oil was 0.02%. After 20 days of storage at 60 ºC, FFA contents were 0.07, 0.12, 0.19, 0.26 and 0.39% (as oleic acid) for SO+200 mg/kg BHT, SO+600 mg/kg ginger extract, SO+400 mg/kg ginger extract, SO+200 mg/kg ginger extract, SO (control), respectively. The addition of 600 mg/kg ginger extract and BHT caused a reduction in FFA content from 0.39% (control) to 0.12 and 0.07%, respectively after storage for 20 days at 60 ºC. With the increase of
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Ginger extract concentration in SO, there was an observed decrease in FFA. The increase in FFA values of sunflower oil samples is mainly due to the formation of acidic compounds and free fatty acids as a result of secondary product cleavage formed during oxidation and frying (Bheemreddy, Chinnar, Pannu & Reynolds, 2002).

b) Peroxide value (PV)

Table 6 shows the effect of the addition of different concentrations of ginger extract on the PV of sunflower oil during storage for 20 days at 60 °C. Results revealed that the control had the highest PV (55.3 meqO2/kg oil). On the other hand, sunflower oil with 200 mg/kg BHT had the lowest PV (30.2 meqO2/kg oil). These results agreed with Jorge and Andreo (2013). Data also showed that the higher the concentrations of ginger extract, the lower the PVs. Oil containing ginger extract at 600 mg/kg exhibited the lowest PV (34.5 meqO2/kg) after storage for 20 days at 60 °C. It is apparent from these results that the addition of ginger extract and BHT retarded the development of rancidity in sunflower oil, but BHT gave better protection than ginger extract. PV indicates the content of primary products of lipid oxidation (i.e. hydroperoxides). Therefore, it seems reasonable to determine the concentration of peroxides as a measure of the extent of oil oxidation (Gray, 1978).

c) Thiobarbituric acid content (TBA)

Besides an increase in free fatty acid content and peroxide value, a marked increase in TBA was observed during the storage of sunflower oil at 60 °C for 20 days (Table 7). The TBA test measures a secondary product of lipid oxidation, malonaldehyde. It was assumed that the accumulation of these products during consecutive days of storage affected the oil quality and was responsible for the development of rancid odor and off-flavor of the oil (Elsorady & Abdl Aziz, 2011). Sunflower oil (control) had the highest TBA values (0.86 mg malonaldehyde/kg oil), while sunflower oil with BHT (200 mg/kg) and with ginger extract (600 mg/kg) ended up with the lowest TBA values of 0.30 and 0.37 mg malonaldehyde/kg oil, respectively.

d) Content of conjugated dienes (CD)

Peroxide values may not indicate the actual magnitude of oil deterioration (Yaghmur, Aserin, Mizrahi, Nerd & Garti, 2001). Conjugated dienes (CD) at 232 nm are considered an important parameter for the investigation of primary oxidative deterioration of the oils (Elsorady & Abdl Aziz, 2011). The oxidation of polyunsaturated fatty acids is accompanied by increased UV absorption. Fatty acids with unsaturated diene strongly absorb waves in the band 232 nm. The changes in the UV spectrum of a given substance can be used as a relative measurement of oxidation (Gray, 1978). Table 8 indicated that the CD was increased during storage for 20 days at 60 °C. The highest increase in CD was observed in the control sample (9.1), compared to those oil samples containing different levels of ginger extract. On the other hand, sunflower oil with 200 mg/kg BHT had the lowest increase in CD (3.4) after storage at 60 °C for 20 days. Again, results revealed the same trend as for FFA, PV and TBA.

### Table 5.

Free fatty acid (FFA) content of sunflower oil (SO) without and with added different concentrations of ginger extract during storage at 60 °C for 20 days

| Storage time (days) | SO (control sample) | SO+200 mg/kg ginger extract | SO+400 mg/kg ginger extract | SO+600 mg/kg ginger extract | SO+200 mg/kg BHT |
|---------------------|---------------------|-----------------------------|-----------------------------|-----------------------------|------------------|
| 0                   | 0.02±0.00<sup>a</sup> | 0.02±0.00<sup>a</sup> | 0.02±0.00<sup>a</sup> | 0.02±0.00<sup>a</sup> | 0.02±0.00<sup>a</sup> |
| 5                   | 0.19±0.01<sup>b</sup> | 0.15±0.02<sup>b</sup> | 0.09±0.02<sup>b</sup> | 0.06±0.01<sup>b</sup> | 0.04±0.00<sup>b</sup> |
| 10                  | 0.26±0.03<sup>c</sup> | 0.19±0.02<sup>c</sup> | 0.13±0.01<sup>c</sup> | 0.09±0.01<sup>c</sup> | 0.05±0.00<sup>c</sup> |
| 15                  | 0.31±0.01<sup>d</sup> | 0.23±0.00<sup>d</sup> | 0.17±0.01<sup>d</sup> | 0.11±0.00<sup>d</sup> | 0.06±0.00<sup>d</sup> |
| 20                  | 0.39±0.02<sup>e</sup> | 0.26±0.01<sup>e</sup> | 0.19±0.00<sup>d</sup> | 0.12±0.01<sup>d</sup> | 0.07±0.00<sup>d</sup> |

<sup>a-e</sup>Different superscripts indicate significant differences (p<0.05)
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DEJSTVO EKSTRAKTA ĆUMBIRA (ZINGIBER OFFICINALE) KAO PRIRODNOG ANTIOKSIDANSA NA SMANJENJE OKSIDACIONIH PROMENA SUNCOKRETOVOG ULJA

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Sažetak: Oksidacione promene značajno utiču na prihvatljivost, nutritivni kvalitet i toksičnost jestivih ulja. Dodatak antioksidanasa je osnovna i uobičajena strategija za poboljšanje oksidativne stabilnosti ulja i produženje vremena indukcionih faza oksidacije. Ćumbir sadrži prirodne antioksidanse kao što su jedinjenja fenola i flavonoida. U radu su ispitivani ekstrakti čumbira dobijeni pomoću različitih rastvarača (metanol, etanol, aceton i voda). Najveći prinos imao je etanolni ekstrakt čumbira (10,52%), dok je najmanji prinos imao vodeni ekstrakt (8,10%). Takođe, etanolni ekstrakt je imao najveći sadržaj fenolnih jedinjenja i flavonoida (75,17 i 19,55 mg/g, respektivno), a zatim metanolni ekstrakt (67,24 i 17,46 mg/g, respektivno). Dalja ispitivanja su nastavljena s etanolnim ekstraktom. Ekstrakt čumbira je pokazao sposobnost da ukloni DPPH slobodne radikale. Sa povećanjem koncentracije ekstrakta čumbira, primećeno je povećanje sposobnosti eliminisanja slobodnih radikala. Kao što je i očekivano, ekstrakt čumbira je imao nižu sposobnost uklanjanja DPPH slobodnih radikala u odnosu na BHT (sintetički antioksidans). Za procenu antioksidacionog efekta ekstrakta čumbira u suncokretovom ulju praćeno je nekoliko parametara kvaliteta: peroksidni broj (PV) sadržaj slobodnih masnih kiselina (FFV), konjugovanih diena (CD) i triobarbiturne kiseline (TBA). Rezultati ove studije su pokazali da ekstrakt čumbira može da se dodaje u suncokretovo ulje u koncentraciji ispod 600 mg/kg kako se ne bi narušila senzorska svojstva ulja. Ekstrakt čumbira može da se preporuči kao prirodni antioksidans u usporavanju oksidacije suncokretovog ulja.

Ključne reči: flavonoidi, fenolna jedinjenja, eliminacija slobodnih radikala, rastvarači, skladištenje, oksidaciona stabilnost

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