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ORIGINAL ARTICLE

Storage quality of walnut oil containing lycopene during accelerated oxidation

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Abstract The purpose of investigation was to assess the effect of lycopene on the peroxide value, acid value, fatty acids, total phenolic content and ferric-reducing antioxidant power of walnut oil. Walnut oil was extracted from Xinjiang walnut variety using cold pressing method. Our study reported that after 45 days of accelerated oxidation at 60 °C (Schaal oven test), 0.005% lycopene exhibited the greatest antioxidant effect than other addition levels of lycopene. Therefore, under ambient storage conditions, the shelf-life of walnut oil could be extended up to 16 months by 0.005% lycopene. Moreover, 0.005% lycopene added to walnut oil had a significantly higher content of saturated fatty acid, unsaturated fatty acid, total phenol, reducing ability of the polar and non-polar components than the blank sample (walnut oil without any addition of lycopene). In conclusion, lycopene improved the quality of walnut oil because of its antioxidant effect against lipid oxidation.

Keywords Walnut oil · Lycopene · Antioxidant · Oxidation stability

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Introduction

Walnut (*Juglans* regia L.) is a very popular tree nut and has high economic values especially for the food industry because of its various phytochemical compounds and nutritional properties (Shah et al. 2017). China is the largest walnut producing country in the world, accounting for 48% of the world's total production of walnut (Fu et al. 2016). Other major producers of walnut are the United States of America, Iran and Turkey (Gharibzahedi et al. 2014; Fu et al. 2016). Walnut is rich in tocopherols and essential fatty acids such as omega-3 and omega-6 (Amin et al. 2017).

Walnut oil, as one of important edible oils, has been widely used as a source of essential fatty acids (Tapia et al. 2013). However, the high concentration of polyunsaturated fatty acids (PUFAs) (> 70%) in walnut oil make oxidation a significant problem (Tapia et al. 2013). When PUFAs are exposed to light and air, they undergo oxidation reactions and produce undesirable flavours (Martínez et al. 2013). Oxidation of walnut oil has raised concerns about food safety and quality issues of products because food degradation can result in a shorter product shelf life and cause harmful effects on consumers' health (Fu et al. 2016).

Increasing worldwide walnut production and demand for walnut oil have encouraged and facilitated the development of new methods to improve the shelf life of walnut oil. Walnut oil is usually stored in transparent containers and exposed to fluorescent light at ambient temperature. The shelf life for walnut oil without the addition of antioxidants under such conditions is rather short (i.e. about 2 months) (Martínez et al. 2011). In China, majority of the small packaging barrels of oil are usually added with relatively cheap synthetic antioxidants such as tertiary butylhydroquinone (TBHQ), butylated hydroxyanisole

(BHA) and butylated hydroxytoluene (BHT) in order to extend their shelf life (Taghvaei and Jafari 2015). However, these synthetic antioxidants are associated with adverse health effects and consumers are aware of the toxicity of such synthetic antioxidants (Falowo et al. 2014). Some developed countries such as Japan and Canada have restricted the use of TBHQ and BHA in food products because of their carcinogenicity (Chen et al. 2014; Goli et al. 2005). Therefore, there is an urgent need to find a safe alternative to replace such harmful synthetic antioxidants. One of the alternatives is lycopene because of its solubility in edible oils and it is safe for human consumption (Srivastava and Srivastava 2015).

As a natural red pigment, lycopene is produced by plants such as tomatoes during photosynthesis (Srivastava and Srivastava 2015). Due to its strong antioxidant property, lycopene is able to quench singlet oxygen, remove superoxide free radicals, inhibit lipid peroxidation and slow the oxidative rancidity of oil. Therefore, the evidence of the effectiveness of lycopene as an antioxidant is well-established. However, to our knowledge, there are limited studies that have investigated the effect of lycopene on the shelf life of food products (Nour et al. 2015; Kim et al. 2011; Sánchez-Escalante et al. 2003) especially the data related to walnut oil are scarce. Therefore, the aim of our study was to evaluate the effect of lycopene on the storage quality of walnut oil during accelerated oxidation.

Materials and methods

Materials

Walnuts used were from Xinjiang Hotan region and picked in late October every year. Walnuts were processed to produce walnut oil in the laboratory of the Department of Food Science and Pharmacy, Xinjiang Agricultural University, China. Walnut oil extraction was carried out with a Komet screw press (IBG Monforts, Germany) with a screw speed of 20 rpm and a restriction die of 5 mm. The pressing temperature was set at 55 °C. Pressed-extracted walnut oil was centrifuged at 3000 r/min for 30 min to remove impurities.

Lycopene (with a purity of 95%) was purchased from Zhengzhou Linuo Connaught Biological Technology Co. (Zhengzhou, China) and 2, 4-tri (2-pyridyl)-s-triazine (TPTZ) (purity > 98%) was purchased from Sigma Company (USA). Oleic acid (purity > 98%), linolenic acid (purity > 98%) and Folin's phenol reagents were purchased from Beijing Solaibao Technology Co. (Beijing, China). TBHQ, BHA and BHT were purchased from Hainan Shupu Biotechnology Ltd Company (Hainan, China). All reagents were of analytical grade.

Pre-treatment of walnut oil

Lycopene (0.1 g) was weighed using an electronic analytical balance and it was added to 10 g walnut oil and labelled as stock solution. The stock solution was sonicated for 5 min at 25 °C and stirred for 5 min using a constant temperature magnetic stirrer. The concentration of lycopene added to walnut oil ranged from 0.001 to 0.015% was prepared by using the stock solution. A blank sample (i.e. only walnut oil) was prepared similarly without the addition of lycopene. Three chemical reference treatments (i.e. 0.005% TBHQ, 0.005% BHA and 0.005% BHT) were also included in order to evaluate the efficiency of lycopene.

Preparation of samples for Schaal oven test (accelerated oxidation test)

Each sample was placed in a 10 mL test tube and wrapped with aluminium foil to avoid light exposure. Samples were then stored at 60 ± 1 °C in the electric blast drying oven and samplings for analyses were measured once every 3 days for 45 days.

Peroxide and acid values

Peroxide values of samples were determined using the modified method of National Standard of the People's Republic of China GB/T5538-2005 (General Administration of Quality Supervision Inspection and Quarantine of the People's Republic of China 2005a). Walnut oil (3 g) was dissolved in 30 mL glacial acetic acid-chloroform and then 1 mL saturated potassium iodide was added to the solution. After the solution was shaken at least three times, 30 mL distilled water and 1 mL starch indicator were added into it. The solution was titrated against 0.1 M sodium thiosulfate until the blue colour disappeared.

Acid values of samples were determined using the modified method of National Standard of the People's Republic of China GB/T5530-2005 (General Administration of Quality Supervision Inspection and Quarantine of the People's Republic of China 2005b). Five drops of phenolphthalein indicator were added to anhydrous ethanol (50 mL) and heated to boiling. The solution was titrated against 0.1 M potassium hydroxide until the pink colour was seen. After that, 3 g of walnut oil was dissolved in the solution and the solution was heated to boiling. The solution was again titrated against 0.1 M potassium hydroxide until the pink colour was seen. The peroxide and acid values were calculated to reflect the oxidation of walnut oil samples.

Fatty acid composition

Fatty acid methyl esters were prepared by dissolving 0.1 g walnut oil in 2 mL *n*-hexane. Two mL of anhydrous methanol and 2 mL of 0.8 M potassium hydroxide-methanol were added to the solution. Then, the solution was shaken at 3000 r/min for 5 min and topped up to 10 mL with deionised water. The solution was allowed to stand and the upper layer of the solution was collected for subsequent analysis using gas chromatography (Wei et al. 2015.

Fatty acids were determined using the method by Jin et al. (2015). The conditions for gas chromatography were set as follows: Agilent DB-WAX capillary column (30 m × 0.25 mm × 0.25 μm); the initial column temperature was programmed at 130 °C (held for 1 min) then ramped at 6 °C/min to 170 °C, further ramped to 215 °C at a rate of 2.75 °C/min (held for 12 min) and kept at 230 °C at a rate of 4 °C/min (held for 3 min); the carrier gas was nitrogen; the flow rate of hydrogen and air used as fuel gas was 40 mL/min and 400 mL/min, respectively; tail-blowing flow rate was 30 mL/min; the split ratio was 50:1; and injection volume was 1 μL. Fatty acids in samples were identified by comparing the relative retention times of peaks standards.

Total phenolic content

The total phenolic contents of oil samples were determined using the modified method by Lianhe et al. (2012). Briefly, 100 mg of samples were mixed with 0.5 mL of Folin-Ciocalteu phenol reagent and 2 mL of methanol. After that, 1.5 mL of 15% Na_2C0_3 solution was added to the mixture and vortexed for 30 s. The solution was diluted to 7 mL by adding distilled water and placed in a water bath at 50 °C for 20 min followed by centrifuging at 3000 r/min for 10 min. The supernatant was measured for absorbance at 750 nm and total phenolic content calculated as gallic acid equivalent (GAE).

Ferric-reducing antioxidant power (FRAP)

Polar and non-polar components of samples were prepared by dissolving 4 g walnut oil in 5 mL methanol. The solution was mixed in the dark for 30 min and centrifuged at 3000 r/min for 15 min. After stratification, the supernatant was taken as polar components. Non-polar components were extracted by dissolving the remaining supernatant in 15 mL ethyl acetate. The above extraction method was repeated three times in order to obtain the maximum amount of polar and non-polar components.

The antioxidant capacity was determined using the modified method of Szydłowska-Czerniak et al. (2008). Briefly, 10 μL of the sample was placed in a 96-well quartz plate, and 200 μL of FRAP working solution was added. The temperature was maintained at 37 °C for 4 min and the absorbance read at 593 nm. Deionized water was used as blank.

Statistical analysis

Analysis was performed using SPSS 17 (SPSS Inc, Chicago, IL, USA). All experiments were conducted in triplicate and the results were reported as mean ± standard deviation (SD). Differences between samples were determined using ANOVA at the 5% level ($P < 0.05$) of significance.

Results and discussion

Peroxide value

Our study used Schaal oven test to study the oxidation of lipid substrates in walnut oil at 60 °C. Peroxide values are used to measure the concentration of hydroperoxides and peroxides produced during lipid oxidation (Chen et al. 2014). Peroxide values of all samples increased with the duration at 60 °C regardless of the addition of lycopene (Table 1). Peroxide values of lycopene added to walnut oil samples were less than the blank, suggesting that lycopene exhibits antioxidant effect on the walnut oil with the strong effect at 0.005% lycopene. In addition, our study reported that the peroxide values of 0.005% lycopene were significantly lower than 0.010 and 0.015% lycopene ($P < 0.05$) after 45 days, suggesting that higher concentration of lycopene may not improve peroxide values. The possible reason for this effect is that when higher concentration of lycopene is used, lycopene may interact with other minor components such as phenolic compounds, phytosterols, squalene and fatty acids to cause antagonistic effect towards the protection against antioxidants (Le Grandois et al. 2017). There was a rapid increase in peroxide values from 21 days to 30 days for 0.005 and 0.01% lycopene because during the early stage of oxidation, walnut oil was at the oxidation induction period in which peroxide value increased slowly (Xiao et al. 2000). However, after the oxidation induction period, peroxide value increased rapidly (Xiao et al. 2000). Our study reported that the strongest antioxidant effect after 45 days was observed at 0.005% TBHQ (15.94 mmol/kg) followed by 0.005% lycopene (25.83 mmol/kg), 0.005% BHA (30.77 mmol/kg) and 0.005% BHT (32.04 mmol/kg).

Table 1 Effect of lycopene on peroxide values of walnut oil over 45 days

Days	Peroxide values[1] (mmol/kg)									
	Blank	0.001% lycopene	0.0025% lycopene	0.003% lycopene	0.005% lycopene	0.010% lycopene	0.015% lycopene	0.005% TBHQ	0.005% BHA	0.005% BHT
0	0.85 ± 0.028^{a}	0.85 ± 0.028^{a}	0.85 ± 0.028^{a}	0.85 ± 0.028^{a}	0.85 ± 0.028^{a}	0.85 ± 0.028^{a}	0.85 ± 0.028^{a}	0.85 ± 0.028^{a}	0.85 ± 0.028^{a}	0.85 ± 0.028^{a}
9	8.25 ± 0.042^{g}	5.88 ± 0.025^{d}	6.69 ± 0.148^{e}	7.62 ± 0.035^{f}	1.68 ± 0.049^{a}	3.40 ± 0.070^{c}	1.67 ± 0.063^{a}	1.85 ± 0.063^{a}	2.95 ± 0.367^{b}	3.34 ± 0.212^{c}
18	12.77 ± 0.028^{i}	10.69 ± 0.040^{g}	10.49 ± 0.141^{f}	12.04 ± 0.063^{h}	3.36 ± 0.063^{b}	9.30 ± 0.134^{e}	3.38 ± 0.056^{b}	2.75 ± 0.148^{a}	3.73 ± 0.169^{c}	4.02 ± 0.035^{d}
27	18.72 ± 0.021^{h}	14.62 ± 0.130^{e}	17.47 ± 0.060^{f}	18.02 ± 0.070^{g}	8.51 ± 0.056^{b}	14.62 ± 0.120^{e}	9.51 ± 0.134^{c}	5.48 ± 0.205^{a}	9.95 ± 0.120^{d}	9.94 ± 0.155^{d}
36	23.83 ± 0.035^{h}	19.57 ± 0.125^{f}	20.42 ± 0.007^{g}	25.94 ± 0.070^{i}	15.32 ± 0.063^{b}	19.72 ± 0.127^{f}	18.42 ± 0.162^{d}	9.61 ± 0.700^{a}	17.23 ± 0.311^{c}	18.79 ± 0.176^{e}
45	40.83 ± 0.014^{h}	35.83 ± 0.050^{g}	27.50 ± 0.106^{c}	45.83 ± 0.042^{i}	25.83 ± 0.077^{b}	30.83 ± 0.261^{d}	32.50 ± 0.226^{f}	15.94 ± 0.084^{a}	30.77 ± 0.311^{d}	32.04 ± 0.332^{e}

[1]Mean (all such values)

[a–i]Within each row, means of samples that have different superscripts are significantly different at $P < 0.05$

Our study found that the oxidation rate of walnut oil was relatively slow during the early period of accelerated oxidation (i.e. 0–18 days). After 18 days, the oxidation rate of walnut oil started to increase gradually. However, the peroxide value of the blank did not increase gradually but show alterations. This is because hydroperoxides are unstable at high temperature and start to decompose into carbonyl compounds, aldehydes and other secondary oxidation products (Chen et al. 2014). Similarly, Vieira and Regitano-D'Arce (2001) also reported that the peroxide values of refined, bleached and deodorised canola oil did not increase in a clear way under microwave heating.

Our study reported that 0, 0.001, 0.0025, 0.003, 0.005, 0.010 and 0.015% lycopene added to walnut oil samples required 12, 18, 18, 15, 30, 21 and 27 days, respectively for the peroxide values to reach 10 mmol/kg at 60 °C when using Schaal oven test. In addition, our study also demonstrated that 0.005% lycopene required the longest duration (about 30 days) to achieve 10 mmol/kg than other addition levels of lycopene. According to the National Standard of the People's Republic of China GB/T 22327-2008, the upper limit of peroxide value of walnut oil is 10 mmol/kg (General Administration of Quality Supervision Inspection and Quarantine of the People's Republic of China 2008). Therefore, taking into consideration that each day of accelerated oxidation at 60 °C is equivalent to 16 days of storage at 20 °C, our study suggested that 0.005% lycopene added to walnut oil is expected to have about 16 months of shelf life under ambient storage conditions.

Acid value

During the accelerated oxidation at 60 °C, walnut oil is broken down into free fatty acids due to high temperature (Choe and Min 2007). Consequently, this increases the concentration of free fatty acids, which elevates the acid value of walnut oil (Choe and Min 2007). Therefore, a high acid value indicates that the oil sample is not stored under proper conditions.

Our study reported that acid value of the blank increased from 0.098 mg/g to 0.496 mg/g after 45 days (Table 2). None of the addition levels of lycopene added to the walnut oil samples had an acid value > 0.496 mg/g after 45 days. Similar to peroxide values, acid values of lycopene added to the walnut oil samples were significantly less than the blank ($P < 0.05$), suggesting that lycopene could effectively delay the formation of free fatty acids in walnut oil and exhibits antioxidant effect with the strong effect at 0.005% lycopene. Acid value of 0.005% lycopene was significantly lower than 0.005% BHA and 0.005% BHT ($P < 0.05$) but it was significantly higher than 0.005% TBHQ ($P < 0.05$).

Table 2 Effect of lycopene on acid values of walnut oil over 45 days

Days	Acid values[1] (mg/g)									
	Blank	0.001% lycopene	0.0025% lycopene	0.003% lycopene	0.005% lycopene	0.010% lycopene	0.015% lycopene	0.005% TBHQ	0.005% BHA	0.005% BHT
0	0.098 ± 0.008^{a}	0.098 ± 0.008^{a}	0.098 ± 0.008^{a}	0.098 ± 0.008^{a}	0.098 ± 0.008^{a}	0.098 ± 0.008^{a}	0.098 ± 0.008^{a}	0.098 ± 0.008^{a}	0.098 ± 0.008^{a}	0.098 ± 0.008^{a}
9	0.195 ± 0.007^{f}	0.174 ± 0.004^{e}	0.183 ± 0.004^{f}	0.190 ± 0.004^{f}	0.118 ± 0.005^{b}	0.150 ± 0.013^{d}	0.123 ± 0.023^{bc}	0.105 ± 0.005^{a}	0.120 ± 0.004^{c}	0.132 ± 0.013^{c}
18	0.232 ± 0.012^{e}	0.213 ± 0.003^{d}	0.225 ± 0.009^{e}	0.212 ± 0.005^{d}	0.153 ± 0.015^{b}	0.198 ± 0.006^{c}	0.153 ± 0.002^{b}	0.125 ± 0.009^{a}	0.158 ± 0.005^{b}	0.163 ± 0.019^{b}
27	0.305 ± 0.014^{f}	0.249 ± 0.006^{d}	0.275 ± 0.008^{e}	0.278 ± 0.012^{e}	0.190 ± 0.010^{b}	0.232 ± 0^{c}	0.203 ± 0.019^{b}	0.161 ± 0.012^{a}	0.201 ± 0.015^{b}	0.221 ± 0.002^{c}
36	0.326 ± 0.013^{f}	0.282 ± 0.008^{d}	0.297 ± 0.016^{e}	0.346 ± 0.009^{g}	0.225 ± 0.012^{b}	0.278 ± 0.005^{d}	0.271 ± 0.007^{cd}	0.201 ± 0.002^{a}	0.233 ± 0.019^{b}	0.257 ± 0.019^{c}
45	0.496 ± 0.015^{i}	0.453 ± 0.009^{h}	0.363 ± 0.005^{f}	0.452 ± 0.012^{h}	0.301 ± 0.014^{b}	0.335 ± 0.009^{e}	0.430 ± 0.004^{g}	0.241 ± 0.011^{a}	0.313 ± 0.002^{bc}	0.322 ± 0.018^{cd}

[1]Mean (all such values)

[a–j]Within each row, means of samples that have different superscripts are significantly different at $P < 0.05$

Fatty acids

Formation of free fatty acid is also an important indicator to measure rancidity in foods (Chen et al. 2014). This is because the hydrolysis of triglycerides forms free fatty acids and the reaction between oil and moisture accelerates the formation of fatty acids (Ardestani and Yazdanparast 2007). The content of fatty acids in our study was determined by gas chromatography. Our study reported that the ratio of linoleic acid (n-6) to linolenic acid (n-3) in walnut oil was about 3:1. There were five fatty acids in walnut oil of which palmitic and stearic acids are saturated fatty acids (Lim et al. 2013). In our study, the highest amount of fatty acids in walnut oil before accelerated oxidation was linoleic acid (54.92 g/100 g) followed by linolenic acid (18.50 g/100 g), oleic acid (12.25 g/100 g), palmitic acid (5.41 g/100 g) and stearic acid (1.91 g/100 g). After 45 days of accelerated oxidation, 0.005% lycopene had significantly lower contents of palmitic acid, stearic acid and oleic acid than other addition levels of lycopene ($P < 0.05$) (Table 3). On the other hand, 0.005% lycopene had significantly higher contents of linoleic acid and linolenic acid than other addition levels of lycopene ($P < 0.05$), suggesting that 0.005% lycopene could be used to delay the degradation of unsaturated fatty acids in walnut oil.

In our study, the contents of palmitic acid, stearic acid and oleic acid were reported to be the highest at 0.005% BHT followed by 0.005% BHA, 0.005% lycopene and 0.005% TBHQ. While the contents of linoleic acid and linolenic acid were found to be the highest at 0.005% TBHQ, 0.005% lycopene, 0.005% BHA and 0.005% BHT, respectively. Therefore, 0.005% lycopene is more stable and beneficial than other addition levels of lycopene in maintaining the quality of walnut oil in terms of fatty acids. Our study findings were consistent with the results reported by Li et al. (2010). During the oxidation process, the content of saturated fatty acids increases while the concentration of unsaturated fatty acids decreases. Although oleic acid is unsaturated fatty acid, its content increased during the accelerated oxidation after 45 days because it is monounsaturated fatty acid (Lim et al. 2013). Another possibility is that the oxidation rate of polyunsaturated fatty acids into monounsaturated fatty acids was greater than that of monounsaturated fatty acids into saturated fatty acids (Li et al. 2010).

Total phenolic content

Phenolic compounds are associated with health benefits because of their antioxidant activity (Balasundram et al. 2006). In our study, total phenolic contents of different addition levels of lycopene added to oil samples were

Table 3 Effect of lycopene on fatty acid content of walnut oil after 45 days

Fatty acids	Fatty acid contents[1] (g/100 g)									
	Blank	0.001% lycopene	0.0025% lycopene	0.003% lycopene	0.005% lycopene	0.010% lycopene	0.015% lycopene	0.005% TBHQ	0.005% BHA	0.005% BHT
Palmitic acid	6.44 ± 0.02^{f}	6.42 ± 0.07^{f}	6.31 ± 0.09^{e}	6.42 ± 0.03^{f}	6.02 ± 0.05^{b}	6.25 ± 0.06^{d}	6.14 ± 0.03^{c}	5.84 ± 0.07^{a}	6.11 ± 0.03^{c}	6.33 ± 0.07^{e}
Stearic acid	2.58 ± 0.08^{h}	2.47 ± 0.04^{g}	2.50 ± 0.03^{g}	2.41 ± 0.04^{f}	2.17 ± 0.03^{b}	2.29 ± 0.03^{d}	2.18 ± 0.12^{b}	2.05 ± 0.03^{a}	2.24 ± 0.04^{c}	2.38 ± 0.04^{f}
Oleic acid	14.13 ± 0.03^{d}	14.02 ± 1.33^{cd}	14.08 ± 1.06^{cd}	13.93 ± 1.02^{cd}	13.38 ± 0.09^{bc}	13.79 ± 1.79^{bcd}	13.77 ± 1.37^{bcd}	12.89 ± 2.31^{a}	13.45 ± 1.02^{b}	14.02 ± 1.33^{cd}
Linoleic acid	51.44 ± 0.65^{e}	51.46 ± 1.48^{e}	51.52 ± 0.02^{e}	51.61 ± 1.56^{de}	53.26 ± 0.08^{ab}	52.04 ± 1.77^{cd}	52.06 ± 1.20^{cd}	53.83 ± 0.02^{a}	53.01 ± 1.56^{b}	52.44 ± 1.48^{c}
Linolenic acid	16.29 ± 0.01^{i}	16.49 ± 0.01^{h}	16.83 ± 0.01^{g}	16.85 ± 0.05^{g}	17.92 ± 0.006^{b}	17.10 ± 0.01^{e}	17.60 ± 0.04^{c}	18.12 ± 0.01^{a}	17.38 ± 0.03^{d}	16.94 ± 0.05^{f}

[1]Mean ± SD (all such values)

[a–i]Within each row, means of samples that have different superscripts are significantly different at $P < 0.05$

Table 4 Effect of lycopene on total phenolic content of walnut oil over 45 days

Days	Total phenolic content[1] (mg GAE/kg)									
	Blank	0.001% lycopene	0.0025% lycopene	0.003% lycopene	0.005% lycopene	0.010% lycopene	0.015% lycopene	0.005% TBHQ	0.005% BHA	0.005% BHT
0	468.38 ± 7.44^{a}	472.41 ± 3.62^{a}	467.75 ± 2.91^{a}	468.63 ± 7.27^{a}	465.38 ± 0.98^{a}	466.04 ± 3.45^{a}	467.51 ± 1.63^{a}	465.78 ± 2.13^{a}	467.39 ± 2.10^{a}	466.42 ± 0.94^{a}
9	157.69 ± 2.78^{g}	182.30 ± 2.66^{f}	189.34 ± 1.06^{e}	210.12 ± 2.20^{c}	257.54 ± 9.81^{b}	198.15 ± 2.20^{d}	177.37 ± 3.71^{f}	290.86 ± 0.38^{a}	208.15 ± 2.20^{c}	177.37 ± 3.71^{f}
18	90.86 ± 0.38^{de}	93.34 ± 2.11^{ef}	91.03 ± 1.61^{cde}	94.98 ± 1.61^{e}	120.83 ± 0.60^{b}	102.29 ± 1.06^{cd}	108.34 ± 4.00^{b}	165.35 ± 1.57^{a}	102.57 ± 0.98^{c}	92.37 ± 1.28^{f}
27	72.71 ± 2.37^{d}	72.62 ± 1.17^{d}	73.08 ± 1.97^{d}	75.72 ± 1.67^{c}	91.12 ± 1.01^{b}	74.69 ± 1.22^{d}	83.00 ± 3.81^{c}	112.60 ± 0.70^{a}	88.00 ± 0.80^{b}	75.98 ± 1.85^{d}
36	47.49 ± 0.66^{f}	51.31 ± 1.83^{e}	48.84 ± 1.61^{ef}	54.66 ± 3.33^{d}	63.33 ± 2.45^{bc}	62.22 ± 1.61^{c}	65.75 ± 0.61^{b}	81.89 ± 1.64^{a}	62.22 ± 1.61^{c}	55.75 ± 0.61^{d}
45	19.56 ± 3.21^{e}	27.08 ± 1.06^{d}	26.65 ± 1.61^{d}	31.78 ± 0.87^{c}	37.54 ± 1.22^{b}	35.46 ± 0.00^{bc}	35.15 ± 3.50^{bc}	47.36 ± 0.81^{a}	33.79 ± 1.53^{bc}	25.15 ± 3.50^{d}

[1]Mean ± SD (all such values)

[a–f]Within each row, means of samples that have different superscripts are significantly different at $P < 0.05$

Table 5 Effect of lycopene on FRAP of walnut oil over 45 days

Days	FRAP[1] (mmol/L)									
	Blank	0.001% lycopene	0.0025% lycopene	0.003% lycopene	0.005% lycopene	0.010% lycopene	0.015% lycopene	0.005% TBHQ	0.005% BHA	0.005% BHT
(a) Polar components										
0	0.595 ± 0.008^{d}	0.599 ± 0.002^{d}	0.606 ± 0.013^{d}	0.604 ± 0.007^{d}	0.597 ± 0.015^{d}	0.600 ± 0.002^{d}	0.603 ± 0.008^{d}	3.246 ± 0.140^{a}	1.471 ± 0.010^{b}	1.340 ± 0.036^{c}
9	0.558 ± 0.009^{f}	0.565 ± 0.003^{f}	0.584 ± 0.001^{e}	0.585 ± 0.003^{de}	0.593 ± 0.005^{de}	0.589 ± 0.001^{de}	0.593 ± 0.004^{d}	2.860 ± 0.069^{a}	1.316 ± 0.008^{b}	1.116 ± 0.001^{c}
18	0.532 ± 0.000^{e}	0.535 ± 0.001^{e}	0.556 ± 0.004^{de}	0.557 ± 0.001^{de}	0.578 ± 0.011^{de}	0.592 ± 0.061^{d}	0.563 ± 0.002^{de}	2.158 ± 0.181^{a}	1.045 ± 0^{b}	0.934 ± 0.020^{c}
27	0.472 ± 0.002^{g}	0.477 ± 0.003^{g}	0.507 ± 0.003^{f}	0.537 ± 0.004^{e}	0.560 ± 0.002^{d}	0.539 ± 0.007^{e}	0.539 ± 0.008^{e}	1.703 ± 0.089^{a}	0.930 ± 0.023^{b}	0.857 ± 0.016^{c}
36	0.463 ± 0.002^{g}	0.468 ± 0.009^{g}	0.476 ± 0.003^{ef}	0.485 ± 0.010^{f}	0.502 ± 0.010^{d}	0.493 ± 0.006^{e}	0.502 ± 0.001^{e}	1.309 ± 0.007^{a}	0.818 ± 0.024^{b}	0.718 ± 0.013^{c}
45	0.433 ± 0.001^{g}	0.431 ± 0.001^{g}	0.463 ± 0.011^{fg}	0.452 ± 0.001^{ef}	0.498 ± 0.001^{d}	0.472 ± 0.009^{de}	0.467 ± 0.001^{d}	1.146 ± 0.001^{a}	0.731 ± 0.025^{b}	0.597 ± 0.045^{c}
(b) Non-polar components										
0	1.069 ± 0.003^{g}	1.089 ± 0.005^{g}	1.263 ± 0.004^{e}	1.484 ± 0.008^{d}	1.608 ± 0.015^{c}	1.878 ± 0.006^{b}	2.055 ± 0.006^{a}	1.210 ± 0.038^{f}	0.910 ± 0.002^{h}	0.860 ± 0.009^{i}
9	0.921 ± 0.002^{e}	0.912 ± 0.008^{e}	1.111 ± 0.012^{d}	1.346 ± 0.111^{c}	1.404 ± 0.038^{c}	1.493 ± 0.003^{b}	1.605 ± 0.020^{a}	1.119 ± 0.032^{d}	0.866 ± 0.004^{e}	0.767 ± 0.028^{f}
18	0.874 ± 0.010^{d}	0.872 ± 0.026^{d}	0.932 ± 0.014^{d}	1.111 ± 0.024^{c}	1.228 ± 0.010^{b}	1.283 ± 0.016^{b}	1.357 ± 0.106^{a}	1.081 ± 0.004^{c}	0.803 ± 0^{e}	0.678 ± 0.015^{f}
27	0.868 ± 0.009^{dec}	0.849 ± 0.011^{e}	0.913 ± 0.018^{cd}	0.946 ± 0.033^{bc}	0.966 ± 0.064^{bc}	0.996 ± 0.006^{b}	1.051 ± 0.053^{a}	0.950 ± 0.007^{bc}	0.713 ± 0.005^{f}	0.578 ± 0.013^{g}
36	0.686 ± 0.033^{e}	0.767 ± 0.005^{d}	0.815 ± 0.002^{c}	0.850 ± 0.012^{bc}	0.857 ± 0.025^{b}	0.873 ± 0.004^{ab}	0.901 ± 0.046^{a}	0.814 ± 0.008^{c}	0.585 ± 0.011^{f}	0.525 ± 0.026^{g}
45	0.662 ± 0.013^{d}	0.674 ± 0.013^{d}	0.738 ± 0.049^{c}	0.807 ± 0.026^{b}	0.831 ± 0.008^{b}	0.856 ± 0.006^{ab}	0.906 ± 0.071^{a}	0.742 ± 0.016^{c}	0.519 ± 0.025^{e}	0.455 ± 0.009^{f}

[1]Mean ± SD (all such values)

[a–g]Within each row, means of samples that have different superscripts are significantly different at $P < 0.05$

significantly decreased after 45 days (Table 4). Total phenolic content of 0.005% lycopene was significantly higher than other addition levels of lycopene ($P < 0.05$). Similar results were reported by Valenzuela-Melendres et al. (2014) who reported a higher phenolic content in 10% tomato paste-added pork frankfurter than the control. Although our study found no difference between 0.005, 0.010 and 0.015% lycopene, 0.005% lycopene had a higher total phenolic content than 0.010 and 0.015% lycopene, suggesting that increasing addition levels of lycopene may decrease total phenolic content. This may be due to the reactions between the presence of high concentration of lycopene and some components such as squalene that promote oxidation in walnut oil (Ren et al. 2015). Ren et al. (2015) reported that the contents of monounsaturated fatty acids, polyunsaturated fatty acid, squalene and ß-sitosterol in walnut oil were 22.5%, 68.9%, 1.3 mg/100 g and 199.9 mg/100 g, respectively. The highest total phenolic content was found at 0.005% TBHQ (47.36 mg GAE/kg) followed by 0.005% lycopene (37.54 mg GAE/kg), 0.005% BHA (33.79 mg GAE/kg) and 0.005% BHT (25.15 mg GAE/kg). Therefore, 0.005% lycopene could be used to reduce the loss rate of total phenolic content.

FRAP

Polar and non-polar components of oil samples have the ability to reduce (Fe^{3+})TPTZ to (Fe^{2+})TPTZ. A higher FRAP value indicated a higher antioxidant capacity (Birasuren et al. 2013). Non-polar components of lycopene added to walnut oil had significantly higher FRAP values than their polar components after 45 days ($P < 0.05$) (Table 5). Since our study was to determine the effect of lycopene on Fe^{3+} reduction ability of walnut oil, we also compared the FRAP values of polar components. This is because lycopene is a fat-soluble antioxidant which is soluble in ethyl acetate and consequently this increases the ferric ion reducing capacity of the non-polar components (Lucini et al. 2012). Although 0.005% lycopene had a significantly lower FRAP value of polar components than 0.005% TBHQ, 0.005% BHA and 0.005% BHT ($P < 0.05$), 0.005% lycopene still had the highest FRAP value of polar components than other addition levels of lycopene ($P < 0.05$). This is because TBHQ, BHA and BHT are soluble in methanol and consequently these increase FRAP values of polar components. However, lycopene has low solubility in methanol (Barba et al. 2006).

In conclusion, our study results indicated that lycopene could be used as an antioxidant to improve the quality of walnut oil. The 0.005% lycopene added to walnut oil exhibited the greatest antioxidant effect, which could extend the shelf life of walnut oil up to 16 months under ambient storage conditions. After 45 days of accelerated oxidation, the contents of unsaturated fatty acid, total phenol, reducing ability of the polar and non-polar components in 0.005% lycopene added to walnut oil were higher than the blank sample by 2.7 g/100 g, 17.98 mg GAE/kg, 0.065 mmol/L and 0.169 mmol/L, respectively.

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Compliance with ethical standards

Conflict of interest declaration All authors have no conflicts to disclose.

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