Stabilizing corn oil using the lemon balm (*Melissa officinalis*) antioxidants extracted by subcritical water

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Abstract This research was set up to identify the impact of the antioxidant compounds present in lemon balm extract (LBE) as obtained by the subcritical water (SBCW) method on the oxidative stability of corn oil. An extraction yield of 28.52% was obtained for the SBCW and rosmarinic acid was identified to be the predominant phenolic compound present in the LBE. The total phenolic content of the LBE was found to be 212.74 mg gallic acid/g extract. Subsequently, 200, 400, 800, 1600 and 3200 ppm of the LBE were added to corn oil and its peroxide value (PV), acid value (AV), conjugated diene (CD), carbonyl value (CV), oxidative stability index (OSI), total polar compound and total phenolic compounds were compared to control and the sample containing 200 ppm of the BHA throughout the 16-day Schaal oven test at 70 °C. Our findings indicated that the corn oil containing greater LBE concentration had lower PV, AV, CD, and CV but greater OSI than the control sample. Evaluation of total polar compounds confirmed lower extent of the compounds with high polarity in the greater levels of the LBE. Finally, the LBE was able to slow down the rancidity of corn oil and the samples with higher LBE exhibited gentle oxidation rate.

Keywords Corn oil · Lemon balm extract · Primary oxidation product · Secondary oxidation product · Subcritical water

Introduction

The complex series of chemical reactions consisting of oxidation, hydrolysis, and polymerization of unsaturated fatty acids occur during processing and storage of lipid systems (Asnaashari et al. 2016). Such unfavorable reactions could alter the fatty acid composition of oils and also generate volatile and nonvolatile oxidation products as well as dimeric, polymeric, or cyclic substances. The oxidation is considered as the major reaction in the oil systems, which is able to deteriorate their quality. This mechanism has been recognized as a basic problem in the edible oil industry and may lead to production of poisonous compounds and off-flavors as well as to the reduced nutritional value of fat products. To decrease the undesirable effects and rate of oxidation, antioxidants are the best option, because such compounds could delay oil rancidity. In fact, by donating the hydrogen atom to free radicals caused by the oxidation mechanism, antioxidants can postpone the degradation of edible oils. Today, the use of synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tertbutyl hydroquinone (TBHQ) has caused the food industry to face a great challenge and safety concern. To solve this problem, the use of phenolic antioxidants from natural resources has been extended as bioactive compounds in recent years due to their health beneficial effects (Farahmandfar et al. 2017).

Lemon balm (*Melissa officinalis*) belongs to the Lamiaceae family and is native to South-Central Europe, Central Asia and Iran, which can have some applications in

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culinary, medicine and aromatic industry. In Iranian traditional medicine, lemon balm is known locally by the name of Badranjbouyeh. It could be used for the treatment of headaches, flatulence, indigestion, colic, nausea, nervousness, anaemia, vertigo, syncope, malaise, asthma, bronchitis, amenorrhea, cardiac failure, arrhythmias, insomnia, epilepsy, depression, psychosis, hysteria, ulcers and wounds (Dastmalchi et al. 2008). Rosmarinic acid, flavonoids, ascorbic acid, carotenoids, monoterpene, sesquiterpenes and tannins are the phytochemical compounds found in lemon balm (Carnat et al. 1998). Such constituents donate antioxidant, anti-inflammatory, antiviral, sedative, analgesic, spasmolytic and hypotensive properties into lemon balm (Gromball et al. 2014; Ross 2015; Yui et al. 2017). Therefore, one of the natural resources for antioxidant extraction is lemon balm. In this way, Dastmalchi et al. (2008) extracted the leaves material of lemon balm with solvent (450 mL/L aqueous ethanol) through medium pressure liquid–solid extraction. This research exhibited that the LBE had good antioxidant activity, even more than gallic and caffeic acids, which was statistically indistinguishable from quercetin and the BHA.

Conventional extraction techniques such as steam distillation and maceration using dangerous liquid solvents such as dichloromethane and methanol are labor-intensive and usually require substantial volumes of solvents and several hours to reach a complete extraction. Subcritical water (SBCW) has been demonstrated to be an effective, environmentally friendly solvent for the extraction of hydrophobic organic compounds from plants, soils and foods. When exposed to high temperatures (100–374 °C) and sufficiently high pressure to keep water in the liquid state, water as polar compound behaves similar to non-polar ones; this phenomenon can be attributed to the decrease of polarity resulted from increasing the temperature of water. This condition is called the SBCW, pressurized hot water or pressurized low polarity water. The decrease in the polarity of water could be explained by the decrease of the dielectric constant; hence, it finds polarity like organic solvents such as ethanol. The ability to tune the dielectric constant of water has been exploited to selectively extract a large number of both polar and non-polar compounds. The SBCW extraction method as green technology has a number of advantages over conventional methods such as being non-toxic, non-flammable and available (Carr et al. 2011). Recently, the SBCW has been used to extract the nutraceutical compounds from citrus pomace (Kim et al. 2009), antioxidant compounds from sea buckthorn leaves (Kumar et al. 2011), onion peels (Lee et al. 2011), pomegranate seed residues (He et al. 2012), marigold (*Tagetes erecta* L.) flower residues (Xu et al. 2015), sea buckthorn (*Hippophaë rhamnoides* L.) seed residue (Gong et al. 2015) and XiLan olive fruit dreg (Yu et al. 2015).

As mentioned above, due to the harmful effects of synthetic antioxidants on human health, in this paper, we investigated the use of the SBCW extraction technique as green technology to extract the lemon balm antioxidants as well as addition of them to corn oil to improve oxidative stability. It should be mentioned that such a research has not been conducted yet.

Materials and methods

Materials

Lemon balm leaves (called Badranjbouyeh in Iran) were collected from Sari Province (Iran) in February 2016. All other chemicals and solvent were of analytical grade and were provided from Merck (Darmstadt, Germany) and Sigma Aldrich (St. Louis, MO) companies. The BHA used as standard antioxidant was provided from TITRAN (Tehran, Iran).

Preparation of lemon balm leaves

The lemon balm leaves shade-dried for 3 days, and then the dried leaves were milled using Cuisinart SG-10 electric Spice-and-Nut grinder. The samples were kept in polyethylene bags until extraction.

Subcritical water extraction

An SBCW extraction device consisting of a distilled water tank, a pump (Comet type: MTP AX 2/70 m), a 140 mL-extraction cell having thick wall to withstand the applied pressure, pressure gauge, a heating coil and a temperature control system were used in the extraction step. After placing the powdered sample in the cell, the extraction process was set up. The extraction was conducted according to Sharifi et al. (2013) and Shaddel et al. (2014) methods. Accordingly, the extraction included the temperature of 160 °C and the pressure of 6.89 MPa for 30 min. Finally, after removing impurities, the filtered extract was weighed to calculate the yield and then was cooled and kept at – 18 °C in dark before use.

Determination of total phenolic content

By using Folin–Ciocalteu's reagent according to the method described by Farahmandfar et al. (2015), total phenolic content of the LBE was determined. For this purpose, 50 mL volumetric flask was poured by 1 mL of a standard solution of gallic acid, 6 mL of methanol, 2.5 mL of the Folin–Ciocalteu's reagent and 5 mL of 7.5% Na₂CO₃. Finally, in order to reach the final volume, the

purified water was added. After storing the solutions overnight, the analysis was conducted at 765 nm wavelength using a spectrophotometer (PG Instrument, Ltd). Calibration curve (0–700 mg/mL) was plotted and the total phenolic content of the samples was expressed as gallic acid from the calibration curve. Results were expressed in milligram of gallic acid per gram of extract.

HPLC analysis of individual extract compounds

A high performance liquid chromatography apparatus (HPLC) was applied to identify the individual compounds according to Dastmalchi et al. (2008) method. The HPLC system (Waters 600) had an in-line degasser pump and a controller coupled to a 2996 PDA detector equipped with a 717 autosampler (20 μ l injection volume) interfaced to a PC running Millennium 32 software (Waters Corporation, Milford, MA, USA). The separations were performed on a reverse-phase Hypersil BDS-C18 analytical column (250 $\times$ 4.6 mm id, particle size 5 μ m) (Agilent Technologies, Santa Clara, CA, USA) operating at room temperature with a flow rate of 1 mL/min. Detections were conducted by a sensitivity of 0.1 AUFS (another union file system) between the wavelengths of 200 and 550 nm. Elution was carried out by using a binary non-linear gradient of the solvent mixture 0.2 g/L aqueous trifluoroacetic acid (solvent A) and MeOH (solvent B). The composition of the mobile phase 80:20 (A/B) remained unchanged for 5 min, changed to 70:30 (A/B) in 5 min, changed to 55:45 (A/B) in 10 min, held for 5 min, changed to 20:80 (A/B) in 20 min, held for 10 min, and then returned to the initial condition in 5 min. A 20 min equilibrium time was allowed between the injections. The components were identified by comparison of their retention times to those of authentic standards under identical analysis conditions and by comparison of their UV spectra with an in-house PDA library. Stock solutions of the extract and standards were prepared in 450 mL/L aqueous ethanol and 700 mL/L aqueous methanol to final concentration of 10 and 1 mg/mL, respectively. The concentration range used for calibration of the standard compounds was 0.001–0.100 mg/mL. The standards and samples were injected in duplicate.

Evaluations of radical scavenging activity

DPPH assay

Radical scavenging activity of the extracts was evaluated using the DPPH (1,1-Diphenyl 1-2-picrylhydrazyl radical) method (Farahmandfar et al. 2015). The BHA was used as control antioxidant. Concisely, after adding the different concentrations of each extract to the DPPH solution which is methanolic (100 μ M), the samples were put in darkness

and after 30 min their absorbance was measured at 517 nm. Inhibition of free radical DPPH in percent was determined by the decrease in its absorbance induced by extracts.

β -carotene/linoleic acid bleaching assay

The antioxidant capacities of the LBE were determined using the β -carotene/linoleic acid bleaching method (Farahmandfar et al. 2015). The BHA was used as control antioxidants. The absorbance of samples was read using a spectrophotometer at 470 nm at zero and 120 min. Antioxidant activity in β -carotene bleaching model expressed as percentage was determined by the decrease in its absorbance.

Analysis of fatty acids profile by gas chromatography

Fatty acid methyl esters of corn oil were prepared according to Firestone (2009). Identification and quantification of *trans* and other fatty acid methyl esters were performed using a HP-5890 chromatograph (Hewlett-Packard, CA, USA) equipped with a CP-SIL 88 (Supelco, Bellefonte, PA, USA) capillary column of fused silica, 60 min length 0.22 mm i.d., 0.2 mm film thickness, and a flame ionization detector (FID). The oven and injector temperature were 195 and 250 $^{\circ}$ C, respectively. The carrier gas was Helium with a flow rate of 1 mL/min. The analysis of fatty acid composition of corn oil is shown in Table 1.

Oxidative stability determination of LBE-containing corn oil by Schaal oven test

At first, with the use of a magnetic stirrer (5 min at 300 rpm), the different concentration levels (200, 400, 800 and 1600 ppm) of the extract were added to corn oil. Canola oil without antioxidants and with 200 ppm of the BHA was considered as blank and control samples, respectively. To evaluate oxidative stability of the corn oil samples, Schaal oven test was conducted within 16 days (with 4-day interval in the days of 0, 4, 8, 12, and 16 at 70 $^{\circ}$ C). In the following, progress of oxidation was monitored on the mentioned days by the determination of peroxide value (PV), carbonyl value (CV), acid value (AV), conjugated dienes (CD), oxidative stability index (OSI) at 120 $^{\circ}$ C, total polar compounds (TPC) and total phenolic content method.

Peroxide value

The PV of all the corn oil samples was determined in accordance with the AOCS method Cd 8-53 (Firestone

Table 1 The fatty acid composition of corn oil

Fatty acids composition	% ^a
Lauric acid (C12:0)	0.17 ± 0.03
Myristic acid (C14:0)	0.2 ± 0.12
Palmitic acid (C16:0)	13.03 ± 0.83
Stearic acid (C18:0)	1.34 ± 0.04
Palmitoleic acid (C16:1)	0.39 ± 0.02
Oleic acid (C18:1)	27.4 ± 0.62
Linoleic acid (C18:2)	54.1 ± 0.01
α-Linolenic acid (C18:3)	1.19 ± 0.01
Arachidic acid (C20:0)	0.46 ± 0.48
Others	1.72 ± 0.52
Saturated fatty acids (SFA)	15.2 ± 1.03
Monounsaturated fatty acids (MUFA)	27.79 ± 0.53
Polyunsaturated fatty acids (PUFA)	55.29 ± 0.01
PUFA/SFA	3.64 ± 0.07
MUFA/PUFA	0.5 ± 0.05
USFA/SFA	5.46 ± 0.01
C18:2/C18:3	45.46 ± 0.1

^aMeans ± SD

2009). In brief, 3 g of the corn oil sample was dissolved in 50 mL of acetic acid-chloroform solution (3:2 v/v), then, 1 mL of the saturated KI solution was added. The mixture was kept in dark for 1 min. After that, 50 mL of distilled water was added. At the end, the mixture was titrated against sodium thiosulfate (0.01 N). The PV (meqO₂/kg) was calculated using the following equation:

$$\text{Peroxide value} = \frac{N \times S}{W} \times 1000 \quad (1)$$

where N is the normality of sodium thiosulfate solution S is the volume of sodium thiosulfate solution (blank corrected) in mL and W is the weight of the corn oil sample (g).

Carbonyl value

The CV of the corn oil samples was determined according to the method developed by Farahmandfar et al. (2015), using 2-propanol and 2,4-decadienal as solvent and standard, respectively and the absorbance of the samples was measured at 420 nm. The results were expressed in μmol of 2,4-decadienal per g of oil.

Acid value

The AV was determined as percentage of oleic acid according to the AOCS official method Ca 5a-40 (Firestone 2009).

Conjugated diene

The CD of the oil samples was determined using an absorption spectrophotometer, at 234 nm. For this purpose, the corn oil sample was diluted (1:600) with hexane (HPLC grade). An extinction coefficient of 29,000 mol/L was utilized to quantify the concentration of CDs formed during oxidation using the following equation:

$$\text{Conjugated dienes value} = \frac{\text{Absorbance} \times 600 \times 1000}{29000} \quad (2)$$

Oxidative stability index (Rancimat test)

A Metrohm Rancimat instrument, model 743 (Herisau, Switzerland) was used to determine the OSI (hour) according to the AOCS method Cd 12b-92. A stream of air was bubbled into 2.5 g oil samples contained in a reaction vessel placed in an electric heating block. Effluent air containing volatile organic acids from the oil sample were collected in a measuring vessel containing 60 mL distilled water. The conductivity of the water was measured automatically as oxidation proceeded. Filtered, cleaned, dried air was allowed to bubble through the hot oil at rate of 2.5 mL/s. The OSIs of the oil samples were automatically recorded at 120 °C, based on determining the time (usually termed “induction period”) before the maximum rate change of oxidation by measuring the increase in conductivity of deionized water.

Total polar compounds

The TPC was determined according to Farahmandfar et al. (2015) method. At first, silica gel 60 (63–100 μm), dried (12 h) at 160 °C was added five parts of water to 95 parts of it, and was shaken vigorously for about 1 min and stayed overnight. Then, the silica gel 60 (1 g) was compressed and filled between two cotton wool balls into a pipette tip. The oil sample (500 mg) and toluene (4 mL) was mixed and finally the solution (1 mL) was pipetted on top of the pipette tip and it used toluene as the eluent. After the solvent was eliminated, the percentage of the TPC (%) was calculated by the equation 100 (w – w₁)/w, in which w and w₁ are the sample weight and weight of nonpolar compounds in milligrams, respectively.

Total phenolic compounds

Total phenolic compounds of the corn oil samples were determined by the Folin–Ciocalteu method (as mentioned in the section “Determination of total phenolic content”) at 4-day intervals over a 16-day course of storage at 70 °C.

Statistical analysis

Statistical analysis including analysis of variance (ANOVA) and significance mean level (Duncan) was carried out at the significant level of $p < 0.05$ by SAS software version 9.

Results and discussion

Extract yield, total phenolic content and individual compounds of lemon balm extract

Recently, the successful extraction of health beneficial compounds (such as anti-inflammatory agents and antioxidants) from plants has attracted attention on the use of such compounds for food application. In the present study, an extract yield of $28.52 \pm 0.81\%$ was attained. The total phenolic content of the LBE was found to be 212.74 ± 1.61 mg gallic acid/g extract. As can be seen in Fig. 1, the individual compounds of the LBE (in mg/g dry extract) consisted of caffeic acid (5.61), eriodictyol-7-O-glucoside (2.13), hesperetin (6.81), hesperetin-7-O-rutinoside (hesperidin) (8.64), hydroxycinnamic acid derivatives (64.01), *m*-coumaric acid (0.36), naringenin (0.54), naringenin-7-O-rhamnoglucoside (naringin) (0.54) and rosmarinic acid (101.67). The amounts of the unidentified compounds were found to be 145.61 mg/g dry extract. The major individual components present in the LBE were rosmarinic acid and hydroxycinnamic acid derivatives (101.67 and 64.01 mg/g dry extract, respectively), whereas the *m*-coumaric acid (0.36 mg/g dry extract) was known as the least abundant compound.

Anti-radical activity of lemon balm extract by the DPPH and β -carotene/linoleic acid bleaching assay

To evaluate the anti-radical activity of antioxidants, the DPPH and β -carotene–linoleic bleaching inhibition assay as popular and fast methods were used. As illustrated in Fig. 2, the anti-radical activity of the LBE was concentration-dependent. It was shown that with the increment of the extracts concentration, the scavenging activity of the DPPH significantly increased ($p < 0.05$). This could be attributed to greater extent of the antioxidant compound (such as phenolic and flavonoid compounds) as present in the LBE. Compared to the LBE, 200 ppm of BHA only exhibited superiority over the LBE concentrations of 200 and 400 ppm whereas it was almost equal to 800 ppm of LBE ($p < 0.05$). In fact, the 1600 and 3200 ppm of LBE (65.59 and 82.30%, respectively) showed significantly ($p < 0.05$) higher anti-radical activity than 200 ppm of the BHA (46.62%).

Moreover, the results of the β -carotenes/linoleic acid method also showed an incremental trend with the increase of the extract content ($p < 0.05$). It was observed that 200 ppm of the BHA was better than 200 and 400 ppm of the LBE to prevent bleaching β -carotenes ($p < 0.05$). In other words, at greater concentrations of the LBE (1600 and 3200 ppm) because of more extent of antiradical compounds, β -carotenes were less bleached (Fig. 2). The substantial antioxidant activity of the compounds of the LBE could be due to their ability at the oil–water interface, thus protecting the lipids against oxidation.

Fig. 1 Individual compounds of the lemon balm extract

Fig. 2 The DPPH scavenging activity and β -carotene-linoleic bleaching inhibition of the lemon balm extract

Oxidative stability of the corn oil as affected by lemon balm extract

Peroxide value

Hydroperoxides are known as primary products of oxidation. These compounds have no odor and flavor; however, aldehydes and ketones produced from their breaking down are off-flavor compounds. The PV of the corn oil samples during the 16-day storage period is shown in Table 2. It is obvious that with the increase of time (the increment of the Schaal oven test) from the day 1 to day 16 at 70 °C, the PVs of the samples significantly increased due to the speeding up effect of heat on oxidation ($p < 0.05$). As shown in Table 2, there was a statistically notable difference among the LBE-containing sample and control ($p < 0.05$). In general, the obtained results indicated that the addition of the LBE into corn oil caused the rate of oxidation to decrease significantly ($p < 0.05$). In fact, the progressive increase was significantly higher in the PVs of the control sample throughout the storage period than in those of the LBE-containing corn oil samples ($p < 0.05$). The PV of the control samples increased from 0.23 to 35.34 meqO₂/kg oil, whereas 200 ppm of the LBE showed an increase from 0.24 to 27.37 meqO₂/kg oil followed by 400, 800, 1600 and 3200 ppm of LBE with the increase from 0.24 to 22.49 meqO₂/kg oil, 0.23 to 20.76 meqO₂/kg oil, 0.24 to 16.77 meqO₂/kg oil, and 0.22 to 14.88 meqO₂/kg oil, respectively. Moreover, PV of BHA increased from 0.23 to 16.25 meqO₂/kg oil during the storage period. In other words, the corn oil including the higher content of the extract was oxidized more slowly. Totally, the order of the

rising rate in hydroperoxides among the LBE-containing samples throughout test is as follows: 3200 < 1600 < 800 < 400 < 200 ppm. In fact, the sample including lower concentrations of the LBE exhibited faster oxidation trend. The corn oil containing 200 ppm BHA could suppress oxidation more effectively than the samples including the concentrations of 200, 400, 800 and 1600 ppm. However, the sample containing 3200 ppm extract had better antioxidant performance as compared to the BHA. The observed results could be explained by increasing concentrations of the phenolic compounds as added more LBE concentration.

Acid value

In the structure of lipids, fatty acids are originally incorporated to glycerol molecule. Exposing oils to poor conditions such as elevated temperatures and/or existence of moisture lead to the release of fatty acids from glycerol molecule (free fatty acids), and therefore losing oil quality and aroma. Hence, the AV is considered as a good indicator for storage quality of oils. The changes in the AV of the corn oil samples during the Schaal oven test are shown in Table 2. As seen in Table 2, significant difference was observed between the AV of the LBE-containing samples and control ($p < 0.05$). The increase in the AV over the Schaal oven time can be attributed to hydrolysis of triacylglycerols and also to the components with carboxyl groups during the autoxidation process. With the increase of time from the day 1 to day 16 at 70 °C, the AV of all the samples presented an incremental trend ($p < 0.05$). The control sample had the highest AV among all the samples.

Table 2 The peroxide value (PV), acid value (AV) and conjugated diene (CD) of the corn oil as affected by the different concentrations of the lemon balm extract during the 16-day storage period at 70 °C

Time (day)	Control	Extract concentration					BHA (200 ppm)
		200 ppm	400 ppm	800 ppm	1600 ppm	3200 ppm	
PV (meqO ₂ /kg)							
0	0.23 ± 0.00 ^{eAB}	0.24 ± 0.00 ^{eA}	0.24 ± 0.00 ^{eA}	0.23 ± 0.00 ^{eAB}	0.24 ± 0.00 ^{eAB}	0.22 ± 0.00 ^{eC}	0.23 ± 0.00 ^{eAB}
4	3.54 ± 0.02 ^{dB}	3.75 ± 0.01 ^{dA}	3.67 ± 0.02 ^{dA}	3.38 ± 0.01 ^{dC}	2.84 ± 0.02 ^{dD}	2.00 ± 0.20 ^{dE}	2.83 ± 0.05 ^{dD}
8	8.41 ± 0.02 ^{cA}	7.69 ± 0.11 ^{cB}	6.76 ± 0.02 ^{cC}	5.70 ± 0.02 ^{cD}	4.62 ± 0.39 ^{cE}	4.32 ± 0.39 ^{cF}	3.96 ± 0.07 ^{cG}
12	26.87 ± 1.55 ^{bA}	18.90 ± 0.00 ^{bB}	18.78 ± 0.14 ^{bB}	16.89 ± 0.91 ^{bC}	13.65 ± 0.17 ^{bD}	10.66 ± 0.03 ^{bE}	13.23 ± 0.73 ^{bD}
16	35.34 ± 0.11 ^{aA}	27.37 ± 0.09 ^{aB}	22.49 ± 0.05 ^{aC}	20.76 ± 0.22 ^{aD}	16.77 ± 0.02 ^{aE}	14.88 ± 0.16 ^{aG}	16.25 ± 0.85 ^{aF}
AV (%)							
0	0.12 ± 0.01 ^{eA}	0.14 ± 0.03 ^{eA}	0.12 ± 0.01 ^{eA}	0.13 ± 0.01 ^{eA}	0.15 ± 0.04 ^{eA}	0.15 ± 0.04 ^{eA}	0.13 ± 0.02 ^{eA}
4	0.94 ± 0.03 ^{dA}	0.70 ± 0.04 ^{dB}	0.54 ± 0.04 ^{dC}	0.37 ± 0.01 ^{dD}	0.33 ± 0.02 ^{dE}	0.26 ± 0.02 ^{dF}	0.22 ± 0.02 ^{dG}
8	1.55 ± 0.14 ^{cA}	1.16 ± 0.08 ^{cC}	1.24 ± 0.04 ^{cB}	0.87 ± 0.04 ^{cDE}	0.80 ± 0.02 ^{cE}	0.59 ± 0.20 ^{cG}	0.76 ± 0.04 ^{cEF}
12	2.84 ± 0.02 ^{bA}	1.60 ± 0.05 ^{bB}	1.49 ± 0.03 ^{bC}	1.45 ± 0.02 ^{bCD}	1.24 ± 0.03 ^{bE}	1.22 ± 0.03 ^{bE}	1.41 ± 0.04 ^{bD}
16	3.54 ± 0.02 ^{aA}	2.81 ± 0.05 ^{aB}	2.00 ± 0.20 ^{aC}	2.06 ± 0.08 ^{aC}	1.68 ± 0.04 ^{aE}	1.61 ± 0.04 ^{aE}	1.72 ± 0.09 ^{aD}
CD (mmol/L)							
0	3.14 ± 0.13 ^{eAB}	3.35 ± 0.12 ^{eAB}	3.47 ± 0.21 ^{eAB}	3.33 ± 0.19 ^{eAB}	3.19 ± 0.28 ^{eAB}	3.63 ± 0.20 ^{eA}	3.28 ± 0.28 ^{eAB}
4	6.95 ± 0.09 ^{dA}	6.19 ± 0.33 ^{dB}	5.28 ± 0.03 ^{dC}	4.80 ± 0.63 ^{dCD}	4.37 ± 0.22 ^{dED}	4.08 ± 0.07 ^{dE}	5.17 ± 0.29 ^{dC}
8	13.60 ± 0.12 ^{cA}	11.50 ± 0.12 ^{cC}	10.34 ± 0.44 ^{cD}	8.57 ± 0.20 ^{cD}	8.18 ± 0.24 ^{cDE}	7.63 ± 0.83 ^{cE}	8.08 ± 0.46 ^{cDE}
12	18.61 ± 0.49 ^{bA}	16.99 ± 0.69 ^{bB}	15.47 ± 0.99 ^{bC}	13.96 ± 0.61 ^{bD}	12.20 ± 1.38 ^{bE}	10.69 ± 0.14 ^{bF}	12.98 ± 0.33 ^{bDE}
16	24.10 ± 0.85 ^{aA}	23.67 ± 0.98 ^{aA}	18.72 ± 1.31 ^{aB}	18.16 ± 0.44 ^{aB}	13.61 ± 0.63 ^{aC}	13.29 ± 0.72 ^{aC}	13.96 ± 0.70 ^{aC}

Mean ± SD within a column with the same lowercase letters are not significantly different at $p < 0.05$

Mean ± SD within a row with the same uppercase letters are not significantly different at $p < 0.05$

It is attributable to the disconnection of fatty acid from glycerol molecule at high temperatures. Since there was no moisture in the LBE-containing samples and control, the increase in AV cannot be attributed to hydrolytic rancidity. In accordance with the PV results, the samples containing richer content of LBE had lower the AV ($p < 0.05$). The AV orders of the LBE-containing samples at the end of the test were as follows: 3200 < 1600 < 800 < 400 < 200 ppm. At the end of the Schaal oven test, although, the BHA-containing sample possessed significantly higher AV than the samples including 1600 and 3200 ppm of the LBE, it exhibited significantly lower AV than the samples with concentrations of 800, 400 and 200 ppm ($p < 0.05$). Thus, the LBE extract at 1600 and 3200 ppm could considerably inhibit corn oil oxidation. The control sample showed the highest AV among all the treatments. These results could be explained by the LBE antiradical activity. Indeed, the antioxidant compounds present in the LBE donated H⁺ atoms to free radicals, generated during oxidation, and were able to lower efficiently lipid oxidation. Therefore, rancidity occurred more slowly. It should be mentioned

that the free fatty acids were taken into account as secondary products of oxidation. Consequently, further decrease of the PV caused less production of free fatty acids.

Conjugated dienes (CDs)

CDs are the compounds produced immediately after forming hydroperoxides. The CDs as primary oxidation products break to form secondary oxidation products. Table 2 shows changes in the CDs of the corn oil samples during the storage. However, no significant difference was observed among the control and sample containing the LBE at all concentrations (on the first day). It is concluded that as the time passed, the sample with higher content of the LBE exhibited less content of CDs ($p < 0.05$). Such results were in accordance with those of the PV. In general, the BHA-containing corn oil as compared to the 200 and 400 ppm LBE, presented less CDs; however, these was no significant difference with samples containing 800, 1600 and 3200 ppm of the LBE ($p < 0.05$).

Carbonyl value

Secondary products of oxidation of lipids are estimated by the CV. After degradation of hydroperoxides, carbonyl compounds consisting of aldehydes and ketones will be generated, which are more stable than hydroperoxides. Such a compound is considered to be a contributor to rancidity of oils accompanied by off-flavors. The CVs of the samples over the 16-day Schaal oven test are presented in Fig. 3a. A notable increase in the CV of all samples was observed throughout the Schaal oven test ($p < 0.05$). Similar to the results of the PV and CDs, increase in the CVs of the corn oil samples with higher extent of the LBE occurred more gently (Fig. 3a). The CV of the control samples reached $27.59 \mu\text{mol/g}$ after 16 days, whereas 200, 400, 800, 1600 and 3200 ppm of the LBE had the CVs of 23.89, 19.57, 18.56, 15.49 and $14.38 \mu\text{mol/g}$, respectively, after 16 days. Indeed, because the corn oils including high

Fig. 3 The changes in the carbonyl value **a** and oxidative stability index **b** of corn oil during the 16-day storage period at 70 °C. Black square, control; white square, 200 ppm of LBE; diamond, 400 ppm of LBE; circle, 800 ppm of LBE; triangle, 1600 ppm of LBE; cross, 3200 ppm of LBE; dashed line, 200 ppm of BHA

extent of the LBE had moderate oxidation rate; so, hydroperoxides and also CDs were less produced (having greater scavenging activity). It was shown that the corn oil sample with 200 ppm of the BHA compared to 200, 400, 800 and 1600 ppm of the LBE concentrations, had less CVs ($p < 0.05$). The CV of corn oil samples including 200 ppm of the BHA after 16 days reached $14.03 \mu\text{mol/g}$, which was almost equal to 3200 ppm of the LBE ($p < 0.05$).

Oxidative stability index

The Rancimat test is a well-known method and widely-used to evaluate the OSI of edible oils. In this test, oil is exposed to elevated temperature (mostly higher than 100 °C) and also certain air-flow and its induction period of oxidation are expressed in hours. These factors facilitate oil rancidity and are considered as intensifying oxidation. Other factors such as the unsaturation degree of fatty acids also affect the results of the Rancimat test. Formic acids are generated as a result of the breakdown of oxidation in secondary products (e.g., aldehyde and ketone), account for tertiary oxidation products and by increasing electrical conductivity of oil cause the Rancimat test to be terminated (indicator of the ending test). In Fig. 3b, the OSI of the samples is presented within 16 days with 4-day interval. While the OSI of the samples had no notable difference on the first day of the test ($p < 0.05$), it exhibited a markedly significant decline ($p < 0.05$) with the increase of time. At higher concentrations of the LBE, the observed decrease had a mild trend (Fig. 3b). Indeed, the corn oil including higher content of antiradical compounds than that of without any protective compounds or lower content of LBE, possesses greater IP. In this way, decrease rate of the OSI for the sample containing 3200 ppm LBE was the lowest following the concentration of 1600, 800, 400 and 200 ppm (Fig. 3b). At the end of the Schaal oven test, the OSI of the BHA-containing sample presented superiority over the LBE-containing samples at the concentration of 200 and 400 ppm; however, it was almost equal to 800, 1600 and 3200 ppm of the LBE ($p < 0.05$). Greater OSI of corn oil with higher content of the LBE could be explained by declining of oxidation rate; therefore, the generation of lower amount of primary and secondary products was occurred. Overall, further quick production of the primary and secondary products leads to lower IP.

Total polar compounds

Polar compounds exhibit the quality of edible oils used in this study. These compounds have more polarity as compared to triacylglycerols. It has been proved the polar compounds have a toxic effect on laboratory animals; thus,

it should be attempted to prevent their increase in oils. It is necessary to mention that if oil includes more than 27% TPC, it should be rejected (Shahidi 2005). As shown in Table 3, all the samples on the first day of the experiment had the same TPC ($p < 0.05$). By passing the time, the samples with higher anti-radical activity exhibited less TPC content. In particular, the presence of the LBE caused the TPC to be significantly reduced ($p < 0.05$); this could be explained by increasing the IP of the oil samples. On the last day, when comparing the BHA-containing oil with other samples, its prohibitive effect was greater than 200 ppm of the LBE whereas it was almost equal in effect with the samples containing 400, 800, 1600 and 3200 ppm of LBE ($p < 0.05$). Totally, 400, 800, 1600 and 3200 ppm of the LBE could remarkably inhibit lipid oxidation.

Total phenolic compounds of the LBE-containing corn oil

As presented in Table 3, an increment in the total phenolic content of all the LBE-containing samples was obviously observed. The total phenolic compounds increased with the increase of the LBE level ($p < 0.05$); this can be attributed to the existence of the compounds such as flavonoids in the LBE. It is necessary to mention that flavonoids are the most abundant polyphenol in plants. Unlike the results of PV, AV, CD, CV, OSI and TPC, with the increase of time (from the day 1 to day 16), a decrease was observed in total phenolic compounds of all the samples. As the level of the LBE increased in corn oil, the drop of total phenolic compounds was lower. It can be concluded that corn oils with greater levels of the LBE have a gentle oxidation rate; therefore, free radicals are less generated. The failure in producing such compounds leads to the reduced use of phenolic compounds. In other words, phenolic compounds at higher levels of the LBE are healthier ($3200 > 1600 > 800 > 400 > 200$ ppm). All the LBE-containing corn oils exhibited more total phenolic compounds than the corn oil with 200 ppm of the BHA.

Conclusions

The DPPH and β -carotene–linoleic acid bleaching inhibition assays confirmed the existence of high content of the antioxidant compounds in the LBE. The results of the PV, AV, CD, and CV of corn oil indicated an increasing rate within the 16-day Schaal oven test at 70 °C. In general, proportional to the extract concentrations, the LBE-containing samples had gentle oxidation and higher IP. The addition of the LBE to corn oil could decrease the content of the TPC, which has harmful effects on consumers' health. The corn oils containing 1600 and 3200 ppm of the LBE reduced oxidation as compared to the sample with

Table 3 The total polar compounds (TPCs) and total phenolic compounds of corn oil as affected by the different concentrations of the lemon balm extract during the 16-day storage period at 70 °C

Time (day)	Control	Extract concentration					BHA (200 ppm)				
		200 ppm	400 ppm	800 ppm	1600 ppm	3200 ppm	200 ppm	400 ppm	800 ppm	1600 ppm	3200 ppm
TPC (%)											
0	4.18 ± 0.11 ^{eA}	4.50 ± 0.38 ^{eA}	4.34 ± 0.39 ^{eA}	4.75 ± 0.43 ^{eA}	4.31 ± 0.18 ^{eA}	4.24 ± 0.10 ^{eA}	4.39 ± 0.27 ^{eA}				
4	7.72 ± 0.19 ^{fA}	6.32 ± 0.26 ^{dB}	5.51 ± 0.24 ^{dCD}	5.85 ± 0.24 ^{dBC}	5.39 ± 0.28 ^{dCD}	5.16 ± 0.04 ^{dD}	4.94 ± 0.68 ^{dD}				
8	9.56 ± 0.09 ^{fA}	7.52 ± 0.65 ^{EB}	6.58 ± 0.57 ^{eBC}	6.95 ± 0.63 ^c	6.86 ± 0.24 ^{eBC}	6.25 ± 0.30 ^{eC}	7.19 ± 1.00 ^{eBC}				
12	10.69 ± 0.38 ^{fA}	8.86 ± 0.61 ^{BB}	8.08 ± 1.01 ^{bBC}	8.41 ± 0.21 ^{BB}	8.22 ± 0.32 ^{BB}	7.19 ± 0.15 ^{BC}	9.04 ± 0.53 ^{BB}				
16	14.20 ± 0.36 ^{fA}	12.91 ± 0.64 ^{AB}	11.36 ± 0.73 ^{AC}	12.04 ± 0.76 ^{ABC}	12.20 ± 0.91 ^{ABC}	11.00 ± 0.91 ^{AC}	11.30 ± 0.16 ^{AC}				
Total Phenolic compounds (mg/g)											
0	103.30 ± 0.56 ^{gG}	236.71 ± 1.03 ^{EE}	259.69 ± 0.79 ^{AD}	287.25 ± 1.65 ^{AC}	321.60 ± 1.11 ^{AB}	339.63 ± 0.39 ^{AA}	177.93 ± 3.17 ^{AF}				
4	101.25 ± 0.22 ^{gG}	233.33 ± 1.02 ^{BE}	251.60 ± 0.72 ^{BD}	282.20 ± 1.65 ^{BC}	320.37 ± 1.13 ^{AB}	324.89 ± 1.61 ^{BA}	162.19 ± 1.99 ^{BF}				
8	86.93 ± 11.53 ^{bF}	227.57 ± 1.13 ^{CD}	243.92 ± 1.21 ^{CC}	272.78 ± 1.64 ^{EB}	318.01 ± 0.57 ^{BA}	311.58 ± 0.95 ^{DA}	150.64 ± 1.50 ^{CE}				
12	43.64 ± 6.03 ^{fF}	222.83 ± 0.88 ^{DD}	232.11 ± 0.74 ^{DC}	264.01 ± 1.45 ^{DB}	315.91 ± 0.17 ^{CA}	313.95 ± 0.37 ^{CA}	142.23 ± 0.99 ^{DE}				
16	30.87 ± 0.08 ^{dG}	198.72 ± 1.38 ^{EE}	206.58 ± 0.85 ^{ED}	247.50 ± 1.36 ^{EC}	307.18 ± 1.32 ^{DB}	314.28 ± 0.37 ^{CA}	132.25 ± 2.93 ^{EF}				

Mean ± SD within a column with the same lowercase letters are not significantly different at $p < 0.05$

Means ± SD within a row with the same uppercase letters are not significantly different at $p < 0.05$

200 ppm of the BHA. Totally, the results of this paper strongly endorsed that the LBE is able to slow down the rate of oxidation in corn oil. Hence, the LBE can be used in food products especially edible oils as a suitable substitution for synthetic antioxidants.

Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest.

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