Evaluation of antioxidant activity of loquat fruit (*Eriobotrya japonica* Lindl.) skin and the feasibility of their application to improve the oxidative stability of soybean oil

Mojtaba Delfanian¹ · Reza Esmailzadeh Kenari² · Mohammad Ali Sahari³

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Abstract The effects of ultrasound-assisted, supercritical CO₂ and solvent extraction techniques on antioxidant activity of loquat (*Eriobotrya japonica* Lindl.) skin extract in oxidation stability of soybean oil was evaluated. The antioxidant efficacy of extracts was determined using 2, 2-diphenyl-1-picrylhydrazyl (DPPH[•]) radical scavenging capacity, β-carotene/linoleic acid, and Rancimat test system. Results showed that solvent extract of loquat fruit skin at 400 ppm had the highest antioxidant activity compared to ultrasound-assisted and supercritical CO₂ extracts. Further, solvent extraction was the most effective method for extraction of phenolic compounds. Protective effects of extracts in stabilization of soybean oil during both frying and storage conditions were tested and compared to tert-butyl hydroquinone (TBHQ) by measuring their

peroxide value, free fatty acids, conjugated dienes and trienes value. During frying process solvent extract of skin at 400 ppm (SOEA) exhibited stronger antioxidant activity in oil compared to other oil samples, but this antioxidant potential was lower than TBHQ in storage conditions. Present study showed that loquat fruit skin is a good source of natural antioxidant compounds, and has the potential to be used as a substitute for synthetic antioxidants in vegetable oils.

Keywords Antioxidant · Deep frying · *Eriobotrya japonica* · Lipid peroxidation · Soybean oil

Research highlights

- The solvent extraction method had the better effect on antioxidant potential of loquat fruit skin extract compared to ultrasound-assisted and supercritical CO₂ extraction techniques.
- The skin extracts at 400 ppm had the highest antioxidant activity compared to other concentrations.
- During frying process thermal stability of soybean oil enriched with 400 ppm of solvent extract was higher than oil containing TBHQ, but despite the appropriate effects of extracts in decrease of oil degradation during storage conditions their efficiency was lower than TBHQ.

✉ Mojtaba Delfanian
makan.delfan@yahoo.com

¹ Faculty of Food Science and Technology, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran

² Department of Food Science and Technology, Sari Agriculture and Natural Resources University, Sari, Mazandaran, Iran

³ Department of Food Science and Technology, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran

The degradation of edible oils and fats is a subject of great interest because it provokes the formation of new compounds, some of them toxic and associated with different diseases such as cancer, Alzheimer and Parkinson among others (Delfanian et al. 2015). The oxidation reactions are the major causes of deterioration in fats and oils during storage or heat conditions. Autoxidation reaction occurs through an auto-catalytic process (free radical chain mechanism) between unsaturated fatty acids and oxygen (Gunstone 2011). Primary antioxidants (natural or synthetic) prevent autoxidation by giving their hydrogen to free radicals formed during initial stages of autoxidation (Shahidi and Zhong 2005). Therefore, addition of these antioxidants is one of the major treatments used to maintain the quality of fats and oils (Terpinc et al. 2012). However, recent reports reveal that synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and tertiary butylhydroquinone (TBHQ) may be implicated in many health risks, including cancer and carcinogenesis (Martínez et al. 2013). Therefore, the most powerful synthetic

antioxidant (TBHQ) is not allowed for food application in Japan, Canada and Europe (Mohdaly et al. 2010). In recent years, extensive researches have been conducted on plants, because plants are rich sources of antioxidant compounds such as phenolic diterpenes, flavonoids, tannins and phenolic acids (Moure et al. 2001).

Loquat (*Eriobotrya japonica*) is an evergreen large shrub or small fruit tree with a rounded crown, short trunk and woolly new twigs in the family of Rosaceae (Hong et al. 2008). Loquat is unusual among other fruits such that they flower in autumn or early winter, and the fruits ripen in late winter or early spring (Lin et al. 1999). The species is native to southeastern China and mainly grows on subtropical and mild temperate regions in the world. Currently it is also cultivated in other areas namely in South Africa, South America, Australia and California (Ercisli et al. 2012). Phenolic compounds of loquat fruits have been characterised in few studies which described the antioxidant activity of loquat fruits as being due to the presence of hydroxycinnamic and benzoic acids derivatives and cyanidine glycoside (Tosun et al. 2009). Recently, various methods such as microwave-assisted, ultrasound-assisted and supercritical CO₂ extraction are used for the extraction of bioactive compounds of plants, because different methods have different abilities in extraction (Yasoubi et al. 2010).

Therefore, the objective of this study was to compare the influence of solvent extraction, ultrasound-assisted and supercritical CO₂ extraction methods on antioxidant activity of loquat fruit skin extracts on the stability of soybean oil during storage and frying conditions.

Materials and methods

Materials

Loquat fruits (*Eriobotrya japonica* Lindl.) were collected from fields in Sari in the Mazandaran province, Iran. Refined, bleached, and deodorized soybean oil with no added antioxidant was supplied by Rana (Gorgan, Iran) and stored at −20 °C until analysis. All reagents used in the experiments were of analytical grade and obtained mostly from Sigma Chemical Co. (St. Louis, MO). Solvents used for extraction of plant samples were purchased from Merck (Darmstadt, Germany).

Preparation of plant extract

The skin of fruits was manually removed, sun-dried, powdered in a grinder to reach 40-mesh and then were packed and stored at −20 °C until extraction of antioxidant. In solvent extraction, dried powders of skin (20 g) were mixed with 100 ml of ethanol. The mixture was stirred in a shaker at

160 rpm away from light at room temperature for 48 h. After extraction, the extracts were filtered and solvent evaporated using a rotary evaporator (Heidolph, Germany) at 50 °C. The concentrated extract was stored until testing at −20 °C (Tachakittirungrod et al. 2007). An Elma Transsonic model 690/H ultrasonic bath (Germany) was used for extraction of phenolic compounds from mixture of ethanol (100 ml) and powdered skin (20 g). The mixture was sonicated for 30 min at 35 °C. The mixture was filtered and solvent evaporated and extract stored in a freezer (Albu et al. 2004). A Suprex MPS/225 Multipurpose system (Pittsburg, USA) in the supercritical CO₂ extraction mode was used for the extraction of phenolic compounds. In this method, extractions of 20 g of dried powder from the skin were accomplished with a 100 ml of ethanol at 35 °C, 100 bar, for 30 min. The mixture was filtered and solvent evaporated and extract stored at −20 °C until testing (Luengthanaphol et al. 2004).

Extraction yield

The extraction yield (%) according to the method described by Tian et al. (2012) is calculated Eq. (1):

$$\text{Yield } (w/w\%) = \frac{\text{Weight of dried crude extract}}{\text{Weight of loquat powder}} \times 100 \quad (1)$$

Total phenolic compounds

Total phenolic content (TPC) of extracts were estimated spectrophotometrically using Folin–Ciocalteu assay described by Vajić et al. (2015) with some modifications. Briefly, 0.5 ml of different extracts was mixed with 2.5 ml of 10-fold-diluted Folin–Ciocalteu reagent. The solution was mixed thoroughly and allowed to stand at room temperature. After 4 min, 2 ml of 7.5 % sodium carbonate solution was added and then incubated at 25 °C for 2 h. The estimation of phenolic compounds was done at 760 nm using a UV–vis spectrophotometer and calculated by a calibration curve performed with gallic acid. The results were expressed as µg of gallic acid equivalents per g of dried sample (µg GAE/g DW).

DPPH[•] radical scavenging activity

The antioxidant activity of the extracts was measured using the DPPH[•] method described by Delfanian et al. (2015). This parameter was assessed according to ability of the extracts to reduce free radicals. Five milliliter of a 0.004 % ethanolic DPPH[•] solution was mixed with 50 µl of extract and the reaction mixture was shaken vigorously and incubated in the dark at 25 °C for 30 min. The absorbance of the mixtures was estimated at 517 nm against a blank. The radical scavenging activity of the extracts was expressed as a percentage of

DPPH[•] radical attraction calculated according to Eq. (2) below:

$$\% \text{ Inhibition} = \frac{A^0 - A^1}{A^0} \times 100 \quad (2)$$

where A^0 is absorbance of blank (DPPH[•] solution with no sample) and A^1 is absorbance of sample.

β -carotene/linoleic acid assay

Antiradical capacity of the extracts was measured according to method by Morelli and Prado (2012). One milligram of β -carotene, 200 mg of Tween 20, and 20 μ l of linoleic acid were added to 5 ml of chloroform and placed in a round-bottom flask. Chloroform was evaporated in a rotary vacuum evaporator at 40 °C. Oxygenated distilled water (50 ml) was added to the flask and mixture was stirred vigorously. Then, 6 ml of the mixture was transferred to tubes containing 50 μ l of extract. Finally, the absorbance of samples (Abs_0) was evaluated at 470 nm, and the tubes kept in a water bath at 50 °C for 2 h. The absorbance of samples (Abs_{120}) was determinate again to calculate the decreased absorbance in each sample. The antioxidant activity index (AAI) was carried out according to Eq. (3) below:

$$AAI(\%) = \left(1 - \frac{(Abs_0 - Abs_{120})_{\text{sample}}}{(Abs_0 - Abs_{120})_{\text{control}}} \right) \times 100 \quad (3)$$

Oxidative stability (rancimat)

Oxidative stability was estimated by measuring the oxidation induction time, on a Rancimat apparatus (Metrohm 743 Rancimat instrument, Herisau, Switzerland). Air (15 L/h) was bubbled through the oil (5 g) heated at 110 ± 0.5 °C, with the volatile compounds being collected in water, and the increasing water conductivity continually measured. The time taken to reach the conductivity inflection was recorded (Farhoosh and Tavassoli-Kafrani 2011).

Storage experiment

The ethanol extract of loquat fruit skin that extracted by different extraction techniques at 400 and 1000 ppm and tert-butyl hydroquinone (TBHQ) at 100 ppm were added to refined soybean oil. Soybean oil with no added antioxidants (SBO) was used as a control oil sample. Oil samples were stored at 25 °C in dark for 60 days. At the end of each 15 days (0, 15, 30, 45 and 60), about 20 g of the oil samples were filtered into a screw-cap vial and promptly stored in freezer at -20 °C until use.

Frying

The loquat fruit skin extracts at 400 and 1000 ppm and TBHQ at 100 ppm were added to soybean oil. Soybean oil without antioxidant addition was used as a negative control. Oil sample (2.5 L) was placed in a fryer oven of 2.5 L capacity (Tefal model 1250, France) and heated at 180 ± 5 °C for 24 h. A batch of 20 g of potato pieces ($7.0 \text{ cm} \times 0.5 \text{ cm} \times 0.3 \text{ cm}$) was fried for 7 min. After time of heating (0, 4, 8, 12, 16, 20, 24 h), 20 g of frying oil samples was stored until testing at -20 °C (Farhoosh and Tavassoli-Kafrani 2010).

Peroxide value (PV)

The spectrophotometric method described by Shantha and Decker (1993) was used to determine peroxide value. 0.2 g of oil samples was dissolved in 9.8 ml chloroform-methanol (7:3 v/v). Then, 50 μ L of ammonium thiocyanate solution (30 % w/v) and 50 μ l of iron (II) chloride solution ([0.5 g barium chloride dihydrate dissolved in 50 ml dH₂O] + [0.5 g FeSO₄·7H₂O dissolved in 50 ml dH₂O] + [2 ml 10 M HCl, with the precipitate barium sulphate, filtered off to produce a clear solution]) were added, and the sample was mixed on a vortex mixer for 2–4 s. After 5 min incubation at room temperature, the absorbance of reaction mixture was measured at 500 nm against a blank. Results were expressed in millequivalents of oxygen per kilogram of oil.

Free fatty acids content (FFA)

FFA contents of samples were determined using AOCS official method Cd 3a-63 (AOCS 1998). Briefly, 10 g of oil samples were dissolved in a 50 ml mixture of neutral ethanol-chloroform (50:50 v/v). Then mixture was shaken for 1 min. Mixture was titrated against potassium hydroxide (0.1 N) using phenolphthalein solution (10 g/l) as an indicator. FFA content (% Oleic acid) was calculated according to Eq. (4) below:

$$[\text{FFA}] = (V \times C \times 56.11)/m \quad (4)$$

Where V is the volume of potassium hydroxide exhausted by samples (ml); C is the concentration of potassium hydroxide (M); m is the mass of soybean oil (g).

Conjugated dienes and trienes

The contents of the conjugated dienes (CDV) and conjugated trienes (CTV) were calculated according to the method described by Fathi et al. (2013), which is based on the measurement of absorbance solution (5 mg of the oil sample dissolved in 10 ml cyclohexane) at 234 nm and 270 nm for CDV and CTV, respectively.

Statistical analysis

All experiments and measurements were carried out in triplicate, and data were subjected to analysis of variance (ANOVA). Significant statistical differences among treatments ($p < 0.05$) were assessed by a Duncan test. The SPSS software version 19.0 (SPSS, Chicago, IL) was used for statistical determinations.

Results and discussion

Extraction yield

The extraction yield of loquat fruit skin was ranged from 10.5 to 14.75 %. The extraction yields of different methods in descending order were: solvent extraction (14.75 %) > supercritical CO₂ (12.5 %) > ultrasound-assisted (10.5 %). This shows that solvent extraction was the best techniques for the extraction of compounds from the loquat fruit skin. Our results concurred with previously published results of Plándier et al. (2012) and Sánchez-Vioque et al. (2013), that reported classic extraction method was more effective in extraction of plant material compared to other techniques.

Total phenolic content

Concentration of phenolics in the extracts, expressed in µgGAE/g dry extract depends on the method used for extraction. There were significant differences ($P < 0.05$) between phenolic compounds of extracts of three mentioned methods. The amounts of phenolic compounds in the extracts of ultrasound-assisted, supercritical CO₂ and solvent extraction were 394.67, 425.02 and 664.53 (µg/g), respectively. Luengthanaphol et al. (2004) and Plándier et al. (2012) reported similar results that the solvent extraction was the most effective in extraction of phenolic compounds in comparison with ultrasound-assisted and supercritical CO₂ extraction techniques. Ferreres et al. (2009) calculated that the total phenolic content of various varieties of loquat fruit skin range from 13.1 to 1349.8 (µg/g). The phenolic content and composition of fruits depend on the genetic and environmental factors as well as post-harvest processing conditions (Rop et al. 2011); (Milivojevic et al. 2012).

DPPH[•] radical scavenging activity

Table 1 shows the ability of loquat fruit skin extracts to scavenge DPPH[•] radical as inhibition percentage at concentrations of 100 to 1000 ppm. In this assay, scavenging free radicals increased as concentrations of the extracts increased which due to increasing amount of phenolic compounds at higher concentrations of the extracts. Similar to previously published

results of Chaillou and Nazareno (2006) and Zhang et al. (2010). With increasing concentrations of phenolic compounds the number of hydroxyl groups available in the reaction medium increased. So, the possibility of hydrogen donation to free radicals is increased (Sánchez-Vioque et al. 2013). The result showed ultrasound-assisted extract of loquat skin (UELS) at concentrations of 100 to 300 ppm had the highest DPPH[•] radical-scavenging capacity, but solvent extract of loquat skin (SELS) had a better performance at 400 and 1000 ppm compared to other extracts. Our result also demonstrated that skin extracts have good antioxidant activity, but the highest inhibitory effect was observed in TBHQ. These results concurred with previously published results by Silva et al. (2004), in which the antioxidant effect of quince fruit skin and pulp extracts compared to TBHQ.

β-carotene bleaching assay

The antioxidant activities of the loquat fruit skin extracts were measured by bleaching of β-carotene which is showed in Table 1. The CELS indicated the lowest significant antioxidant activity at 100, 400 and 1000 ppm, while the SELS showed better performance to prevent β-carotene oxidation in all the tested concentrations. The SELS at 400 ppm exhibited antioxidant activity which was comparable to that of standard synthetic antioxidant TBHQ. Results of antioxidant activities were in agreement with previously published results by Koba et al. (2007) that examined the antioxidant effect of loquat fruit skin by β-carotene bleaching assay.

Rancimat analysis

The Rancimat analysis was performed at 110 °C and the induction period (h) was evaluated for soybean oils with or without loquat fruit skin extracts at 400 and 1000 ppm. Our result showed extracts and TBHQ had a strong antioxidant activity in soybean oil. As shown in Fig. 1, the presence of extracts retarded the oxidation of soybean oil. Generally, the lowest thermal stability in oils treated with extracts was for CELS (CE) sample (3.92 h), which was higher compared to control oil (3.32 h). On the other hand the highest thermal stability in oils containing loquat fruit skin extracts was for SELS (SE) at 400 and 1000 ppm (4.69 and 4.49 h), but the best protection effect was observed in soybean oil containing 100 ppm of TBHQ (6.48 h). These results concurred with the results of Sun and Ho (2005) and Aladedunye and Matthäus (2014) who reported the induction period of oils containing buckwheat (*Fagopyrum esculentum* Möench), rowanberry (*Sorbus aucuparia*) and crabapple (*Malus baccata*) extracts was higher than control oil, but the most antioxidant effect was for TBHQ. Moreover, from the findings of Rancimat assay it is understood that the samples extracted by solvent

extraction method exhibited strong antioxidant activity under the Rancimat conditions compared to other techniques.

Changes in peroxide value (PV)

Changes in PV of the oil samples during deep frying and storage conditions are shown in Table 2. The PV of fresh oil should be less than 2 meqO₂/kg (Man and Hussin 1998). The initial PV of oil samples was 0.65 (meqO₂/kg). It was generally observed that the presence of extracts significantly ($P < 0.05$) reduced the PV compared to control oil (SBO) in both conditions. The PV of SOEA, SOSB and SO-TBHQ treatments has no significant differences during 4 h of frying. The peroxide levels during frying after 8 h in SOUB, after 12 h in SOEA, after 16 h in SOUB, after 20 h in SO-TBHQ, after 24 h in SOEA and SOUA were lower than the other oil samples.

The SOEA showed greater ability to prevent augmentation in PV compared to other oil samples. There was an initial increase in PV for SOUB from 0 to 12 h, for SOSB, SOUA, SO-TBHQ and SBO from 0 to 16 h, for SOEA, SOEB and SOSA from 0 to 20 h and after which the rate decelerated. Peak values for PV were obtained as follows: SBO (6.58 meq/kg), SO-TBHQ (5.11 meq/kg) after 16 h, SOEA (4.55 meq/kg), SOEB (5.43 meq/kg), SOSA (5.84 meq/kg), SOSB (5.66 meq/kg), SOUA (4.72 meq/kg), SOUB (5.42 meq/kg) after 20 h. The PV decreased in oil samples after the peak point. Results were in agreement with Abdulkarim et al. (2007) and Casal et al. (2010).

During storage conditions (Table 2) the PV of SO-TBHQ, SOEA, SOEB, SOUA, SOUB, SOSB and SOSA were 9.42, 9.72, 10.06, 11.11, 11.82, 12.05 and 12.33 meq O₂ per kg,

Fig. 1 Antioxidant activity of loquat fruit skin extracts analyzed by Rancimat method. SE: solvent extract of loquat fruit skin, UE: ultrasound-assisted extract of loquat fruit skin, CE: supercritical CO₂ extract of loquat fruit skin, Blank: soybean oil without additives

respectively. It was generally observed that the extracts reduced PV compared to control oil significantly ($P < 0.05$). Results also showed that the PV increased linearly with increasing of storage days. This results concurred with the results of Suja et al. (2005); Goli et al. (2005). Trend of increase in PV of oils consisting solvent extract of skin was lower than other oils treated with extracts. There was no significant difference between the PV level of SOEB at day 15 and SOEA at days 30, 45 with SO-TBHQ. Therefore, SOEA showed a suitable antioxidant activity, but the best protection was observed in SO-TBHQ. Sikwese and Duodu (2007) compared antioxidant effects of sorghum crude phenolic extract with TBHQ

Table 1 Effects of loquat skin extracts on DPPH[•] and β -carotene/linoleic acid free radicals

Sample	Concentration				
	100 ppm	200 ppm	300 ppm	400 ppm	1000 ppm
DPPH [•] assay			% Inhibition		
SELS	45.68 ± 0.15 ^c	49.88 ± 0.1 ^b	50.96 ± 0.07 ^b	59.13 ± 0.07 ^a	66.71 ± 0.04 ^a
UELS	49.44 ± 0.07 ^b	53.88 ± 0.07 ^a	54.35 ± 0.11 ^a	56.44 ± 0.09 ^b	57.28 ± 0.11 ^b
CELS	44.26 ± 0.01 ^d	43.07 ± 0.03 ^c	47.22 ± 0.06 ^c	50.58 ± 0.07 ^c	53.65 ± 0.07 ^c
TBHQ	67.63 ± 0.01 ^a				
β-carotene assay					
SELS	33.33 ± 2.2 ^b	55.41 ± 5.95 ^a	78.33 ± 3.33 ^a	85.58 ± 3.51 ^a	64.58 ± 2.08 ^a
UELS	24.16 ± 2.19 ^c	34.58 ± 1.43 ^c	39.16 ± 5.51 ^c	77.08 ± 3.91 ^b	56.25 ± 3.61 ^b
CELS	21.74 ± 5.86 ^d	41.66 ± 2.66 ^b	55.69 ± 4.17 ^b	64.73 ± 2.63 ^c	51.24 ± 1.27 ^c
TBHQ	89.58 ± 1.04 ^a				

Mean value $\pm$ SD (values followed by different letters are significantly different within the same column, $P < 0.05$)

SELS solvent extract of loquat skin, UELS ultrasound-assisted extract of loquat skin, CELS supercritical CO₂ extract of loquat skin

in sunflower oil in the presence of ferric ions. They showed that TBHQ made a higher inhibitory effect on primary oxidation of the oil than the extract. Mohdaly et al. (2011) compared antioxidative effects of sesame cake extract with BHT, BHA and TBHQ in soybean and sunflower oil. They showed that sesame cake extract exhibited stronger antioxidant activity in oils than BHT and BHA, while its antioxidant activity was less than that of TBHQ.

Changes in free fatty acids content (FFA)

Table 3 shows change in FFA content of oil samples during deep frying and storage process. There was no significant difference between the initial FFA (0 h) of oil samples. Similar to results reported by Kim and Choe (2008) and Aladedunye and Przybylski (2013) it was observed that the FFA content increased with the frying time. At the end of the deep frying process, the FFA contents of the SOEA, SO-TBHQ, SOSA, SOUA, SOEB, SOSB, SOUB and SBO were 3.55 %, 3.85 %, 4.22 %, 4.22 %, 4.35 %, 4.44 %, 4.52 % and 7.44 %, respectively. The FFA contents of the SOSB, SOUB, and SO-TBHQ after 4 h, SOEA, SOUB, and SO-TBHQ after 8 h with no significant difference were lower than other oil samples. The low level of FFA for SOEA during frying time (at 8, 12, 16, 20, 24 h) indicated high ability to reduce oxidation of unsaturated fatty acids. The results also showed that the natural antioxidants in the loquat skin extracts better protected the oils from hydrolysis compared to TBHQ and control oil sample. These results concurred with the results of Casarotti

and Jorge (2014) and Urbančič et al. (2014) which examined the antioxidant effect of rosemary extract in soybean and sunflower oil.

In storage conditions the FFA content for SO-TBHQ, SOEA, SOEB, SOUA, SOSA, SOSB, SOUB and SBO after 65 days of storage was 0.34, 0.44, 0.52, 0.52, 0.56, 0.65, 0.65 and 1.22 %, respectively. Therefore, the FFA in SOEA was lower than other oils containing extracts, but it could not make reduction compared to TBHQ. The FFA results concurred with Habib and Shah (2004) and Zia-ur-Rehman (2006) which examined the antioxidant effect of citrus peel extract and potato peels extract in soybean oil at 25 °C.

Change in conjugated dienes and trienes values

The changes in CDV and CTV of oil samples during frying and storage conditions are shown in Fig. 2. The CDV and CTV were initially present in the fresh oil in small amounts (2.85 and 0.64 mmol/l), that increase with increased time, as this is reported in other studies such as the works by Abdulkarim et al. (2007) and Bou et al. (2012). The CDV and CTV of soybean oil treated with skin extracts were significantly different from the control oil and from the oils with the addition of the TBHQ. During frying process the CDV (Fig. 2a) of the SOEA, SO-TBHQ, SOUA, SOEB, SOUB, SOSA, SOSB and SBO after 24 h of frying were 16.58, 16.66, 17.44, 17.61, 17.82, 18.05, 18.43 and 23.5 mmol/l, respectively. The CDV for SO-TBHQ after 4 h, for SOEA, SOUB, and SO-TBHQ (with no significant difference) after

Table 2 Changes in peroxide value (meq/kg) of the oil samples during frying and storage conditions

Time	Frying							
	SOEA	SOEB	SOSA	SOSB	SOUA	SOUB	SO-TBHQ	SBO
0	0.65 ± 0.01 ^a	0.65 ± 0.01 ^a	0.65 ± 0.01 ^a	0.65 ± 0.01 ^a	0.65 ± 0.01 ^a	0.65 ± 0.01 ^a	0.65 ± 0.01 ^a	0.65 ± 0.01 ^a
4	1.64 ± 0.03 ^{ab}	2.04 ± 0.05 ^c	2.22 ± 0.03 ^d	1.65 ± 0.07 ^{ab}	2.24 ± 0.04 ^d	1.72 ± 0.06 ^b	1.58 ± 0.14 ^a	3.82 ± 0.08 ^e
8	2.56 ± 0.04 ^c	2.52 ± 0.07 ^{de}	2.34 ± 0.04 ^b	2.44 ± 0.07 ^{cd}	2.53 ± 0.03 ^e	2.11 ± 0.07 ^a	2.43 ± 0.02 ^c	4.54 ± 0.02 ^f
12	3.58 ± 0.06 ^a	3.74 ± 0.05 ^b	4.15 ± 0.05 ^c	4.24 ± 0.02 ^d	3.75 ± 0.03 ^b	4.42 ± 0.03 ^e	4.12 ± 0.04 ^c	5.76 ± 0.04 ^f
16	4.32 ± 0.04 ^b	4.51 ± 0.03 ^c	5.27 ± 0.02 ^e	5.66 ± 0.04 ^f	4.72 ± 0.03 ^d	4.05 ± 0.07 ^a	4.75 ± 0.02 ^d	6.58 ± 0.04 ^g
20	4.55 ± 0.03 ^b	5.43 ± 0.03 ^e	5.84 ± 0.02 ^f	5.06 ± 0.03 ^d	4.68 ± 0.06 ^c	5.42 ± 0.02 ^e	4.04 ± 0.02 ^a	5.88 ± 0.03 ^f
24	4.06 ± 0.02 ^a	5.12 ± 0.03 ^c	5.23 ± 0.02 ^d	5.48 ± 0.04 ^e	4.05 ± 0.01 ^a	4.76 ± 0.04 ^b	5.11 ± 0.05 ^c	6.34 ± 0.02 ^f
	Storage							
0	0.65 ± 0.01 ^a	0.65 ± 0.01 ^a	0.65 ± 0.01 ^a	0.65 ± 0.01 ^a	0.65 ± 0.01 ^a	0.65 ± 0.01 ^a	0.65 ± 0.01 ^a	0.65 ± 0.01 ^a
15	2.12 ± 0.03 ^c	1.45 ± 0.02 ^a	2.35 ± 0.03 ^c	2.14 ± 0.03 ^c	2.22 ± 0.02 ^d	1.85 ± 0.04 ^b	1.42 ± 0.02 ^a	5.85 ± 0.03 ^f
30	4.63 ± 0.03 ^a	5.37 ± 0.06 ^b	6.54 ± 0.03 ^d	6.88 ± 0.03 ^e	5.42 ± 0.04 ^b	5.68 ± 0.04 ^c	4.57 ± 0.05 ^a	12.77 ± 0.05 ^f
45	6.75 ± 0.01 ^a	7.24 ± 0.01 ^b	8.52 ± 0.02 ^e	8.84 ± 0.02 ^f	7.32 ± 0.03 ^c	7.77 ± 0.02 ^d	6.73 ± 0.03 ^a	20.64 ± 0.04 ^g
60	9.72 ± 0.03 ^b	10.06 ± 0.03 ^c	12.33 ± 0.03 ^g	12.05 ± 0.04 ^f	11.11 ± 0.03 ^d	11.82 ± 0.02 ^e	9.45 ± 0.03 ^a	31.15 ± 0.03 ^h

Means ± SD within each row followed by different letters (a, b, c, etc.) are significantly different ($P < 0.05$)

Soybean oil with 400 (SOEA) and 1000 ppm (SOEB) of solvent extracts of skin, soybean oil with 400 (SOSA) and 1000 ppm (SOSB) of skin extracts by supercritical CO₂ method, soybean oil with 400 (SOUA) and 1000 ppm (SOUB) of skin extracts by sonication method, soybean oil with 100 ppm of TBHQ (SO-TBHQ), soybean oil with no antioxidant added (SBO)

Table 3 Changes in free fatty acids content (%) of the oil samples during frying and storage conditions

Time	Frying							
	SOEA	SOEB	SOSA	SOSB	SOUA	SOUB	SO-TBHQ	SBO
0	0.05 ± 0.01 ^a	0.05 ± 0.01 ^a	0.05 ± 0.01 ^a	0.05 ± 0.01 ^a	0.05 ± 0.01 ^a	0.05 ± 0.01 ^a	0.05 ± 0.01 ^a	0.05 ± 0.01 ^a
4	1.22 ± 0.06 ^b	1.18 ± 0.03 ^b	1.22 ± 0.06 ^b	1.05 ± 0.04 ^a	1.22 ± 0.06 ^b	1.05 ± 0.04 ^a	1.05 ± 0.04 ^a	1.44 ± 0.02 ^c
8	1.44 ± 0.02 ^a	1.58 ± 0.03 ^c	1.74 ± 0.02 ^d	1.74 ± 0.02 ^d	1.52 ± 0.04 ^b	1.44 ± 0.02 ^a	1.44 ± 0.02 ^a	2.52 ± 0.04 ^e
12	1.88 ± 0.03 ^a	2.08 ± 0.03 ^b	2.45 ± 0.03 ^e	2.58 ± 0.03 ^f	2.45 ± 0.03 ^e	2.34 ± 0.03 ^d	2.28 ± 0.06 ^c	3.64 ± 0.02 ^g
16	2.44 ± 0.02 ^a	3.05 ± 0.04 ^d	3.24 ± 0.04 ^e	3.44 ± 0.02 ^f	2.98 ± 0.03 ^c	3.05 ± 0.04 ^d	2.85 ± 0.03 ^b	4.67 ± 0.06 ^g
20	3.05 ± 0.04 ^a	3.32 ± 0.04 ^b	3.52 ± 0.03 ^d	4.32 ± 0.04 ^e	3.48 ± 0.04 ^{cd}	4.45 ± 0.03 ^f	3.44 ± 0.02 ^c	6.72 ± 0.03 ^g
24	3.55 ± 0.03 ^a	4.35 ± 0.03 ^d	4.22 ± 0.06 ^c	4.44 ± 0.02 ^e	4.22 ± 0.06 ^c	4.52 ± 0.03 ^f	3.85 ± 0.03 ^b	7.44 ± 0.02 ^g
Storage								
0	0.06 ± 0.01 ^a	0.06 ± 0.01 ^a	0.06 ± 0.01 ^a	0.06 ± 0.01 ^a	0.06 ± 0.01 ^a	0.06 ± 0.01 ^a	0.06 ± 0.01 ^a	0.06 ± 0.01 ^a
15	0.15 ± 0.01 ^c	0.15 ± 0.02 ^c	0.22 ± 0.02 ^d	0.11 ± 0.02 ^b	0.22 ± 0.02 ^d	0.11 ± 0.02 ^b	0.07 ± 0.01 ^a	0.28 ± 0.03 ^e
30	0.22 ± 0.02 ^b	0.34 ± 0.02 ^c	0.34 ± 0.03 ^c	0.44 ± 0.02 ^e	0.38 ± 0.03 ^{cd}	0.44 ± 0.02 ^e	0.11 ± 0.03 ^a	0.42 ± 0.02 ^{de}
45	0.34 ± 0.01 ^b	0.44 ± 0.01 ^c	0.44 ± 0.04 ^c	0.52 ± 0.02 ^d	0.44 ± 0.01 ^c	0.52 ± 0.03 ^d	0.22 ± 0.02 ^a	0.75 ± 0.01 ^e
60	0.44 ± 0.04 ^b	0.52 ± 0.01 ^c	0.56 ± 0.03 ^c	0.65 ± 0.02 ^d	0.52 ± 0.02 ^c	0.65 ± 0.01 ^d	0.34 ± 0.01 ^a	1.22 ± 0.02 ^e

Means ± SD within each row followed by different letters (a, b, c, etc.) are significantly different ($P < 0.05$)

Soybean oil with 400 (SOEA) and 1000 ppm (SOEB) of solvent extracts of skin, soybean oil with 400 (SOSA) and 1000 ppm (SOSB) of skin extracts by supercritical CO₂ method, soybean oil with 400 (SOUA) and 1000 ppm (SOUB) of skin extracts by sonication method, soybean oil with 100 ppm of TBHQ (SO-TBHQ), soybean oil with no antioxidant added (SBO)

8 h and for SOEA after 12 to 24 h, were lower than other oil samples.

The CTV (Fig. 2b) for the control oil at the end of 24 h of frying was greater than that for the oils treated with skin extracts and TBHQ. For the all treatments of the SOEA, SOEB, SOSA, SOSB, SO-TBHQ, SOUA, SOUB and SBO, the levels of the CTV at the end of the frying time were

8.14, 8.55, 8.94, 9.45, 9.48, 9.48, 9.77 and 13.65 (mmol/l), respectively. The CTV of the SO-TBHQ after 8 h and SOEA after 12 to 20 h were lowest. Therefore, the low levels of both conjugated dienes and trienes in SOEA are indications of good oxidative stability of the oil. The conjugated dienes and trienes results are approved by other studies such as Casarotti and Jorge (2014) and Urbančič et al. (2014), that

Fig. 2 Changes in conjugated dienes and trienes of the oil samples during frying (a, b) and storage (c, d) conditions. Soybean oil with 400 (SOEA) and 1000 ppm (SOEB) of solvent extract of loquat fruit skin, soybean oil with 400 (SOSA) and 1000 ppm (SOSB) of supercritical CO₂ extract, soybean oil with 400 (SOUA) and 1000 ppm (SOUB) of ultrasound-assisted extract, soybean oil with 100 ppm of TBHQ (SO-TBHQ), soybean oil without any antioxidant added (SBO)

showed the antioxidant effect of rosemary extract in prevention of conjugated compounds augmentation in vegetable oil was higher than TBHQ.

During storage conditions the CDV and CTV values (Fig. 2C and D) for the SBO at the end of the 60 days of storage were greater than that of the oils treated with extracts and TBHQ. The CDV and CTV values of soybean oils consisting solvent extract of loquat fruit skin was lower in comparison with ultrasonic and supercritical fluid CO₂ extracts. The SOEA with no significant difference with SO-TBHQ indicated a greater ability to reduce the production of conjugated compounds, compared to other oil samples during storage conditions. Our results were in agreement with previously published results of Mohdaly et al. (2010) and Mohdaly et al. (2011).

Conclusion

The present study focused on effects of different extraction techniques on the antioxidant activity of loquat fruit skin extracts. Evaluation of antioxidant activity of these extracts in vitro by DPPH radical scavenging, β -carotene/linoleic acid, and Rancimat tests showed that the solvent extraction method had the most anti-radical activity. This subject also confirmed by the assessment of influence extracts in oxidation stability of soybean oil in terms of peroxide value, free fatty acids, conjugated dienes and trienes value during storage (60 day at 25 °C) and frying process (24 h at 180 °C). The SOEA showed the highest antioxidant activity during frying process of oil compared to other extracts and TBHQ, but during storage conditions TBHQ was more effective.

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