Influence of extraction techniques on antioxidant properties and bioactive compounds of loquat fruit (*Eriobotrya japonica* Lindl.) skin and pulp extracts

Mojtaba Delfanian¹, Reza Esmaeilzadeh Kenari¹ & Mohammad Ali Sahari²

¹Department of Food Science and Technology, Sari Agriculture and Natural Resources University, Sari, Mazandaran, Iran
²Department of Food Science and Technology, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran

**Abstract**

In this study, the bioactive compounds of loquat fruit (*Eriobotrya japonica* Lindl.) skin and pulp extracted by two extraction methods (solvent and ultrasound-assisted) with three solvents (ethanol, water, and ethanol–water) were compared to supercritical fluid extraction. The antioxidant activities of skin and pulp extracts were evaluated and compared to tertiary butylhydroquinone (TBHQ) using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, β-carotene bleaching, and the Rancimat assays. In DPPH assay solvent extracts of skin by ethanol (SSE) and ethanol–water (SSEW) showed strong inhibitory activity. The SSEW also showed the highest inhibition percentage of 85.58% by the β-carotene bleaching assay and longest induction time of 4.78 h by the Rancimat method. The large amount of tocopherols and phenolics contained in the skin extract may cause its strong antioxidant ability. The results indicated that the solvent extraction with ethanol–water produced the maximum extraction yield of phenolic and tocopherol compounds from loquat fruit skin and pulp. Furthermore, solvent extraction was the most effective in antioxidant activity of the extracts compared to other extraction techniques.

**Introduction**

To prevent oxidation of oils and fats, the use of antioxidants is mandatory because compounds produced from the oxidation of oil, such as hydroperoxide, hydroxyl radical, and a single oxygen capacity can damage biological molecules (Mohdaly et al. 2010). This causes cellular damage and the development of physiological abnormalities such as premature aging, neurological, and heart disease (Suja et al. 2004).

Therefore, synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and tertiary butylhydroquinone (TBHQ) are often used to increase the oxidative stability of oils. Studies have shown that these antioxidants are toxic and carcinogenic. Therefore, the use of natural antioxidants is required as alternatives to synthetic antioxidants (Zhang et al. 2010). Various studies have shown plants to be a rich source of natural antioxidants. Compounds with antioxidant properties found in plants include the vitamins A, E, and phenolic compounds, flavonoids, tannins, and lignins (Razali et al. 2012).

Loquat (*Eriobotrya japonica*) is an Asian fruit in the family of Rosaceae. The species is native to southeastern China and mainly grow in subtropical and mild temperate regions (Cha et al. 2011). Currently, it is also cultivated in other areas, namely in South Africa, South America, Australia, and California. Loquat is an evergreen tree with short branches that flower in late autumn or early winter (Hong et al. 2008). Its fruits ripen in late winter or early spring. Loquat is a round yellow fruit with a smooth and downy form that has a white pulp which has three to five brown seeds. Its sweet or sour taste depends on the area where it grows (Ercisli et al. 2012). Loquat fruit has good antioxidant properties due to the presence of phenolic (benzoic acid and hydroxy cinnamic...
derivatives) and tocopherol compounds (Tosun et al. 2009).

Nowadays, various techniques such as ultrasound-assisted and supercritical fluid extraction are used to extract bioactive compounds from plants. Ultrasonic waves with frequency of more than 20 KHz are in two forms of the probes and bath. Ultrasound has high ability to extract phenolic compounds and that is the reason it is being used frequently (Shirsath et al. 2012). Ultrasound cavitation effect can cause damage to the cell wall and thus improve the extraction yield. Sometimes ultrasound waves may possibly destroy the antioxidant compounds and reduce the extraction yield (Cao et al. 2009). Thus, implementation of this method for any plant material requires further investigation.

The supercritical fluids were also used for the extraction of antioxidant compounds. Supercritical state was achieved when the temperature and the pressure of a substance rose over its critical value, in which the fluid has the characteristics of both liquids and gases (Wang and Weller 2006). Selection of supercritical fluids is important to develop a supercritical fluid extraction method. Mostly, supercritical CO₂ is used because it is inexpensive, nonflammable and nontoxic (Martinez-Correa et al. 2011). Supercritical CO₂ is a suitable solvent for the extraction of nonpolar compounds such as hydrocarbons. Many polar compounds such as phenols, tocopherols, glycosides and alcohols have poor solubility in supercritical CO₂ (Hamburger et al. 2004). The use of modifier solvents such as ethanol, acetone, and methanol can effectively increase the solubility and extraction of polar compounds. Although methanol has greater ability to extract polar compounds, but due to its toxicity ethanol is used more frequently (Lang and Wai 2001). Recently this method has been used extensively because there are various nonpolar compounds in plants which have relatively good antioxidant effects.

Solvent extraction method is a traditional method for extraction and is more frequently used for the isolation of bioactive compounds. In this method, extraction yield of bioactive compounds is dependent on conditions of extraction and the solvent polarity. Since there was no study reporting any kind of damaging effect to plant bioactive compounds in the solvent extraction method, hence it is being used extensively for extraction, but the long process time in this method caused scientists to search for an alternative method (Rodriguez-Rojo et al. 2012).

Therefore, the aim of this work was to compare the effects of solvent extraction and ultrasound-assisted techniques on antioxidant activity of loquat fruit skin and pulp extracts with supercritical CO₂ extraction.

Materials and Methods

Materials

Loquat fruits were collected from fields in Sari in the Mazandaran province, Iran. All reagents used in the experiments were of analytical grade and obtained mostly from Sigma Chemical Co. (St. Louis, MO). Solvents used for the extraction of plant samples were purchased from Merck (Darmstadt, Germany).

Extraction Methods

Solvent extraction

The skin and pulp of the loquat fruit were separated and dried in the shade in natural conditions. Dried powder of the samples (20 g) were mixed with 100 ml of each solvent (ethanol, water, ethanol–water (1:1)). The mixture was stirred in a shaker (LABTRON Ls-100) at 160 rpm away from light at room temperature for 48 h. The extracts were filtered and solvents evaporated using a rotary evaporator (Heidolph, Schwabach, Germany) at 50°C. The extracts were stored at −20°C until testing (Tachakittirungrod et al. 2007).

Ultrasound-assisted extraction

Dried powders of samples (20 g) were mixed with 100 mL of each solvent (ethanol, water, ethanol-water (1:1)). All the samples were placed in dark brown-colored reagent bottles with narrow necks and mixed for 30 min in an ultrasonic water bath (KQ-250DB; Kunshan Ultrasound Co. Ltd., Elma, model 690/H, Cottbus, Germany) of 35°C at 35 KHz (100% power). The extracts were filtered and solvents evaporated using a rotary evaporator. The concentrated extracts were stored in a freezer (Albu et al. 2004).

Supercritical CO₂ extraction

A Suprex MPS/225 Multipurpose system (Roth Scientic, Basingstoke, Hampshire, U.K.) was used for the extraction of bioactive compounds. In this method, extractions of 20 g of dried powder from the skin and pulp were accomplished with 100 mL ethanol at 35°C, 100 bar for 30 min. The extracts were filtered and concentrated using a rotary evaporator. The concentrated extracts were stored under refrigeration until further analysis (Luengthanaphol et al. 2004).

Determination of extraction yield

The extraction yield (%) according to the method described by Tian et al. (2012) is calculated as follows:
Yield (w/w%) = \frac{\text{Weight of dried crude extract}}{\text{Weight of loquat powder}} \times 100

**Determination of total tocopherol content**

The content of tocopherol compounds was determined according to the colorimetric method described by Wong et al. (1988). A calibration curve of pure \( \alpha \)-tocopherol in toluene was performed in a concentration range of 0–240 \( \mu \)g/mL. The extract (0.2 g) was dissolved in 5 mL of toluene and then 3.5 mL of 2, 2’-bipyridine (0.07% w/v in 95% aqueous ethanol) and 0.5 mL of FeCl\(_3\)-6H\(_2\)O (0.2% w/v in 95% aqueous ethanol) were added into that mixture. The solution was made up to 10 mL with 95% aqueous ethanol. After standing for 1 min, the absorption at 520 nm was determined by using a spectrophotometer (Cintra 20; GBC, Dandenong, Australia). The results are expressed as microgram of \( \alpha \)-tocopherol equivalents per gram extract (\( \mu \)g \( \alpha \)-tocopherol/g).

**Determination of total phenolic content**

The content of phenolic compounds in the extracts was determined according to the method of McDonald et al. (2001). Briefly, 0.5 mL of extract was mixed with 2.5 mL of 10-fold-diluted Folin–Ciocalteu reagent and 2 mL of 7.5% sodium carbonate. Then, the mixture was shaken for 1 min and allowed to stand at room temperature for 15 min. Absorbance of the solution was measured at 765 nm using a spectrophotometer (Cintra 20; GBC). The concentration of phenolic compounds was estimated using a calibration curve traced with gallic acid in methanol at concentrations of 0.04–0.4 mg/mL as a polyphenol reference. Results were expressed as \( \mu \)g gallic acid per g extract (\( \mu \)g GAE/g). Each test was repeated three times, and the results were averaged.

**DPPH radical scavenging activity**

The ability of the extracts to scavenge 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) was determined by the method described by Burits and Bucar (2000). Briefly, 50 \( \mu \)L of various dilutions of the test materials (pure antioxidants or loquat extracts) were mixed with 5 mL of a 0.004% methanolic DPPH solution. The reaction mixtures were shaken vigorously and incubated in the dark for 30 min. The absorbance of the solution was measured at 517 nm against a blank. The radical scavenging activity of each solution was calculated as inhibition percentage according to the following equation:

\[
\% \text{Inhibition} = 100 \left( \frac{\text{Abs}_{\text{blank}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{blank}}} \right)
\]

**\( \beta \)-Carotene bleaching assay**

Antioxidant activities of loquat extracts were determined using the \( \beta \)-carotene bleaching method, as described by Amarowicz et al. (2010). Briefly, 5 mg of \( \beta \)-carotene were dissolved in 10 mL of chloroform and 600 \( \mu \)L \( \beta \)-carotene solutions was mixed with 40 mg of linoleic acid and 400 mg of Tween 40 emulsifier in a round-bottom flask. Then, chloroform was removed in a rotary vacuum evaporator. Oxygenated distilled water (100 mL) was added to the flask and the resulting mixture was stirred vigorously. Five milliliters of the emulsion were transferred into tubes containing 200 \( \mu \)L of different concentrations of extracts. After mixing, the absorbance of the samples was measured at 470 nm at initial time \( (T = 0) \) against a blank. The remaining samples were placed in dark for 24 h. Then, the absorbance of each sample was measured at 470 nm \( (\text{Abs}_{24}) \). Antioxidant activity was calculated as inhibition percentage according to the following equation:

\[
\% \text{Inhibition} = \frac{1 - (\text{Abs}_{s(24)} - \text{Abs}_{s(0)})}{(\text{Abs}_{\text{c}(24)} - \text{Abs}_{b(0)})} \times 100
\]

where \( \text{Abs}_{s(24)} \) is the absorbance of the antioxidant at 24 h, \( \text{Abs}_{\text{c}(24)} \) is the absorbance of the blank at 24 h, \( \text{Abs}_{s(0)} \) is the absorbance of the antioxidant at 0 times, and \( \text{Abs}_{b(0)} \) is the absorbance of the blank at 0 time.

**Rancimat analysis**

A Metrohm 743 Rancimat instrument (Herisan, Switzerland) was used in the experiment. Air supply was maintained at 15 L/h and the temperature was kept at 120°C (Farhoosh and Tavassoli-Kafhari 2011).

**Statistical analysis**

All experiments were conducted at three levels of measurements and results reported as mean ± standard deviation (SD). The data were analyzed by analysis of variance (ANOVA), followed by Duncan test to detect significant differences between means of treatments at \( P < 0.05 \). Statistical analysis was performed using SPSS statistical software (version 19; SPSS Inc., Chicago, IL).

**Results and Discussion**

**Extraction yields**

Loquat fruit skin and pulp contains considerable amounts of extractable compounds (Fig. 1). The extraction yield of loquat fruit skin and pulp range from 10.5 to 30 and 17.85 to 44.75%, respectively. ANOVA results indicated that the
published results such as the work by Plánder et al. (2012) and Sánchez-Vioque et al. (2013). The lower capability of supercritical CO2 for extraction of polar compounds and destruction of bioactive compounds against ultrasound waves are the reasons of high extraction yield of solvent method (Tian et al. 2012). Our results indicated under the same conditions the extraction yield of loquat pulp was significantly higher compared to that of skin. Similar results were obtained by Li et al. (2006), which reported the extraction yield in pomegranate skin extract was lower than that of pulp. Moreover, Barros et al. (2011) found the maximum extraction yield in various parts of the Brazilian citrus was in pulp extracts.

**Total tocopherols content**

Tocopherols are a group of antioxidant compounds that prevent oxidation progress by scavenging peroxyl radicals without reacting in further chain-propagating steps (Mussatto et al. 2011). Table 1 shows the total tocopherol compounds found in skin and pulp extracts of loquat fruit. The amount of tocopherols in loquat fruit pulp and skin extracted by different solvents and techniques ranged from 71.15 to 205.42 and 231.23 to 515.68 µg α-tocopherol/g. Choosing the appropriate solvent is one of the most important factors in obtaining extracts with a high content of phytochemical compounds (González-Montelongo et al. 2010). Ethanol, water, and mixtures of these solvents had dissimilar efficiencies in extracting tocopherol compounds from loquat fruit skin and pulp. Water extracted the least amount of tocopherol compounds. The results indicated that the ethanol–water mixture is an effective solvent for extracting tocopherol compounds from various parts of the fruit. This result concurred with previously published results such as the work by González-Montelongo et al. (2010) which found maximum total tocopherols in the methanol–water (1:1) extracts from banana peel, whereas lower values were found for water. In general, the high amount of tocopherol compounds were found in skin extracts compared to pulp extracts under the same extraction conditions. The comparison of the extraction methods showed the solvent extraction was more effective in extraction of tocopherol compounds compared to ultrasound-assisted and supercritical CO2 extraction.

**Figure 1.** Extraction yield (%) of loquat fruit skin and pulp extracts. Note: Solvent extracts of skin by ethanol (SSE), water (SSW), and ethanol–water (SSEW). Solvent extracts of pulp by ethanol (SPE), water (SPW), and ethanol–water (SPEW). Ultrasound-assisted extract of ethanol (ESS), water (WSS) and ethanol–water (EWSS). Ultrasound-assisted extract of ethanol (EPS), water (WPS) and ethanol–water (EWPS). Ethanol extract of skin by supercritical CO2 method (ESC). Ethanol extract of pulp by supercritical CO2 method (EPC).

**Table 1.** Total phenolic and tocopherol compounds of loquat fruit skin and pulp extracts

| Sample    | Total phenol (µg/g) | Total tocopherol (µg/g) |
|-----------|---------------------|-------------------------|
| SSE       | 664.53 ± 2.59c      | 486.53 ± 0.67b          |
| SPE       | 249.96 ± 2.1l       | 162.34 ± 0.78j          |
| SSW       | 272.51 ± 2.19g      | 231.23 ± 1.15f          |
| SPW       | 147.39 ± 0.81m      | 71.15 ± 0.51n           |
| SSEW      | 879.54 ± 3.91a      | 515.68 ± 0.39a          |
| SPEW      | 387.69 ± 2.6f       | 205.42 ± 0.3h           |
| ESS       | 394.67 ± 4.01e      | 388.58 ± 0.25c          |
| EPS       | 209.01 ± 2.42k      | 187.73 ± 0.64i          |
| WSS       | 264.89 ± 1.08h      | 309.86 ± 0.51e          |
| WPS       | 164.96 ± 2.28l      | 107.3 ± 0.51i           |
| EWSS      | 760.71 ± 3.63b      | 353.2 ± 0.5d            |
| EWPS      | 219.40 ± 1.97j      | 98.5 ± 0.53m            |
| ESC       | 425.02 ± 0.00d      | 228.41 ± 0.15g          |
| EPC       | 206.45 ± 0.59k      | 146.36 ± 0.15k          |

Values (Mean ± SD, n = 3) in the same column with different letters are significantly different (P < 0.05).

Note: Solvent extracts of skin by ethanol (SSE), water (SSW), and ethanol–water (SSEW). Solvent extracts of pulp by ethanol (SPE), water (SPW) and ethanol–water (SPEW). Ultrasound-assisted extract of ethanol (ESS), water (WSS) and ethanol–water (EWSS). Ultrasound-assisted extract of ethanol (EPS), water (WPS) and ethanol–water (EWPS). Ethanol extract of skin by the supercritical CO2 method (ESC). Ethanol extract of pulp by the supercritical CO2 method (EPC).
Total phenolic content

Polyphenolic compounds are widely available in plants (Wang et al. 2012) and reports show that there is a positive relation between total phenolic content and antioxidant activity in many plant species (Yasoubi et al. 2010). Phenolic compounds in plants are known as potent in vitro antioxidants due to their ability to donate hydrogen or electrons and to form stable radical intermediates (Li et al. 2006). Table 1 shows the total phenolic content of the various fruit parts, which ranged from 147.39 to 387.69 (μg/g) in pulp and 264.89 to 879.54 (μg/g) in skin extracts. Generally, the total phenolic compound content was higher in ethanol–water extracts compared to water and ethanolic extracts of the same fruit parts. Recently, a wide variety of total phenolic content was reported in loquat fruits cultivars that ranged from 129 to 578 μg GAE per g in Turkey (Polat et al. 2010) and 240 to 572 μg GAE per g in China (Xu and Chen 2011). According to Rop et al. (2011) and Milivojevic et al. (2012), the phenolic content and composition of fruits depend on the genetic and environmental factors as well as postharvest processing conditions. Our results showed the total phenolic in loquat skin was higher than pulp that concurred with the results of Ferreres et al. (2009) which calculated the total phenolic content of different varieties of loquat fruit pulp that was significantly lower compared to that of skin. Similar previously published results such as the work by Luengthanaphol et al. (2004), Goli et al. (2005), and Sánchez-Vioque et al. (2013) it was revealed that the solvent extraction method was more effective to extract phenolic compounds compared to ultrasound-assisted and supercritical CO_2 extraction methods. Based on low levels of extraction yield of phenolic and tocopherol compounds in the supercritical CO_2 extraction method, we realized that these compounds had high polarity since CO_2 has greater tendency to extract nonpolar compounds (Hamburger et al. 2004).

DPPH\(^{\dagger}\) radical scavenging activity

The DPPH\(^{\dagger}\) radical scavenging is widely used as a method to determine antioxidant activity in a relatively short period compared to other methods (Li et al. 2006). Table 2 shows the ability of the loquat fruit skin and pulp extracts to scavenge the DPPH radical as the inhibition percentage at concentrations of 100–1000 ppm. In this assay, scavenge of free radicals increased as concentration of the extracts increased, which is due to the increasing amount of phenolic and tocopherol compounds at higher concentrations of the extracts, similar to the previously published results such as the works of Chaillou and Nazareno (2006) and Zhang et al. (2010). By increasing the concentrations of phenolic compounds, the number of hydroxyl groups available in the reaction medium increased. So, the possibility of hydrogen donation to free radicals will increase (Sánchez-Vioque et al. 2013). The extracts with the highest DPPH radical-scavenging capacity were in this order: SPE at 100 ppm, SPE and ESS at 200 ppm, SPE, SSEW and ESS at 300 ppm (with no significant difference), SSEW at 400 ppm and SSE at 1000 ppm. The inhibition percentage of SSE at 1000 ppm achieved more than 66.7%, which almost matched with TBHQ. Our results clearly showed that skin samples extracted by ethanol–water (1:1) and pulp extracted by ethanol had higher antioxidant activity in different methods. In addition, from these results, we found that antioxidant activity of pulp extracts were less than the skin extracts. This conclusion was confirmed by the results of previously published results such as the works of Koba et al. (2007) and Ferreres et al. (2009), which showed that skin had higher levels of antioxidant compounds compared to the pulp. Also, Silva et al. (2004) and Omena et al. (2012) reported the antioxidant potential of quince and the exotic Brazilian fruit was higher in skin than pulp. Consequently, we found that the samples extracted by the solvent extraction method have better performance against DPPH radical in comparison with ultrasound-assisted and supercritical CO_2 extraction.

\(\beta\)-Carotene bleaching assay

In this assay, \(\beta\)-carotene is oxidized by free radicals derived from the oxidation of linoleic acid, which can better simulate the food system compared to DPPH assay (Razali et al. 2012). The auto-oxidation products of linoleic acid attack the double bonds of \(\beta\)-carotene and in the absence of an antioxidant the \(\beta\)-carotene molecule loses its chromosphere and undergoes rapid discoloration which can be monitored spectrophotometrically (Martinez-Correa et al. 2011). However, in the presence of an antioxidant, \(\beta\)-carotene retains its original yellow color by scavenging the free radicals formed in the system (Razali et al. 2012). Effect of loquat fruit skin and pulp extracts on oxidation of \(\beta\)-carotene/linoleic acid emulsion is shown in Table 3. It was clear that the presence of extracts reduced the oxidation of \(\beta\)-carotene. There were significant differences (\(P < 0.05\)) between the skin and pulp extracts, control (no added antioxidant), and the reference standard (TBHQ). The antioxidant activity of SSEW in levels of 100 and 200 ppm, SSE, SSEW (with no significant difference) in levels 300 and 400 ppm and SSW in level 1000 ppm were higher than other samples. Overall, the antioxidant
Table 2. Comparison of the capacity of loquat fruit skin and pulp extracts to scavenge DPPH radicals.

| Sample | 100 ppm | 200 ppm | 300 ppm | 400 ppm | 1000 ppm |
|--------|---------|---------|---------|---------|----------|
| SSE    | 49.44 ± 0.15de | 49.88 ± 0.1e | 50.96 ± 0.07d | 54.65 ± 0.07b | 66.71 ± 0.04a |
| SPE    | 53.46 ± 0.1b | 53.98 ± 0.13a | 54.36 ± 0.04a | 54.71 ± 0.11b | 56.24 ± 0.11g |
| SSW    | 49.05 ± 0.09f | 49.48 ± 0.11f | 50.1 ± 0.06f | 50.62 ± 0.07g | 56.29 ± 0.09g |
| SPW    | 49.53 ± 0.09d | 50.07 ± 0.04d | 50.59 ± 0.13e | 51.09 ± 0.04f | 58.57 ± 0.03c |
| SSEW   | 49.2 ± 0.05ef | 53.08 ± 0.07b | 54.31 ± 0.14a | 55.08 ± 0.02a | 63.14 ± 0.07b |
| SPEW   | 49.61 ± 0.05d | 49.87 ± 0.1e | 50.2 ± 0.15f | 50.65 ± 0.09g | 57.24 ± 0.07d |
| ESS    | 52.01 ± 0.07c | 53.88 ± 0.07a | 54.3 ± 0.11a | 54.76 ± 0.09ab | 57.28 ± 0.11d |
| EPS    | 49.08 ± 0.17f | 49.53 ± 0.07f | 50.95 ± 0.17d | 53.08 ± 0.11d | 55.8 ± 0.15h |
| WSS    | 49.47 ± 0.15de | 49.91 ± 0.09de | 50.58 ± 0.15e | 51.29 ± 0.16f | 56.47 ± 0.09f |
| WPS    | 49.6 ± 0.55d | 49.87 ± 0.11e | 52.01 ± 0.06c | 52.49 ± 0.1e | 53.11 ± 0.06j |
| EWSS   | 52.02 ± 0.07c | 52.43 ± 0.17c | 52.92 ± 0.05b | 53.96 ± 0.14c | 54.66 ± 0.04i |
| EWPS   | 49.62 ± 0.07d | 49.87 ± 0.11d | 50.27 ± 0.09f | 52.41 ± 0.09e | 56.85 ± 0.05e |
| ESC    | 42.26 ± 0.00g | 43.07 ± 0.03g | 44.46 ± 0.06g | 48.01 ± 0.7h | 49.16 ± 0.00k |
| EPC    | 40.81 ± 0.03h | 41.38 ± 0.02h | 42.56 ± 0.00h | 42.95 ± 0.03i | 45.27 ± 0.04e |
| TBHQ   | 67.63 ± 0.01a |

Values (Mean ± SD, n = 3) in the same column with different letters are significantly different (P < 0.05).

Note: Solvent extracts of skin by ethanol (SSE), water (SSW), and ethanol–water (SSEW). Solvent extracts of pulp by ethanol (SPE), water (SPW) and ethanol–water (SPEW). Ultrasound-assisted extract of ethanol (ESS), water (WSS), and ethanol–water (EWSS). Ultrasound-assisted extract of ethanol (EPS), water (WPS) and ethanol–water (EWPS). Ethanol extract of skin by supercritical CO2 method (ESC). Ethanol extract of pulp by the supercritical CO2 method (EPC).

Table 3. Antioxidant activity of loquat fruit skin and pulp extracts in a β-carotene/linoleate model system.

| Sample | % Inhibition |
|--------|--------------|
| SSE    | 33.33 ± 2.2c | 55.41 ± 5.95b | 78.33 ± 3.33a | 85.58 ± 3.51ab | 64.58 ± 2.08b |
| SPE    | 23.01 ± 1.99ef | 35.83 ± 5.1cd | 45.93 ± 2.08de | 68.75 ± 2.76ef | 54.1 ± 0.06bcd |
| SSW    | 29.08 ± 2.19cd | 32.16 ± 1.43d | 51.69 ± 2.95b | 70.83 ± 3.91e | 68.75 ± 3.61a |
| SPW    | 27.08 ± 3.6de | 33.33 ± 4.17d | 50 ± 4.17cd | 63.33 ± 3.99f | 58 ± 1.66d |
| SSEW   | 46.66 ± 5.86b | 62.91 ± 2.66a | 77.08 ± 4.17a | 83.41 ± 2.63bc | 62.41 ± 0.97bc |
| SPEW   | 28.5 ± 1.64d | 37.66 ± 0.71cd | 54.41 ± 0.19bc | 67.66 ± 4.61ef | 54.16 ± 2.39ef |
| ESS    | 24.16 ± 2.62def | 34.58 ± 1.85d | 39.16 ± 1.64f | 77.08 ± 5.64d | 56.25 ± 2.34d |
| EPS    | 22.91 ± 3.4ef | 35.8 ± 5.95cd | 45.34 ± 2.06de | 55.32 ± 4.56g | 44.16 ± 3.77g |
| WSS    | 22.83 ± 2.11ef | 37.5 ± 2.08cd | 56.66 ± 2.28b | 69.75 ± 1.13ef | 58.75 ± 1.92cd |
| WPS    | 22.58 ± 3.07ef | 34.73 ± 2.58d | 43.72 ± 3.44ef | 52.04 ± 2.82g | 40.27 ± 1.34g |
| EWSS   | 23.08 ± 2.18ef | 36.6 ± 3.25c | 43.93 ± 2.32ef | 79.16 ± 2.08cd | 52.08 ± 2.08f |
| EWPS   | 27.16 ± 3.45de | 36.58 ± 4.14cd | 55.3 ± 3.25b | 65.7 ± 0.77ef | 60.43 ± 5.17bcd |
| ESC    | 21.74 ± 0.01f | 41.66 ± 0.12c | 55.69 ± 0.02b | 64.73 ± 0.02f | 51.24 ± 0.02f |
| EPC    | 20.04 ± 0.03f | 34.34 ± 0.02d | 45.65 ± 0.04de | 50.47 ± 0.04g | 43.47 ± 0.02g |
| TBHQ   | 89.58 ± 0.04a |

Values (Mean ± SD, n = 3) in the same column with different letters are significantly different (P < 0.05).

Note: Solvent extracts of skin by ethanol (SSE), water (SSW), and ethanol–water (SSEW). Solvent extracts of pulp by ethanol (SPE), water (SPW) and ethanol–water (SPEW). Ultrasound-assisted extract of ethanol (ESS), water (WSS), and ethanol–water (EWSS). Ultrasound-assisted extract of ethanol (EPS), water (WPS) and ethanol–water (EWPS). Ethanol extract of skin by supercritical CO2 method (ESC). Ethanol extract of pulp by the supercritical CO2 method (EPC).

activity effect of skin extracts on reducing the oxidation of β-carotene was higher than the control and pulp extracts, but the best antioxidant power was observed in TBHQ. This suggests that the content of phenolic and tocopherol compounds can play a major role in the antioxidant activity of skin extracts compared to pulp extracts. Results also showed that solvent extraction was more effective on antioxidant activity of loquat extracts compared to other methods. These results concurred with Koba et al. (2007) which examined the antioxidant effect of loquat fruit skin and pulp by the β-carotene bleaching assay.
Rancimat analysis

The Rancimat analysis was performed at 120°C and the induction period (h) was evaluated for soybean oils with or without loquat fruit skin and pulp extracts at 400 and 1000 ppm. Our results showed that both skin and pulp extracts had a strong antioxidant activity in soybean oil. As it has been shown in Table 4, the presence of extracts retarded the oxidation of soybean oil, so that the lowest thermal stability in oils treated with extracts was for the EPC sample (3.8 h), which was higher compared to the control oil (3.32 h). On the other hand, the highest thermal stability in oils containing loquat fruit skin and pulp extracts was for SSEW at 400 and 1000 ppm (4.69 and 4.49 h), but the best protection effect was observed in oil containing 100 ppm of TBHQ. Therefore, results also showed that the skin extracts was more effective in prevention of soybean oil oxidation compared to pulp extracts. These results concurred with Sun and Ho (2005) and Aladedunye and Matthäus (2014) that reported the induction period of oils containing buckwheat, rowanberry, and crabapple extracts was higher than control oil, but the most antioxidant effect was in TBHQ. Moreover, the findings of this assay showed that the samples extracted by the solvent extraction method exhibited strong antioxidant activity under the Rancimat test conditions.

Conclusions

Generally, the results of the present study indicated that the solvent extraction was more effective in antioxidant activity of loquat fruit extracts in the DPPH free radical assay, β-carotene bleaching, and the Rancimat methods. So, it is suggested that the best method for the extraction of phenolic and tocopherol compounds from loquat fruit skin and pulp is by the solvent extraction method using the ethanol–water mixture. Furthermore, antioxidant activities of loquat skin extracts were higher than the pulp extracts. The high antioxidant activity of the skin extracts appeared to be attributed to its high tocopherol and phenolics content. Therefore, loquat fruit skin and pulp extracts could be used as natural antioxidants to enhance the antioxidant properties of functional food.

Conflict of Interest

None declared.

References

Aladedunye, F., and B. Matthäus. 2014. Phenolic extracts from *Sorbus aucuparia* (L.) and *Malus baccata* (L.) berries: antioxidant activity and performance in rapeseed oil during frying and storage. Food Chem. 159:273–281.

Albu, S., E. Joyce, L. Paninwyk, J. Lorimer, and T. Mason. 2004. Potential for the use of ultrasound in the extraction of antioxidants from *Rosmarinus officinalis* for the food and pharmaceutical industry. Ultrason. Sonochem. 11:261–265.

Amarowicz, R., I. Estrella, T. Hernández, S. Robredo, A. Troszyńska, A. Kosińska, et al. 2010. Free radical-scavenging capacity, antioxidant activity, and phenolic composition of green lentil (*Lens culinaris*). Food Chem. 121:705–711.

Barros, L., L. Cabrita, M. V. Boas, A. M. Carvalho, and I. C. Ferreira. 2011. Chemical, biochemical and electrochemical assays to evaluate phytochemicals and antioxidant activity of wild plants. Food Chem. 127:1600–1608.

Burits, M., and F. Bucar. 2000. Antioxidant activity of *Nigella sativa* essential oil. Phytother. Res. 14:323–328.

Cao, X., X. Ye, Y. Lu, Y. Yu, and W. Mo. 2009. Ionic liquid-based ultrasonic-assisted extraction of piperine from white pepper. Anal. Chim. Acta 640:47–51.

Cha, D. S., J. S. Eun, and H. Jeon. 2011. Anti-inflammatory and antinoiceptive properties of the leaves of *Eriobotrya japonica*. J. Ethnopharmacol. 134:305–312.

Chaillou, L. L., and M. A. Nazaren. 2006. New method to determine antioxidant activity of polyphenols. J. Agric. Food Chem. 54:8397–8402.
Ercisli, S., S. Gozlekci, M. Sengul, A. Hegedus, and S. Tepe. 2012. Some physicochemical characteristics, bioactive content and antioxidant capacity of loquat (Eriobotrya japonica (Thunb.) Lindl.) fruits from Turkey. Sci. Horticult. 148:185–189.

Farhoosh, R., and M. H. Tavassoli-Kafkani. 2011. Simultaneous monitoring of the conventional qualitative indicators during frying of sunflower oil. Food Chem. 125:209–213.

Ferreres, F., D. Gomes, P. Valentão, R. Gonçalves, R. Pio, E. A. Chagas, et al. 2009. Improved loquat (Eriobotrya japonica Lindl.) cultivars: variation of phenolics and antioxidative potential. Food Chem. 114:1019–1027.

Goli, A. H., M. Barzegar, and M. A. Sahari. 2005. Antioxidant activity and total phenolic compounds of pistachio (Pistacia vera) hull extracts. Food Chem. 92:521–525.

González-Montelongo, R., M. Gloria Lobo, and M. González. 2010. Antioxidant activity in banana peel extracts: testing extraction conditions and related bioactive compounds. Food Chem. 119:1030–1039.

Hamburger, M., D. Baumann, and S. Adler. 2004. Supercritical carbon dioxide extraction of selected medicinal plants—effects of high pressure and added ethanol on yield of extracted substances. Phytochem. Anal. 15:46–54.

Hong, Y., Y. Qiao, S. Lin, Y. Jiang, and F. Chen. 2008. Characterization of antioxidant compounds in Eriobotrya fragrans Champ. leaf. Sci. Horticult. 118:288–292.

Koba, K., A. Matsuoka, K. Osada, and Y. S. Huang. 2007. Effect of loquat (Eriobotrya japonica) extracts on LDL oxidation. Food Chem. 104:308–316.

Lang, Q., and C. M. Wai. 2001. Supercritical fluid extraction in herbal and natural product studies—a practical review. Talanta 53:771–782.

Li, Y., C. Guo, J. Yang, J. Wei, J. Xu, and S. Cheng. 2006. Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract. Food Chem. 96:254–260.

Luengthanaphol, S., D. Mongkholkhajornsilp, S. Douglas, P. L. Douglas, L. I. Pengsopa, and S. Pongamphai. 2004. Extraction of antioxidants from sweet Thai tamarind seed coat—preliminary experiments. J. Food Eng. 63:247–252.

Martínez-Correa, H. A., P. M. Magalhaes, C. L. Queiroga, C. A. Peixoto, A. L. Oliveira, and F. A. Cabral. 2011. Extracts from pitanga (Eugenia uniflora L.) leaves: influence of extraction process on antioxidant properties and yield of phenolic compounds. J. Supercrit. Fluids 55:998–1006.

McDonald, S., P. D. Prenzler, M. Antolovich, and K. Robards. 2001. Phenolic content and antioxidant activity of olive extracts. Food Chem. 73:73–84.

Milivojevic, J., A. Slatnar, M. Mikulic-Petkovsek, F. Stampar, M. Nikolic, and R. Veberic. 2012. The influence of early yield on the accumulation of major taste and health-related compounds in black and red currant cultivars (Ribes spp.). J. Agric. Food Chem. 60:2682–2691.

Mohdaly, A. A. A., M. A. Sarhan, A. Mahmoud, M. F. Ramadan, and I. Smetanska. 2010. Antioxidant efficacy of potato peels and sugar beet pulp extracts in vegetable oils protection. Food Chem. 123:1019–1026.

Musatton, Z. L. F. Ballesteros, S. Martins, and J. A. Teixeira. 2011. Extraction of antioxidant phenolic compounds from spent coffee grounds. Sep. Purif. Technol. 83:173–179.

Omena, C. M. B., I. B. Valentim, G. D. S. Guedes, L. A. Rabelo, C. M. Mano, E. H. Bechara, et al. 2012. Antioxidant, anti-acetylcholinesterase and cytotoxic activities of ethanol extracts of peel, pulp and seeds of exotic Brazilian fruits: antioxidant, anti-acetylcholinesterase and cytotoxic activities in foods. Food Res. Int. 49:334–344.

Pländer, S., L. Gontaru, B. Blazics, K. Veres, Á. Kéry, S. Kareth, et al. 2012. Major antioxidant constituents from Satureja hortensis L. extracts obtained with different solvents. Eur. J. Lipid Sci. Technol. 114:772–779.

Polat, A. A., O. Çalışkan, S. Serçe, O. Saraçoğlu, C. Kaya, and M. Özgen. 2010. Determining total phenolic content and total antioxidant capacity of loquat cultivars grown in Hatay. Pharmacogn. Mag. 6:5–14.

Razali, N., S. Mat-Juniet, A. F. Abdul-Muthalib, S. Subramaniam, and A. Abdul-Aziz. 2012. Effects of various solvents on the extraction of antioxidant phenolics from the leaves, seeds, veins and skins of Tamarindus indica L. Food Chem. 131:441–448.

Rodríguez-Rojo, S., A. Visentin, D. Maestri, and M. Cocero. 2012. Assisted extraction of rosemary antioxidants with green solvents. J. Food Eng. 109:98–103.

Rop, O., T. Juriková, J. Sochor, J. Mléček, and D. Kramarova. 2011. Antioxidant capacity, scavenging radical activity and selected chemical composition of native apple cultivars from Central Europe. J. Food Qual. 34:187–194.

Sánchez-Vioque, R., M. Polisiou, K. Astraka, M. Mozos-Pascual, P. Tarantilos, D. Herrera-Peñalver, et al. 2013. Polyphenol composition and antioxidant and metal chelating activities of the solid residues from the essential oil industry. Ind. Crops Prod. 49:150–159.

Shirsath, S., S. Sonawane, and P. Gogate. 2012. Intensification of extraction of natural products using ultrasonic irradiations—a review of current status. Chem. Eng. Proc. Inten. 53:10–23.

Silva, B. M., P. B. Andreade, P. Valentão, F. Ferreres, R. M. Seabra, and M. A. Ferreira. 2004. Quince (Cydonia oblonga Miller) fruit (pulp, peel, and seed) and jam: antioxidative activity. J. Agric. Food Chem. 52:4705–4712.

Suja, K., J. T. Abraham, S. N. Thamizh, A. Jayalekshmy, and C. Arumugahan. 2004. Antioxidant efficacy of sesame cake extract in vegetable oil protection. Food Chem. 84:393–400.

Sun, T., and C.-T. Ho. 2005. Antioxidant activities of buckwheat extracts. Food Chem. 90:743–749.

Tachakitirungrod, S., S. Okonogi, and S. Chowwanapoonpohn. 2007. Study on antioxidant activity of
certain plants in Thailand: mechanism of antioxidant action of guava leaf extract. Food Chem. 103:381–388.
Tian, Y., H. Zeng, Z. Xu, B. Zheng, Y. Lin, C. Gan, et al. 2012. Ultrasonic-assisted extraction and antioxidant activity of polysaccharides recovered from white button mushroom (Agaricus bisporus). Carbohyd. Polym. 88:522–529.
Tosun, M., S. Ercisli, H. Karlidag, and M. Sengul. 2009. Characterization of red raspberry (Rubus idaeus L.) genotypes for their physicochemical properties. J. Food Sci. 74:C575–C579.
Wang, L., and C. L. Weller. 2006. Recent advances in extraction of nutraceuticals from plants. Trends Food Sci. Technol. 17:300–312.
Wang, Y., X. Chen, Y. Zhang, and X. Chen. 2012. Antioxidant activities and major anthocyanins of myrobalan plum (Prunus cerasifera Ehrh.). J. Food Sci. 77:C388–C393.
Wong, M., R. Timms, and E. Goh. 1988. Colorimetric determination of total tocopherols in palm oil, olein and stearin. J. Am. Oil Chem. Soc. 65:258–261.
Xu, H. X., and J. W. Chen. 2011. Commercial quality, major bioactive compound content and antioxidant capacity of 12 cultivars of loquat (Eriobotrya japonica Lindl.) fruits. J. Sci. Food Agric. 91:1057–1063.
Yasoubi, P., M. Barzegar, M. Sahari, and M. Azizi. 2010. Total phenolic contents and antioxidant activity of pomegranate (Punica granatum L.) peel extracts. J. Agric. Sci. Technol. 9:35–42.
Zhang, Y., L. Yang, Y. Zu, X. Chen, F. Wang, and F. Liu. 2010. Oxidative stability of sunflower oil supplemented with carnosic acid compared with synthetic antioxidants during accelerated storage. Food Chem. 118:656–662.