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Original Article

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Nutrient composition and physicochemical characteristics of Loquat (Eriobotrya japonica) seed oil

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Abstract

Useful medicinal and chemical properties of different parts of Eriobotrya Japonica seeds including flowers, fruits, and leaves have been investigated. In this study, fat content, moisture, unsaponifiable matter, saponification and iodine values, free fatty acid compound, individual sterols, antioxidant activity, total phenol, tannin, and flavonoids content, and detection and quantification of polyphenol compounds of the extracted oil of Loquat seeds were determined. Extracted oil percentage of the seeds was obtained 0.79-1.51 (w/w%) using soxhlet extractor. Moisture of extracting oil was obtained (0.03±0.01) (w/w%). Iodine and saponification values of Loquat seed oil were obtained 92.64±0.18 (mg KOH/g fat) and 196.3±0.2 (mg I2/g fat), respectively. Unsaponifiable matter was too high about 21.44±0.02%. The amount of unsaponifiable matter shows that this oil is rich in tocopherols, sterols and squalene. Predominant fatty acids in Loquat seed oil were oleic acid (C18:1cis) (33.30±0.03%) followed by linoleic acid (C18:2cis) (31.00±0.20%) then palmitic acid (C16:0) (17.50±0.05%). The most abundant sterol of Loquat seed oil was β-Sitosterol (88.61±0.50%). The most content of triglycerides related to ECN48 (71.4±0.40%). In addition, results showed that unsaturated fatty acid content (FAC) in sn-2-position was higher than that of sn-1,3-positions. The antioxidant activity percentage of Loquat seed oil was 94.33±0.05%. Total phenol, tannin, and flavonoid content of Loquat seed oil extract were obtained 30.38±0.60 (mg GAE/g dry matter), 0.493±0.01 (mg GAE/g dry matter) and 6.97±0.10 (mg QE/g dry matter), respectively. In addition, ferulic acid was known the main polyphenol compound in Loquat seed oil extract. Loquat seed oil can be used in the food industry, cosmetics, paint, and soaps.

Keywords: Eriobotrya Japonica, Loquat, Scavenging Activity, Seed Oil

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Introduction

Loquat (Eriobotrya Japonica) is an evergreen fruit tree with edible fruit in the Rosaceae subfamily. It probably has been cultivated in China and Japan since ancient times, but it is also cultivated in different countries, including Cyprus, Egypt, Greece, Israel, Italy, Spain, Tunisia, Iran and Turkey (Taskin, Erdal, 2011). It was reported that about 97% of Loquat fruit production has been supplied from the Mediterranean region, including Australia, South America, California, South Africa, India. This plant is a small tree which has white flowers giving rise to pale-yellow and narrow green leaves (Taskin, Erdal, 2011). The fruits of Eriobotrya Japonica tree can be sold at higher price in the spring, due to there are few competitive fruits on the market in the world. Loquat fruit is usually eaten fresh but may be stewed, syrup, served as a sauce, or made into an excellent jelly. The Loquat fruit has vitamins A, B and C, high amount of carotene, mineral substances, salt, sugar and is a rich source of acid and pectin (Durgac et al., 2006). It was reported that Loquat fruit is composed of, 0.30%-0.37% cellulose, 0.026%-0.029% ash, 0.32%-0.35% protein, 84%-89% water, 0.30%-0.60% lipids, and 9.89% -12.79% sugar and starch. In addition, the leaves of Eriobotrya Japonica tree have been consumed for treatment of coughs, skin diseases and diabetes, cancers, phlegm, chronic bronchitis, and ulcers (Liu et al., 2016). Both Loquat fruits and leaves are well known and often applied in herbal traditional Chinese medicine (Wu et al., 2013). In addition, Loquat flowers have been widely applied for treatment of cough, colds, and sputum in China (Zhou et al., 2011). Each Loquat fruit usually has 4-7 large seeds (Kikuchi et al., 2014). These seeds have useful medicinal properties, therefore, different extracts of these seeds were orally administered to rats and the development of liver fibrosis was inhibited (Nishioka et al., 2002). In addition, Hamada et. al., (2004) have reported that seeds extract of Eriobotrya Japonica had antioxidant activity and effectively reduced oxidative stress of the adriamycin-induced renal disorder in rats. In addition, Eriobotrya Japonica seeds had a hypoglycemic property (Tanaka et al., 2008). In this study, Loquat seed oil was extracted with two different methods, including cold solvent and soxhlet extractor which are cold and hot methods for extraction of fat, respectively. Then, types and quantities of polyphenol compounds, antioxidant activity percentage, total phenol content (TPC), total flavonoid content (TFC), and total tannin content (TTC) of Loquat seed oil were determined. In addition, physico-chemical properties of extracted Loquat seed oil, including fatty acid composition, moisture, individual sterols, triglycerides, iodine value, unsaponifiable matter, and saponification values were analyzed.

Material and methods

Materials

Acetonitrile, 99.9%, orthophosphoric acid, 85%, and methanol, 99.99%, with the HPLC grade were purchased from Fisher Scientific (Lisbon, Portugal). 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma-Aldrich (America). The reference standard of FAME mixture (C4-C24), 5-α-Cholestane, mono-, di-, triglycerides, Cholesterol, β-Sitosterol, Stigmasterol, vanillic acid, vanillin, caffeic acid, quercetin, oleuropein, tyrosol, cinnamic acid, luteolin, catechin, gallic acid, apigenin, and ferulic acid were purchased from Sigma–Aldrich Company. Other materials, including, hexane, n-propanol, glacial acetic acid, chloroform, potassium iodide (KI), Folin–Ciocalteu reagent, and Hanus solution were also purchased from the Merck Company (Germany).

Sampling and preparation of test sample

Samples of Loquat fresh fruits were obtained from Eriobotrya Japonica trees cultivated in Gilan province located in the north of Iran (at the end of spring). The seeds were separated from their fruit pulp and washed with water three times. Then, the seeds were dried at 30ºC under vacuum oven (Memmert VO400 Vacuum Oven, Germany). Dried seeds were crushed in a blender to obtain homogenized powders (Blender, Sanyo, Japan). For obtaining same particle size, powders were passed through the micromesh sieves. Powders were kept in a dark glass at 4-8 ºC until further analysis.

Fat content of Loquat seed

For extraction of oil content of Loquat seeds using the cold solvent method, a flask was put in the oven at 103ºC for 1h and cooled in a desiccator then accurately weighed. The weight of this flask was recorded. 150 g of dried powders of Loquat seeds was weighed in a flask (500 ml) and 350 ml of n-hexane was added to it, then the

flask was put on a shaker (WiseCube, Germany) at 150 rpm in darkness and room temperature for 30 h. Then, the content of the flask was filtered by 0.45μm propylene filter. 100 ml of hexane was used for washing off residue on the filter. Then, the filtrate was collected in the flask which was weighed before. The solvent was evaporated using a rotary evaporator (Heidolph, type: Heizbad WB, Germany) at 35°C for 45-60 minutes under vacuum. Then, the flask was put in the desiccator for 1 hour and weighed again to measure the weight of the extracted oil (ISO 659: 2009). In addition, by another method, the oil content of seeds was determined using a Soxhlet extractor unit.

Moisture content

The moisture content of seeds was determined according to the described method by ISO 665:2000. In addition, determination of water content of seed oil was performed by Karl Fischer method (pyridine free) (ISO 8534: 2017).

Saponification value

The saponification value of Loquat seed oil was determined using the ISO 3657: 2013 method for measuring off the free and esterified acids present in fats and fatty acids.

Unsaponifiable matter

ISO 18609:2000 method was used for determination of the unsaponifiable matter in Loquat seed oil.

Iodine value

The iodine value of Loquat seed oil was determined according to the ISO 3961:2009 method.

Fatty acids profile

Preparation of fatty acid methyl esters was made according to the described method of ISO 5509:2000. Then, fatty acid methyl esters were injected to the gas chromatography (GC) equipped with the flame ionization detector (FID) and peaks were identified according to the described method in ISO 5508:1990. The analyses were performed using a Young Lin 6100 (Korea) gas chromatography with the analytical column Cp-Sil88 (100 m × 0.25 mm × 0.25 μm). The isothermal program was used at 175ºC and the detector and injector temperatures were adjusted at 320 and 280 °C, respectively.

Determination of sterols

The individual sterols content of Loquat seed oil was determined according to the described method in the ISO12228:2014.

Triacylglycerol analysis

Determination of triacylglycerols (TAGs) with equivalent carbon number 42 (ECN42) was obtained by HPLC according to the described method in the COI/T.20/DOC. 20 – 2010.

Extraction of phenolic compounds

Polyphenol compounds from Loquat seed oil were extracted according to the described method in the COI/T.20/Doc No 29 – 2009 using a methanol: water (80: 20) solution for extraction.

Chromatographic analysis and identification of phenolic compounds

Polyphenol compounds of the Loquat seed oil extract were determined using HPLC system according to the COI/T.20/Doc No 29 method. An HPLC system (model Young Lin- 9100, Korea) equipped with UV detector was used for determination and detection of polyphenol compounds at 280 nm wavelength. A solvent was water and 0.2% H3PO4, and B solvent was Acetonitrile and methanol (50: 50). The column was Athena C18-WP (Column: 4.6 mm × 250 mm, 100 A°, 5 μm). The temperature of the column was kept constant at 25 °C. A gradient elution program was applied as follows:

Time (min)	A [%]	B [%]	Flow (ml/min)
Initial	96.0	4.0	1.000
40.0	50.0	50.0	1.000
45.0	40.0	60.0	1.000
60.0	0.0	100.0	1.000
70.0	0.0	100.0	1.000
72.0	96.0	4.0	1.000
82.0.	96.0	4.0	1.000

DPPH assay

The antioxidant activity of the Loquat seed oil extract was determined according to the method developed by Arabshahi-Delouee (2007). For this purpose, 1 ml of DPPH methanolic solution (0.1 mM) was mixed with 3 ml of Loquat seed oil extract. Then, the mixture was then vigorously vortexed and kept for 30 minutes at room temperature in the dark place. The absorbance of the sample was measured at 517 nm by UV/VIS spectrometer (Lambda 25–Perkin Elmer, USA) and the percentage of DPPH scavenging relative to control can be calculated using the

following equation:

DPPH scavenging activity (%) = (Absorbance of Control-Absorbance of the sample)/ (Absorbance of control) × 100

All determinations were made in triplicates.

Determination of Total Phenolic Content (TPC)

TPC of Loquat seed oil extract was determined by colorimetric assay. Briley, 200 μL of an aliquot seed oil extract was mixed with 800 μL deionized water as well as 100 μL of Folin-Ciocalteu reagent, respectively. The mixed solution was then incubated for three minutes at the room temperature. Then, 300 μL of sodium carbonate (Na2CO3) (20% (w/v)) was added to it and incubated for two hours in a dark place at room temperature. The absorbance of the solution was determined at 765 nm with UV/Vis spectrometer. The blank was prepared with distilled water. The calibration curve of gallic acid (GA) standard was obtained in the range 0 - 200 mg/L and calibration curve equation was Y= 0.0154X, R2 = 0.9936. The total phenolic content was expressed in mg GA equivalent to per g dry matter (Mohammed and Manan, 2015). All determinations were made in triplicates.

Determination of Total Tannins Content (TTC)

The TTC was determined using Folin-Ciocalteu reagent. At first, 100 μL of the aliquot extract was added to 750 μL distilled water and then 500 μL Folin-Ciocalteu reagent, as well as 1000 μL of sodium carbonate (Na2CO3) (35% (w/v)), added afterward. Then, the mixture was vigorously shacked and diluted to10 mL with distilled water and then incubated for 30 minutes at the room temperature. The absorbance was determined at 725 nm by UV/ Vis. For this experiment distilled water was selected as the blank. As already mentioned above, a calibration curve of the GA standard solution was prepared in the range 0 - 100 mg/ GA. Finally, TTC was expressed as GAE/g dry matter (Mohammad and Manan, 2015). All determinations were made in triplicates.

Determination of Total Flavonoids Content (TFC)

The colorimetric method was applied for the determination of the total flavonoid content (TFC). 150 μL of sodium nitrite (NaNO2) (5% (w/v)) was added to 200 μL seed extract and mixed, then incubated for 6 min at room temperature. 150 μL AlCl3.6H2O (10% w/v) was added to it and again incubated for another 6 min at room temperature. Then, 800 μL NaOH solution (10% (w/v)) was added to it and incubated again at room temperature for 15 minutes. A blank was distilled water instead of extract. Absorbance was read at 510 nm by using UV/vis. All determinations were made in triplicates. The calibration curve of quercetin dissolved (QE) standard was obtained in the range 0 - 500 μg/mL in 80% ethanol and total flavonoid content was expressed mg Quercetin equivalent (QE)/g dry matter (Mohammad and Manan, 2015).

Statistical analysis

All data were reported as means of triplicate measurements with standard deviation (SD). A one-way analysis of variance (SPSS 11) and Duncan test were applied for the statistical analysis of the results. Statistical significance was declared at $p<0.05$. In addition, all tests were performed in Microsoft Excel.

Result & Discussion

Physicochemical properties of Loquat seed oil

The moisture content of Loquat fresh seeds was obtained 13.51±0.2 %, but after drying it under vacuum at 30ºC reached to 3.5±0.5%. The moisture content of extracted seed oil was 0.03±0.01% (w/w) using soxhlet extractor. Results showed that the fat content of Eriobotrya Japonica seeds was obtained 0.5-0.75 (g/100g) for extraction of seed oil with hexane at room temperature and 0.79-1.51 (g/100g) for extraction of oil by soxhlet extractor. In addition, the oil yield of watermelon seeds was reported 50±0.2g/100g dry matter (Moaddab doost Baboli and Safe Kordi, 2007). The extracted seed oil was observed solid and its color was pale white at room temperature. The melting point of oil was obtained 78±0.04 °C. A study was reported that Loquat seeds from the local Turkey market had the high amount of protein and total carbohydrate content but low-fat content and ash (Taskin, and Erdal, 2011). It was reported that the oil content of these seeds was so lower than those of in common oilseeds, including rapeseed (40-48%), cottonseed (22-24%), soybean (18-22%), safflower (30-35%), and olive (12-50%) (Adinew, 2014). Saponification and iodine values of extracted watermelon seed oil were respectively reported 200±0.1 and 156±0.2, while it was obtained from Loquat seed oil 196.30±0.2 and 92.64±0.18,

respectively. The iodine value of seed oil shows resistance to oxidation, the degree of unsaturation, and shelf life of oil in industrial applications (Kittiphoom, 2013). In addition, saponification value indicates the evaluation of the molecular weight of fatty acids or the chain length occurring in the triacylglycerols in fats and oils, which the lower saponification value shows a higher amount of low molecular weight triacylglycerols content (Kittiphoom, 2013). The unsaponifiable matter was obtained 21.44±0.02 (%g/g). It was found that higher amounts of unsaponifiable matter indicate that squalene, tocopherols, and sterols content of Loquat seed oil are very higher than other common oilseeds. In addition, higher amounts of unsaponifiable matter are very important on properties and stability of fats and oils (Sims, Fioriti, and Kanuk, 1972).

The fatty acid composition of Loquat seed oil

Results of fatty acid composition determination of Loquat seed oil are shown in Table 1. Based on the results, oleic acid and linoleic acid are

Table 1: Fatty acid composition of Loquat seed oil

Fatty acid	(%)	Fatty acid	(%)
C12:0	4.20±0.05	C18:2c	31.00±0.20
C12:1c	0.20±0.01	C18:3c	3.50±0.06
C14:0	0.70±0.01	C20:0	0.50±0.100
C14:1c	0.20±0.01	C20:1c	0.20±0.05
C16:0	17.50±0.05	C22:0	1.10±0.04
C16:1c	0.90±0.05	C22:1c	0.20±0.10
C17:0	0.20±0.01	C24:0	1.60±0.02
C17:1c	0.30±0.01	C24:1c	0.10±0.15
C18:0	4.06±0.03	Other	0.30±0.01
C18:1c	33.30±0.03	SFA	29.86±0.10
		MUFA	35.40±0.12
		PUFA	34.50±0.08

the most abundant fatty acids found in the seed oil of Loquat. The saturated fatty acids (SFA) and unsaturated fatty acids (MUFA) were separately 29.86%and 35.40%, respectively. In addition, palmitic acid was the major SFA (17.50%) in the Loquot seed oil. The quantities of oleic and linoleic acids were respectively obtained 33.30% and 31.00%, while the amount of them in the seed oil of orange, pumpkin, watermelon, mango were reported 24.8% - 29.3%1 and 21.32%- 31.86% (Moura aRaNHa, JoRGe, 2013), 38.42% and 30.84% (Gohari Ardabili, Farhoosh, and Haddad Khodaparast, 2011), 13.3% and 68.3% (Mohammed, and Manan, 2015), 42.60%- 45.76% and 5.70% - 6.78% (Kittiphoom, 2015), respectively. In addition, palmitic, stearic, oleic and linoleic acids content in Loquat seed oil were close to other commercial oils, including corn and peanut oil.

Sterols determination

Results of individual sterols determination of Loquat seed oil were presented in Table 2. Based on the results, β-sitosterol was the highest value among other phytosterols component exists in the Loquat seed oil. The amounts of campesterol, and brassicasterol were in the range of olive oil. In addition, β-sitosterol was the predominant sterol in this oil like as all other vegetable oils, including olive oil. Kawahito et. al. (2008) reported that the amounts of amygdalin

Table 2: Desmethyl sterol composition of Loquat seed oil

Sterols	(%)
Cholesterol	0.14±0.10
Campesterol	4.74±0.30
Brassicasterol	<0.10
Stigmasterol	1.11±0.40
Beta-Sitosterol	88.61±0.50
Delta5,24-Stigmastadienol	0.42±0.05
Delta(7)-Stigmastenol	1.40±0.15
Delta-7-Avenasterol	3.57±0.50

and β-sitosterol using soxhlet extractor were obtained 16.38 and 0.59 mg/g for unroasted ground Loquat seed, and were obtained 11.27 and 0.45 mg/g for the roasted powder seed, respectively. There was not reported data about sterol composition of loquat seed oil in the previous studies by researchers.

Triacylglycerols (TAGs) determination

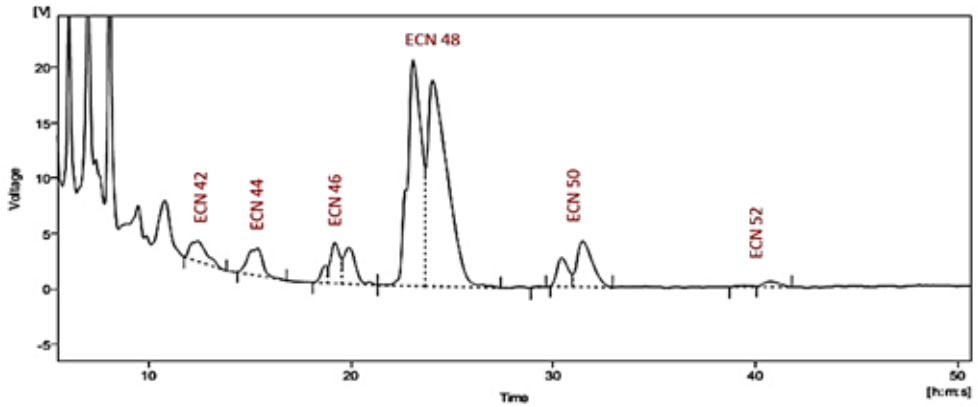
It was well known that the separation of the triglycerides is according to their retention time depending on the carbon number and degree of unsaturation of the fatty acids in the triglycerides. Triacylglycerols (TAGs) with equivalent carbon number 42 (ECN42) were obtained in the Loquat seed oil by HPLC according to the described method in COI/T.20/Doc. No. 20 /Rev. 3. 2010. Chromatograph of TAGs in Loquat seed oil is presented in Fig. 1. There was no data about triacylglycerol composition of Loquat seed oil until now.

The relative percentage of each triglyceride corresponding to TAGs from ECN42 up to ECN54 was obtained by HPLC (Table 3). According to the results, triacylglycerols with ECN48 were dominant (71.4±0.40%).

Table. 4 shows the percentage of fatty acid content (FAC) in the sn- 2- and 1,3-positions of the TAGs in Loquat seed oil. In addition, results showed that the unsaturated fatty acid content

Table 3: The amounts corresponding to TAGs from ECN 42 up to ECN 52ECNs detected in Loquat seed oil by HPLC

ECN	(%)
42	4.20±0.20
44	3.93±0.30
46	8.96±0.30
48	71.4±0.40
50	9.96±0.50
52	1.23±0.30
54	0.00±0.00



Main components of chromatographic peaks include: ECN42: LLL+PoLL, OLLn+PoOLn, PLLn; ECN44: OLL+PoOL, OOLn+PLL, POLn+PPoPo, OOL+PoOO; ECN46: OOL+LnPP, PoOO, SLL+PLO, PoOP+SPoL+SOLn+SPoPo, PLP; ECN48: OOO+PoPP, SOL+POO, POP, ECN50: SOO, POS+SLS. P: Palmitic acid (ECN 16); Po: Palmitoleic acid (ECN 14); S: Stearic acid (ECN 18); O: Oleic acid (ECN 16); L: Linoleic acid (ECN 14), Ln: Linolenic acid (ECN 12).

Figure 1: Peaks corresponding to TAGs from ECN 42 up to ECN 52 of Loquat seed oil.

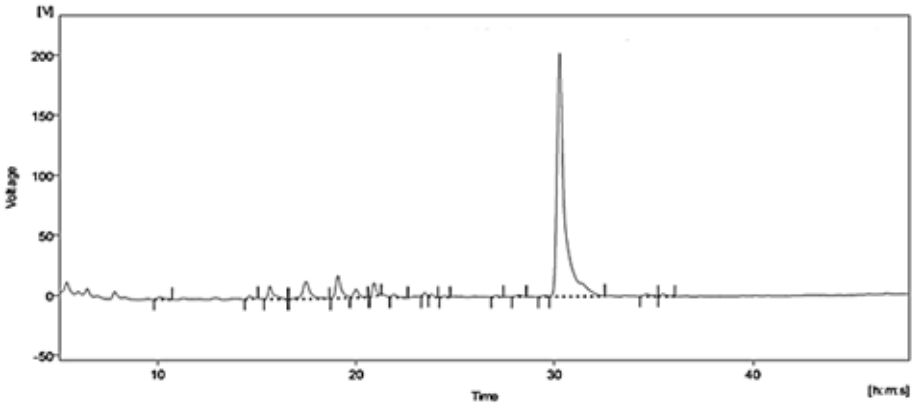


Figure 2: Chromatogram of the detected polyphenol compounds of Loquat seed oil by HPLC

Table 4: Fatty acids composition in sn-2- and sn-1, 3-positions of the TAGs in Loquat seed oil

FA	1,3- Position	2-Position
P	30.70	1.25
S	6.32	0.26
Po	0.91	1.42
O	30.36	47.47
L	28.47	44.53
Ln	3.24	5.06
Sum	100%	100%

(FAC) in sn-2-position (of oleic, linoleic and linolenic acids) was higher than that of sn-1,3-positions. Also, the quantities of TAGs in sn-1,3- positions of palmitic and stearic acids were higher than that those of sn- 2- position. In addition, there are no data on the triacylglycerol composition of Loquat seed oil in the literature for comparative purposes.

Total Phenol, Tannin, and Flavonoid content

TPC of the Loquat seed oil extract was obtained 30.38±0.60 mgGAE/g dry matter and total tannin content (TTC) of Loquat seed oil was estimated 0.493±0.01mg GAE/g dry matter. The quantity of TPC is important in justifying the antioxidative properties of the seed oil. In addition, the total flavonoid content (TFC) was obtained 6.97±0.10 mg QE/g dry matter. The antioxidant activity of the Loquat seed oil extract was obtained 94.33±0.05%. Zhou et. al. (2011) reported that the TPC and TFC of ethanolic extract of Loquat flower were obtained 10.59 and 102.02 mg GA/g dry weight, respectively. Polyphenol compounds can influence the color and sensory characteristics of fruits and vegetables, which have various therapeutic and protective effects. Plant seeds are rich in phenolics, flavonoids, and tannins (Zhou et. al., 2011). Chromatogram of the detected polyphenol compounds of seed oil extract is presented in Fig. 2. The results of the amount of the detected polyphenol compounds are shown in Table 5. Results showed that the dominant polyphenol of seed oil extract was ferulic acid.

The results of the detected polyphenol compounds demonstrated that seed oil extract of Loquat is containing a potential source of bioactive compounds. Ferulic acid is a natural phenolic acid with low toxicity and has many physiological properties including, anti-inflammatory, antioxidant, anti-cancer, antimicrobial, anti-thrombosis activities (Ou, and Kwok, 2004). There is no report about detection of polyphenol compounds in Loquat seed oil in the literature for comparison.

Conclusion

The results showed that Loquat seed oil is a noticeable source of essential fatty acids and has high concentrations of phytosterols, particularly β-Sitosterol and campesterol like olive oil. Loquat seed oil has a large amount of ferulic acid, which is a significant phenolic acid with a considerable treatment and biological properties. Finally, Loquat seed oil is a good source of linoleic and oleic acids, ferulic acid, and phytosterols. Therefore, Loquat seed oil can be applied in the food industry or pharmacological treatment because of containing a very large amount of unsaponifiable matter which demonstrated that the numbers of tocopherols, sterols and squalene content of this oil are too high.

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Reference

Adinew B. (2014). Physico-chemical properties of Trichiliaemetica seed oil and its comparison with some selected oilseed oils. Bulgar Chem Commun, 46(2), 330 – 3.

Arabshahi-Delouee S, Devi DV, Urooj A. (2007). Evaluation of antioxidant activity of some plant extracts and t heir heat, pH and storage stability. Food Chem. 100, 1100–105.

COI/T.20/Doc No 29. (2009). Determination of biophenols in olive oils by HPLC. International Olive Council.

COI/T.20/Doc.No.20/Rev.3. (2010). Determination of the difference between actual and theoretical content of triacylglycerols with ECN42.International Olive Council.

Durgac C, Polat A, Kamilogl O. (2006). Determining performances of some loquat (Eriobotrya japonica) cultivars under Mediterranean coastal conditions in Hatay, Turkey. New Zealand J. Crop and Hort. Sci. 34, 225-30.

Gohari Ardabili A, Farhoosh R, Haddad Khodaparast MH. (2011). Chemical Composition and Physicochemical Properties of Pumpkin Seeds (Cucurbita pepoSubsp. pepo Var. Styriaka) Grown in Iran.J. Agr. Sci. Tech, 13, 1053-63.

Hamada A, Yoshioka S, Takuma D, Yokota J, Cui T, Kusunose M, Miyamura M, Kyotani S, Nishioka Y. (2004). The effect of Eriobotrya japonica seed extract on oxidative stress in adriamycin-induced nephropathy in

rats.Biol. Pharm. Bull, 27, 1961–1964.

ISO 12228. (2014). Determination of individual and total sterols contents -- Gas chromatographic method -- Part 2: Olive oils and olive pomace oils.

ISO 18609. (2000). Animal and vegetable fats and oils-Determination of unsaponifiable matter-Method using hexane extraction.

ISO 3657. (2013(E)). Animal and vegetable fats and oils-Determination of saponification value.

ISO 3961. (2009). Animal and vegetable fats and oils – Determination of iodine value-test method.

ISO 5508. (1990). Animal and vegetable fats and oils -- Analysis by gas chromatography of methyl esters of fatty acids.

ISO 5509. (2000). Animal and vegetable fats and oils -- Preparation of methyl esters of fatty acids.

ISO 659. (2009). Oilseeds -- Determination of oil content (Reference method).

ISO 665. (2000). Oilseeds -- Determination of moisture and volatile matter content.

ISO 8534. (2017). Animal and vegetable fats and oils -- Determination of water content--Karl Fischer method (pyridine free)

Kawahito Y, Kondo M, Machmudah S, Sibano K, Sasaki M, Goto M. (2008). Supercritical CO2 extraction of biological active compounds from loquat seed. Sep Purific Technol. 61(2), 130–5.

Kikuchi S, Iwasuna M, Kobori A, Tsutaki Y, Yoshida A, Murota Y, Nishino E, Sassa H, Koba T. (2014). Seed formation in triploid loquat (Eriobotrya japonica) through cross-hybridization with pollen of diploid cultivars. Breeding Sci, 64, 176–182.

Kittiphoom S. (2013). Sutasinee S. Mango seed kernel oil and its physicochemical properties. Int Food Res J, 20(3), 1145-9.

Liu Y, Zhang W, Xu Ch, Li X. (2016). Biological Activities of Extracts from Loquat (Eriobotrya japonica Lindl.): A Review. Int J Mol Sci, Dec. 17(12), 1-15.

Moaddab doost Baboli Z, Safe Kordi AA. (2010). Characteristics and Composition of Watermelon Seed Oil and Solvent Extraction Parameters Effects. J Am Oil Chem Soc, 87, 667-71.

Mohammed S, Manan FA. (2015). Analysis of total phenolics, tannins and flavonoids fromMoringaoleifera seed extract. J Chem Pharm Res, 7(1), 132-5.

Moura aRaNHa CP, JoRGe N. (2013). Physico-chemical Characterization of Seed Oils Extracted from Oranges (Citrus sinensis).Food Sci. Technol. Res.,19(3), 409 – 415.

Nishioka Y, Yoshioka S, Kusunose M, Cui T, Hamada A, Ono M, Miyamura M, Kyotani S. (2002). Effects of extract derived from Eriobotrya japonica on liver function improvement in rats .Biol. Pharm. Bull, 25(8), 1053-1057.

Ou SH, Kwok K-C. (2004). Ferulic acid: pharmaceutical functions, preparation and applications in foods. J Sci Food Agric, 84(11), 1261–9.

Sims RJ, Fioriti JA, Kanuk MJ. (1972). Sterol additives as polymerisation inhibitors for frying oils. J. Amer. Oil Chem. Soc, 49, 298-301.

Tanaka k, Nishizono S, Makino N, Tamaru S, Terai O, Ikeda I. (2008). Hypoglycemic Activity of Eriobotrya japonica Seeds in Type 2 Diabetic Rats and Mice. Biosci. Biotechnol. Biochem, 72(3), 686–693.

Taskin M, Erdal S. (2011). Utilization of waste loquat (Eriobotryajaponica Lindl.) kernel extract for a new cheap substrate for fungal fermentations. Romanian Biotech Let, 16(1), 5872-80.

Wu L, Jiang X, Huang L, Chen Sh. (2013). Processing Technology Investigation of Loquat (Eriobotrya japonica) Leaf by Ultra-Performance Liquid Chromatography-Quadrupole Time-of-Flight Mass Spectrometry Combined with Chemometrics. PLOS ONE. 8(5), e64178: 1-9.

Zhou CH, Sun C, Chen K, Li X. (2011). Flavonoids, Phenolics, and Antioxidant Capacity in the Flower of Eriobotrya japonica Lindl. Int J Mol Sci, 12(5), 2935–2945.