Journal of Medicinal Plants Research Vol. 5(9), pp. 1735-1740, 4 May, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals

Full Length Research Paper

Oleanolic and ursolic acid in the fruit of *Eriobotrya japonica* Lindl.

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Accepted 2 February, 2011

High performance liquid chromatography (HPLC) method was applied to determine the content of oleanolic acid (OA) and ursolic acid (UA) in different tissues of 'Ruantiaobaisha' and 'Dayeyangdun' loquat (*Eriobotrya japonica* Lindl.) fruits in this research. The results demonstrated that peel contained higher OA and UA contents, while flesh and kernel contained very lower amounts of OA and UA. On the basis of above results, the OA and UA contents in the peel of different developmental stages and different cultivars of loquat fruits were analyzed and compared. It is found that OA and UA contents in the peel varied with different developmental stages and cultivars. The OA and UA content in the peel of ripe fruit of different cultivars were in the range of 0.59-1.68 and 2.82-8.20 mg/g DW, respectively. These results can provide a theoretical basis for the comprehensive utilization of loquat fruit in the future.

Key words: *Eriobotrya japonica*, fruit, oleanolic acid, ursolic acid.

INTRODUCTION

Oleanolic acid (OA) and ursolic acid (UA) are isomeric pentacyclic triterpenoids and widespread in plant kingdom (Figure 1). Based on pharmacological studies, both OA and UA are proved to have bioactivities such as antioxidative (Tsai and Yin, 2008; Yin and Chan, 2007), antibacterial (Fontanay et al., 2008; Kurek et al., 2010), anti-inflammatory (Tsai and Yin, 2008; Vasconcelos et al., 2006), anti-glycative (Wang et al., 2010), anti-tumor (Young et al., 1994), anti-HIV (Kashiwada et al., 1998, 2000), cholesterol-lowering (Lin et al., 2008), diuretic (Shibata, 2001) and hepatoprotective activities (Ma et al., 1982; Saraswat et al., 2000). Both components have been widely used in cosmetics and health foods industry because of their relatively non-toxicity. Loquat (*Eriobotrya japonica* Lindl.) is a Rosaceae plant originated in southeastern China and has been commercially cultivated world-widely in countries such as China, Japan, India, Spain, Brazil, USA, Australia, and South Africa (Lin et al., 1999). Loquat fruits are known to be rich in carbohydrate (Serrano et al. , 2003), organic acid (Chen et al., 2009), phenolics (Ding et al., 2001), carotenoids (Zhou et al., 2007a), and can be consumed fresh or processed into jam, juice, wine, syrup, or candied fruits.

At present, studies of extraction, identification, and pharmacological research of triterpene acids such as OA and UA in loquat (*E. japonica* Lindl.) are mainly focused on leaf (Banno et al., 2005; Komiya et al., 1998; Li et al., 2009; Liang et al., 1990; Taniguchi et al., 2002) and flower (Cheng et al., 2001; Zhou et al., 2007b), with few concerning the fruit. The objective of this work is to analyze OA and UA content in loquat fruit, for a better health evaluation of loquat fruit and comprehensive utilization of loquat fruit processing waste in the future.

MATERIALS AND METHODS

Chemicals

OA and UA standards were purchased from Sigma Chemical Co.

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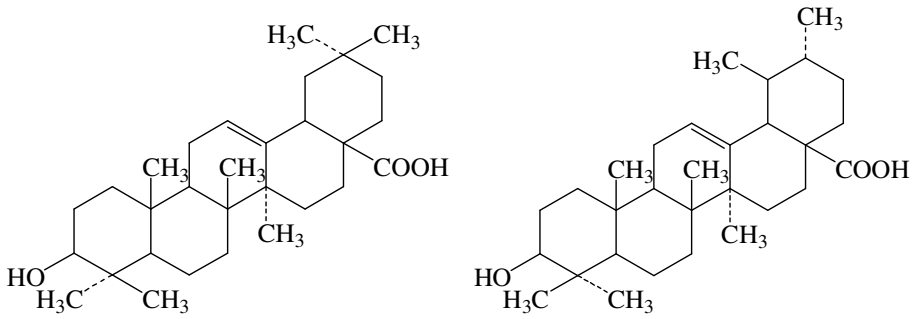

Figure 1. The chemical structure of oleanolic and ursolic acid.

Figure 2. The photos of ripe fruit of loquat ('Ruantiaobaisha' and 'Dayeyangdun').

(St. Louis, MO, USA). Methanol (HPLC grade) was obtained from Caledon Laboratories Co. (Georgetown Ont., Canada). All the other reagents used in the present study were of analytical grade.

Preparation of standard solution

A stock solution of 1 mg/ml was prepared in methanol for OA and UA standards, respectively. A serial dilution was made on each stock solution with methanol to prepare standard solutions at concentrations of 50, 100, 200, 300 and 400 µg/ml, from each of which 20 µl was used for plotting the standard curves for OA and UA, respectively.

Chromatographic apparatus and conditions

Quantifications of OA and UA were performed on a HPLC system (Beckman, USA) equipped with 125 pump, 166 UV detector and an ODS C_{18} column (250 x 4.6 mm, 5 µm). Both compounds were detected at 210 nm at room temperature with an eluent flow rate of 1.0 ml/min. The mobile phase consisted of methanol (A) and 0.03 mol/l phosphate buffer (pH 2.8) (B) with a ratio of 88:12 (A:B, v/v) for simultaneous detection of OA and UA.

Collection of fruit materials

The 'Ruantiaobaisha' (White-flesh type) and 'Dayeyangdun' (Red-fleshed type) ripe fruit were used for OA and UA contents comparison between different parts of fruit, such as peel, flesh and kernel (Figure 2). These two cultivars were also used for OA and UA contents comparison between different developmental stages. The fruits of six developmental stages, including ripe stage, were collected from the orchard of Yuhang Loquat Science Institute (Hangzhou, China), and the number of fruits picked depends on fruit size. The quality index of loquat fruit during development was shown in Table 1. Twenty-four samples were used for OA and UA contents comparison between cultivars, of which 11 cultivars belongs to White-fleshed type ('Baisha'), 13 cultivars belongs to Red-fleshed type ('Hongsha'), and the ripe fruits were harvest in the orchard of Yuhang Loquat Science Institute (Hangzhou, China) and Taihu Technological Popularization Center of Evergreen Fruit (Suzhou, China). Ten fruits of every cultivar were picked from the loquat tree. The fruit was separated into peel, flesh and kernel, and then all the tissues were cut into small pieces, and frozen in liquid nitrogen and stored at -20°C until analysis.

Preparation of extract solution for OA and UA analysis

Fruit material of 1.0 g was ground into powder with liquid nitrogen and solubilized in 20 ml ethanol for 2 h followed by 30 min ultrasonic extraction by TBT/C-YCL 500Tt/3P (D) ultrasonic machine (Sinobest electronic Co. Ltd., Jining, Shangdong Province, and P. R. China). The samples were extracted twice and both extracts were combined and evaporated to dryness at 35°C. The residue was dissolved in 1 ml methanol and transferred to an

Table 1. Changes in quality index of loquat fruit during fruit development.

No	Stage	TSS (ºBrix)	Weight (g)	Vertical diameter (cm)	Horizontal diameter (cm)	Total acid (%)	Total sugar (%)	pH
				Ruantiaobaisha				
1	2006/04/07	-	3.45	2.19	1.89	0.66	1.54	3.90
2	2006/04/17	-	6.20	2.37	2.28	1.03	1.46	3.64
3	2006/04/27	7.00	11.03	2.92	2.78	1.41	1.50	3.48
4	2006/05/07	8.41	21.26	3.25	3.51	1.95	4.52	3.33
5	2006/05/17	13.93	25.73	3.31	3.67	1.85	13.56	3.44
6	2006/05/24	16.63	28.23	3.45	3.73	0.91	14.02	3.83
				Dayeyangdun				
1	2006/04/07	-	3.87	2.20	1.91	0.66	1.51	3.93
2	2006/04/17	-	8.24	2.67	2.52	1.25	1.24	3.53
3	2006/04/27	5.64	14.72	3.22	3.10	1.83	1.33	3.37
4	2006/05/07	5.69	23.17	3.45	3.57	1.57	2.16	3.33
5	2006/05/17	9.56	31.13	3.71	3.81	1.07	7.40	3.48
6	2006/05/24	11.25	33.08	3.76	3.83	0.61	10.92	3.64

Table 2. OA and UA content in different tissues of loquat fruit (mg/g DW).

Component	Tissue	Ruantiaobaisha	Dayeyangdun
OA	Peel	0.87±0.08 ^{b}	1.45±0.02 ^{b}
	Flesh	0.03±0.01 ^{d}	0.04±0.02 ^{d}
	Kernel	0.02±0.01 ^{d}	0.03±0.00 ^{d}
UA	Peel	4.45±0.15 ^{a}	7.50±0.09 ^{a}
	Flesh	0.12±0.00 ^{c}	0.13±0.02 ^{c}
	Kernel	0.18±0.00 ^{c}	0.19±0.00 ^{c}

Different superscript lowercase letters within each column indicate significant differences ($P < 0.05$).

Eppendorf tube. The crude extract was filtered through a 0.30 µm micro-filter before HPLC analysis.

Statistical analysis

Standard deviations (SD) were calculated by Origin (Microcal Software Inc., Northampton, MA, USA). Duncan's new multiple range method test (DPS version 3.11) was calculated for mean separation.

RESULTS AND DISCUSSION

OA and UA contents in different tissues of loquat fruit

The ripe fruit of loquat were separated into peel, flesh and kernel, and the levels of OA and UA of different tissues was shown in Table 2. Triterpenoid composition of loquat fruit showed similarity with its flower (Zhou et al., 2007b) and leaf (Li et al., 2009), and UA is also the main triterpenoid of fruit, which was significantly higher than OA. The contents of OA and UA were highest in the peel, which were significantly higher than those of loquat flower (Zhou et al., 2007b), and similar to those of loquat leaf (our unpublished data). The results demonstrated that OA and UA contents in the peel of 'Dayeyangdun' (Red-fleshed type) were much higher than those of 'Ruantiaobaisha' (White-fleshed type), whether there is a significant difference between White- and Red-fleshed categories is subject to further analysis. Flesh and kernel contained only trace amounts of OA and UA, far lower than the amounts in the peel of fruit, and there is no difference between these two cultivars. Wang et al. (2008) studied the difference of OA and UA content between the peel, flesh and kernel of cornus (*Macrocarpium officinalis*) fruit, and discovered that the two compounds mainly located in peel of the fruit. Zhao

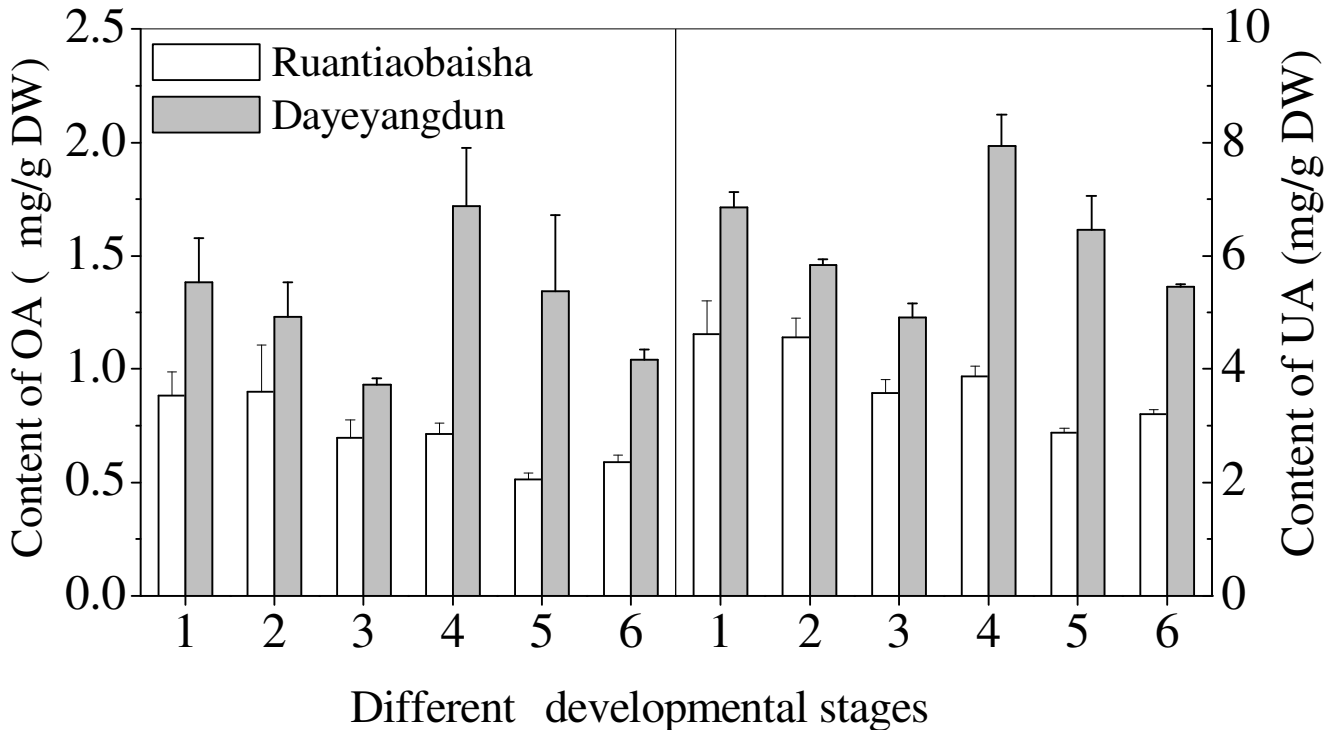

Figure 3. OA and UA content in loquat fruit peel of different developmental stages.

and Li (2008) also found that UA was rich in the peel of cornus fruit, while no UA was found in the flesh. Therefore, the distribution of OA and UA in different parts of loquat fruit was similar with that of cornus fruit. For different development stages and cultivars, the analysis of OA and UA was only carried out in the peel of loquat fruit because of the highest levels of OA and UA in the peel and very low levels of OA and UA in the flesh and kernel.

OA and UA contents in the peel of loquat fruit at different developmental stages

The OA and UA contents in the peel of 'Dayeyangdun' and 'Ruantiaobaisha' varied considerably with fruit development, and both OA and UA levels in 'Dayeyangdun' were higher than those in 'Ruantiaobaisha'. For each cultivar, the OA and UA contents were also significantly different between distinct developmental stages. In the peel of 'Ruantiaobaisha', the level of OA and UA were higher at early developmental stages, and then declined slightly, and finally increased little. The trend of OA and UA levels of 'Dayeyangdun' was not similar to 'Ruantiaobaisha'. There were clear peaks of OA and UA contents on May 7th during fruit development, with OA and UA content 1.71 ± 0.26 and 7.93 ± 0.56 mg / g DW, respectively. From then on, the OA and UA levels continued to decline (Figure 3).

OA and UA contents in the loquat fruit peel of different cultivars

For the ripe loquat fruit, the OA and UA contents in the peel of different cultivars varied greatly, the range of OA and UA contents were 0.59-1.68 and 2.82-8.20 mg / g DW, respectively. The average contents of OA and UA were 1.01 ± 0.29 and 4.98 ± 1.48 mg / g DW, respectively. The differences between the highest and lowest content were 2.85 and 2.91 times for OA and UA, respectively. Our previous study demonstrated that there was a positive or negative correlation between the carotenoids level of the loquat flesh and the fruit color index such as *L*, a*, b*, a*/b** and *H°* (Zhou et al., 2007a). In this research no correlation was found between the levels of OA and UA and fruit color index.

Among the 24 cultivars analyzed, the OA and UA levels in the peel of 'Dazhong' were lowest, with the contents of 0.59 ± 0.00 and 2.82 ± 0.05 mg / g DW, respectively. While the OA and UA levels in the peel of 'Baozhu' were highest, with the contents of 1.68 ± 0.03 and 8.20 ± 0.09 mg / g DW, respectively (Table 3). The reports of Cui et al. (2006) and Guo et al. (2009) also showed that there were great difference of OA and UA contents between cultivars for the fruit of hawthorn (*Crataegus pinnatifida*) and jujube (*Ziziphus jujuba*). However, it remains to be further studied whether the difference of OA and UA contents in the peel of loquat fruit is caused by genetic factors or environmental factors.

Table 3. OA and UA content in loquat fruit peel of different cultivars (mg/g DW).

Group	No.	Cultivar	OA	UA
	1	90-1	1.15±0.08 ^{de}	5.95±0.25 ^{d}
	2	Baiyu	0.79±0.02 ^{jkl}	4.08±0.14 ^{ijk}
	3	Bingtangzhong	0.99±0.04 ^{fgh}	4.17±0.14 ^{ij}
	4	Dazhong	0.59±0.00 ^{m}	2.82±0.05 ^{m}
	5	Guanyu	1.21±0.04 ^{d}	6.41±0.16 ^{c}
White-fleshed	6	Jidanbai	0.67±0.03 ^{lm}	3.39±0.19 ^{l}
	7	Luqiaobaisha	1.04±0.21 ^{efgh}	5.41±0.82 ^{e}
	8	Qingzhong	0.95±0.02 ^{gh}	4.70±0.13 ^{gh}
	9	Ruantiaobaisha	1.09±0.10 ^{ijk}	4.47±0.15 ^{h}
	10	Tianzhong	0.71±0.02 ^{lm}	3.55±0.15 ^{l}
	11	Tongpi	0.91±0.00 ^{hij}	4.16±0.06 ^{ij}
	12	90-2	1.05±0.06 ^{efgh}	4.78±0.19 ^{gh}
	13	Algeie	1.10±0.03 ^{defg}	5.24±0.08 ^{ef}
	14	Baozhu	1.68±0.03 ^{a}	8.20±0.09 ^{a}
	15	Bahong	1.13±0.04 ^{def}	5.42±0.16 ^{e}
	16	Dahongpao I	0.78±0.03 ^{jkl}	4.08±0.11 ^{ijk}
	17	Dahongpao II	0.74±0.10 ^{kl}	3.71±0.16 ^{jkl}
Red-fleshed	18	Dayeyangdun	1.45±0.02 ^{bc}	7.50±0.09 ^{b}
	19	Jiajiao	0.91±0.01 ^{hij}	4.71±0.07 ^{gh}
	20	Jidanhong	1.37±0.07 ^{c}	7.24±0.34 ^{b}
	21	Luoyangqing	0.71±0.00 ^{lm}	3.63±0.03 ^{kl}
	22	Marc	0.72±0.06 ^{klm}	3.49±0.25 ^{l}
	23	Pelusheis	1.06±0.01 ^{efg}	4.93±0.10 ^{fg}
	24	Zaozhong	1.54±0.41 ^{b}	7.58±1.05 ^{b}

Different superscript lowercase letters within each coloum indicate significant differences ($P < 0.05$).

Conclusions

The contents of OA and UA varied with tissues in loquat fruit. Peel contained highest OA and UA contents, and the UA content in the peel were higher than OA, while flesh and kernel was only found very low levels of OA and UA. The OA and UA contents in the peel between different developmental stages and cultivars varied considerably. There was no correlation between triterpenoids (OA and UA) and fruit color index. The average OA and UA contents in the loquat fruit peel of 24 cultivars were 1.01 ± 0.29 and 4.98 ± 1.48 mg / g DW, respectively.

ACKNOWLEDGEMENTS

The authors greatly thank Yuhang Loquat Science Institute, Zhejiang Province, P.R. China and Taihu Technological Popularization Center of Evergreen Fruit, Jiangsu Province, P.R. China for kindly providing the fruit materials used in this study. This research was financially supported by the Special Scientific Research Fund of Agricultural Public Welfare Profession of China (200903044), International Co-orparation Project of the Science and Technology Department of Zhejiang Province (2006C24005) and the 111 project (B06014).

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