

Note

Oleanolic Acid and Ursolic Acid in Commercial Dried Fruits

Feng ZHANG^{1,2}, Eisuke DAIMARU², Masao OHNISHI², Mikiyo KINOSHITA² and Yoshihiko TOKUJI^{2*}

¹ Department of Bioresource Science, United Graduate School of Agricultural Science, Iwate University, Ueda 3-18-8, Morioka, Iwate 020-8550, Japan

² Department of Food Science, Obihiro University of Agriculture and Veterinary Medicine, Inada-cho W2-11, Obihiro, Hokkaido 090-8555, Japan

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Oleanolic acid (OA) and ursolic acid (UA) are natural triterpenoids that have preventive properties such as anti-tumor and anti-hepatitis activities. These triterpenes are known to exist in several medicinal plants and fruit skins. Dried fruits are usually eaten without removing the skin; thus, they may be good resources for oral intake of OA and UA. HPLC analysis of OA and UA contents in a variety of dried fruits showed that raisins contained higher levels of OA than the other fruits investigated, with green raisins containing the highest levels (79.0 mg/100 g). While raisins were found to contain only OA, dried cranberries, blueberries and cherries were shown to contain both OA and UA. The highest level of UA (65.9 mg/100 g) was detected in cranberries, which also exhibited relatively high OA levels (17.8 mg/100 g). Thus, raisins and dried cranberries can be used as rich sources of OA and UA.

Keywords: oleanolic acid, ursolic acid, dried fruits, raisins, cranberries

Introduction

Oleanolic acid (3 β -hydroxy-olea-12-en-28-oic-acid, OA) and its isomer, ursolic acid (3 β -hydroxy-urs-12-en-28-oic-acid, UA), are natural triterpenoids. Both of these compounds have anti-inflammatory, anti-bacterial, anti-tumor, anti-HIV and immunoregulatory activities, in addition to providing hepatoprotection (Liu, 1995; Ovesná *et al.*, 2004; Liu, 2005). These triterpenic compounds are known to exist in many plant species (Jäger *et al.*, 2009), including medicinal herbs such as rosemary, thyme and ginseng. OA and UA are also present on the surface of fruit skins, such as those of grapes, persimmons and olives. However, as the skins of fresh fruits may be removed when eaten, fresh fruits may not be a significant source of OA and UA intake for many people. Because the solubility of OA and UA in wine or juice is quite low, they also remain on the fruit surface following processing, including washing and squeezing steps. As a consequence, fruit juices and wines do not contain significant levels of these compounds either. However, unlike fresh fruits, dried fruits are usually eaten without removing their skins, and

may be a good source for the natural oral intake of OA and UA. Their ingestion could therefore provide beneficial health effects. For instance, it is known that raisins, the dehydrated form of grapes, contain OA, and that extracts from raisins inhibit the growth of *Streptococcus mutans*, the oral pathogenic bacteria that causes tooth decay (Rivero-Cruz *et al.*, 2009). While dried fruits are a popular snack worldwide, with a variety commercially available in any given country, little is known about the OA and UA content of dried fruits other than raisins. The objective of this study is therefore to quantify and compare the levels of OA and UA in some dried fruits in order to identify rich sources of these triterpenes.

Materials and Methods

The 17 types of commercial dried fruit used in this study were purchased from Japanese markets (Table). Dried fruits (20 g) were added to ethanol (100 mL) and homogenized by agitation for 1 h, followed by filtration through filter paper No. 2 (Advantec, Tokyo, Japan). After 3 repetitions of this operation, the combined extracts were concentrated to dryness using a rotary evaporator and dissolved in 10 mL methanol for use in TLC and HPLC analysis. To check the recovery yield, 2 mg of UA was added to 20 g of green raisin, and

*To whom correspondence should be addressed.

E-mail: tokuji@obihiro.ac.jp

Table . Dried fruits used in this study.

Dried fruits	Materials	Skin*	Country of origin
Apple	apple, sugar, citric acid, spice, bleaching agent	–	China
Apricot	apricot	+	Turkey
Banana	banana, sulfite	–	Thailand
Blueberry	blueberry, sugar, melon seed oil	+	USA
Cherry	cherry, sugar, melon seed oil	+	USA
Cranberry	cranberry, sugar, plant oil	+	USA
Green raisin	grape (white)	+	China
Kyoho raisin	grape (red), plant oil	+	Chile
Mango	mango, sugar, citric acid, sulfite, colorant	–	Thailand
Orange	starch syrup, sugar, orange skin, lemon juice	+	Italy
Pear	pear, sugar, pH adjustment agent, vitamin C, sodium thio-sulfate	–	China
Persimmon	persimmon, sulfur dioxide	–	Japan
Pineapple	pineapple, sugar, pineapple juice, citric acid, sulfite	–	Thailand
Prune	prune, plant oil, potassium sorbate	+	USA
Fig	fig	+	Turkey
Strawberry	strawberry, sugar, plant oil, citric acid, sulfite, colorant, spice	+	China
Sultana raisin	grape (red), plant oil	+	Turkey

* Mean presence (+) and absence (–) of skin in dried fruits.

follow-up extraction and HPLC analysis were accomplished. Similarly, 2 mg of OA was added to 20 g of dried cranberry and analyzed. The recovery yields of UA from green raisin and OA from cranberry in this extraction method were $98.2 \pm 1.7\%$ ($n = 3$) and $97.9 \pm 2.3\%$ ($n = 3$), respectively.

The presence of OA and UA were confirmed by TLC prior to HPLC analysis. Sample solutions (about 10 μ L) were applied to a Silica gel 70 plates (0.25 mm thickness, 20 \times 20 cm; Wako, Osaka, Japan) and separated with cyclohexane/acetone/ethyl acetate (4:2:1). After the silica gel plates were dried, samples were visualized by spraying the plates with 50% sulfuric acid and baking.

Quantitative analyses of OA and UA were carried out by HPLC according to a previously reported protocol (Ebisui, 2006), with some modifications. Separation was achieved using a 4.6 \times 250 mm, 5- μ m Wakosil II 5C18 column (Wako) and L-2420 UV-VIS detector (Hitachi, Tokyo Japan) set at 210 nm. The solvent was vacuum filtered and degassed prior to use, and sample extracts were filtered through a 0.2- μ m Dismic-13 filter (Advantec) before injection. Samples (7 μ L) were injected onto the column following equilibration with acetonitrile/methanol/water/phosphoric acid (500:400:100:0.5), and were separated with the same solvent

at a rate of 0.5 mL/min. Analyses were performed at 20°C. Data were processed with a PC-based integrator (Chromato-Pro; Lablab Company, Hachioji, Japan), and the amounts of OA and UA in each dried fruit were calculated from the calibration curves (concentration range: 10 – 2000 μ g/mL) of OA (> 97%, Wako) and UA (> 95%, Wako) standards, respectively (R^2 of calibration curves for OA and UA were 0.9994 and 0.9998). The LOD and LOQ of OA were 0.255 mg/100 g and 0.850 mg/100 g, respectively, while the LOD and LOQ of UA were 0.309 mg/100g and 1.031 mg/100g, respectively (calculated using S/N ratio of the samples nearest to the detection limits). All data are presented as the means of 3 independent extracts with standard deviations calculated using Microsoft Excel (Microsoft, Redmond, WA, USA).

Results and Discussion

Of the 17 types of dried fruit assessed in this study, OA and UA were not detected in apple, banana, mango, pear or pineapple on TLC analysis (data not shown). This is perhaps not surprising, as it has been reported that triterpenic compounds such as OA and UA are mainly distributed in the skins of fruits (Orbán *et al.*, 2009; Frighetto *et al.*, 2008), and these 5 kinds of dried fruit had their skins removed during processing (Table). The dried fruits without skin do not contain – or contain very little – OA and UA, which supports previous studies. In contrast, TLC analysis clearly detected OA and/or UA in 3 varieties of raisin, persimmon, cranberries and cherries (Fig. 1).

However, we were unable to confirm the presence of OA or UA in prunes, oranges, figs or strawberries by TLC under our assay conditions (detection limit by TLC was 0.20 μ g/

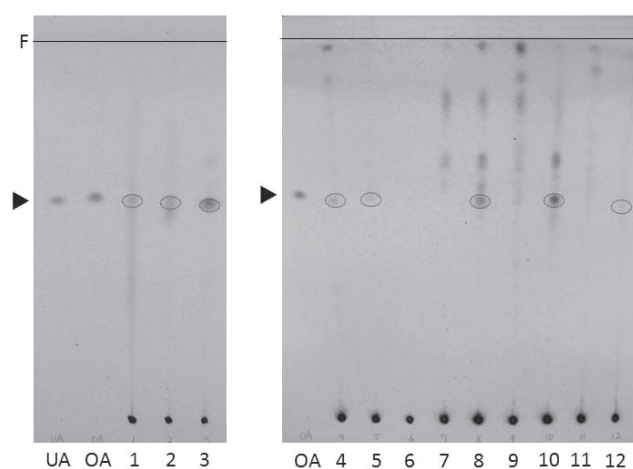


Fig. 1. TLC analysis of dried fruits. OA and UA are standards. Arrow heads and circles indicate the position of OA and UA. F lines indicate the front line of the TLC analysis. Lanes 1 – 12 are as follows: Sultana raisin (1), Kyoho raisin (2), green raisin (3), cherry (4), persimmon (5), orange (6), strawberry (7), cranberry (8), prune (9), blueberry (10), fig (11) and apricot (12).

spot). Because we believed that these fruits contain only trace amounts of UA and OA, we did not analyze them by HPLC.

As OA and UA are isomers with similar characteristics, they cannot be separated by TLC (Fig. 1). We therefore employed HPLC to separate and quantify the individual levels of OA and UA present in the fruits identified to contain these triterpenoids by TLC analysis. Figure 2A shows a representative chromatogram for the HPLC separation of OA and UA, which showed complete separation of the OA and UA peaks under our chromatography conditions.

All 3 varieties of raisin investigated in this study were found to contain higher levels of OA than other dried fruits, with OA contents of 79.0 mg/100 g (green raisins), 65.9 mg/100 g (Sultana raisins), and 38.5 mg/100 g (Kyoho raisins) (Fig. 3). This corresponds to previous findings that have shown that the presence of OA in raisins is independent of whether the grapevine cultivar is red or white (Orbán *et al.*, 2009; Yunoki *et al.*, 2008). We were unable to confirm the reasons for the differences in OA content between the different raisin types in this study, as the OA content in the raw materials and any changes that occurred during manufacture were unknown. No UA was detected in any of the raisin varieties (Fig. 2C, Fig. 3).

In contrast, dried cranberries, blueberries and cherries were found to contain both OA and UA (Fig. 3). Of the dried fruits analyzed in this study, the highest amount of UA (65.9 mg/100 g) was detected in cranberries, which also contained 17.8 mg/100 g OA. Blueberries were found to contain similar levels of OA and UA, at 13.9 mg/100 g and 11.8 mg/100 g, respectively. The level of UA in cherries (9.7 mg/100 g) was similar to that of blueberries, although OA in cherries was detected at significantly lower levels (1.72 mg/100 g). Low

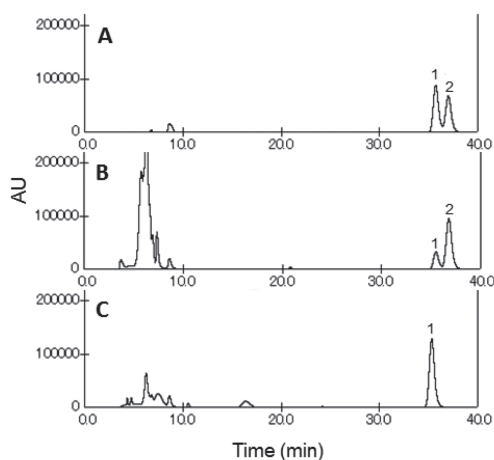


Fig. 2. HPLC chromatogram of OA and UA at 210 nm. Representative chromatograms of OA and UA standard mix (A), cranberry (B) and green raisin (C) are shown. The longitudinal axis shows absorbance at 210 nm in arbitrary units (AU). Peaks: (1) OA; (2) UA.

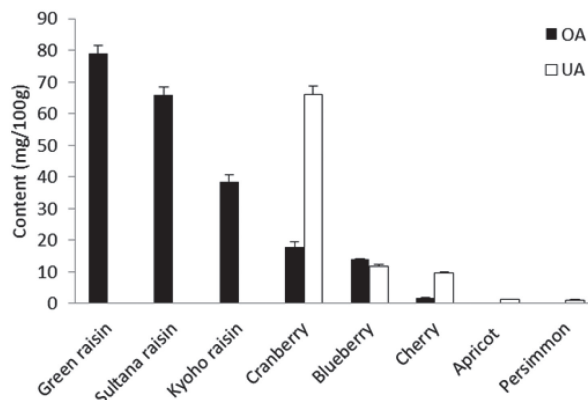


Fig. 3. OA and UA content in dried fruits. Bars indicate standard deviation (n = 3 independent extracts).

levels of UA were also found in apricots, although no OA was detected. While Zhou *et al.* (2010) previously reported that both OA and UA were present in most of the cultivars of persimmon that they investigated, we detected only very low amounts of UA (1.03 mg/100 g) and no OA in dried persimmons. This discrepancy might be explained by the lack of skin present on the dried persimmons used in our study, while the UA that was detected may be derived from the calyces that were retained on the fruit tops.

As OA and UA are known to possess many bioactive properties, identifying sources of these triterpenes is important. Indeed, many studies that have examined the *in vivo* functions of OA and UA have reported health benefits. For instance, Rodríguez *et al.* (2003) found that oral intake of OA can promote healing from acetic acid-induced chronic gastric lesions in rats. OA and UA have also been observed to have a suppressive effect on preneoplastic lesions in rat colon (Furtado *et al.*, 2008), and OA has been found to protect the liver against various hepatotoxicants in mice (Liu, 1995). Finally, OA has also been shown to inhibit the growth of oral pathogenic bacteria *S. mutans* (Rivero-Cruz *et al.*, 2008). Some folk medicines and herbal plants contain remarkable quantities of OA and UA, and their effects may partially be due to these triterpenes. For instance, 18.2 mg/100 g FW OA and 63.8 mg/100 g FW UA are present in fruits from *Ziziphus jujube* (Guo *et al.*, 2009), while the OA and UA contents in Chinese hawthorn (*Crataegus pinnatifida*) fruits are 14.7 mg/100 g FW and 95.2 mg/100 g FW, respectively (Cui *et al.*, 2006). OA and UA contents in dried fruits were lower than in these medicinal fruits. However, our study shows that OA and UA can be readily taken in from raisins and dried cranberries as part of daily food consumption. As raisins and dried cranberries also contain various functional flavonoids (Zhao and Hall, 2007; Vinson *et al.*, 2008), the combination of flavonoids and the triterpenes OA and UA provided by these fruits may be particularly beneficial for

human health.

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