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Full Length Research Paper

Effects of drying methods on the bioactive components in loquat (*Eriobotrya japonica* Lindl.) flowers

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The contents of bioactive components such as flavonoids, phenolics, oleanolic acid (OA), ursolic acid (UA), amygdalin, chlorophylls and carotenoids in loquat (*Eriobotrya japonica* Lindl.) flower with different drying methods were investigated. Results showed that freeze drying can effectively keep the bioactive components, but the drying rate is slow. The microwave drying is rapid, especially at the high power, and the drying effect was better compared with oven drying. The contents of bioactive components varied greatly with drying temperature of oven-dried samples and decreased sharply when the temperature reached 100°C except OA and UA. The effect of natural convective drying was similar to oven drying at 60 and 80°C but with longer time. All the results suggested that microwave drying is more efficient and low-cost, and 60 to 80°C oven drying is recommended to be used under the circumstance of no microwave drying equipment.

Key words: *Eriobotrya japonica*, flower, drying methods, bioactive component.

INTRODUCTION

Loquat flower is the dry inflorescence of the Rosaceae plant *Eriobotrya japonica* Lindl., and has been used as Traditonal Chinese Medicine for long times. According to descriptions of the medical literatures, it can be used for treatment of cold, cough, sputum and so on, and the extracts can be used in cosmetics and health food industry besides medicine. The main bioactive components existed in loquat flower include flavonoids, phenolics, oleanolic acid (OA), ursolic acid (UA), amygdalin, chlorophylls, carotenoids, etc (Hu et al., 2008; Zhou et al., 2007a). All above-mentioned compounds play critical roles in preventing ailments and maintaining human health (Fraser and Bramley, 2004; Ghosh, 2009; Graf et al., 2005; Lanfer-Marquez et al., 2005; Park et al., 2005; Yang et al., 2010). The fresh loquat flower contains lots of water, and is a perishable plant product. Therefore, the flower need drying in the flower processing, because desiccation is a course of water desorption, and it can inhibit the activities of microorganism, enzyme and ferment, thus the product can be stored for a much longer time (AlibaşÖzkan et al., 2001). Different methods can be used in the drying of fruits, vegetables and flowers. Among these methods, oven drying (hot-air drying) is the most frequently used dehydration operation in the food and conventional chemical industry. This method would result in flavor deterioration, color darkness and nutrient loss because of higher temperature and longer drying time (Sharma and Prasad, 2001). Freeze drying is one of the most sophisticated dehydration methods and can maintain much bioactive components, especially various heat-sensitive biological compounds, but the drying rate is relatively slow (Krokida and Philippopoulos, 2006). Microwave drying is a rapid dehydration technique that can be applied to specific foods and has been proved to be effective to much agricultural products (Orsat et al., 2007; Zhang et al., 2006). This method offers many advantages in processing, including less startup time,

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less quality decrease, faster heating, energy efficiency and space savings (Díaz et al., 2003; Schiffmann, 2001; Sumnu, 2001; Wu and Mao, 2008).

Until now, there was no report about the effects of different drying methods on bioactive compounds in loquat flowers. Therefore, different drying methods were applied to loquat flowers, and the influence of these methods to the main bioactive components was investigated, with the aim to pick out the practical drying method. The results may provide theoretical basis for integrative utilization and processing of loquat flower resources derived from flower thinning during loquat cultivation.

MATERIALS AND METHODS

Material

The whole inflorescence of *E. japonica* cv. Ruantiaobaisha flower was used in this study. The materials were collected from Yaojiadai Village, Tanxi Town, Yuhang District, Hangzhou, Zhejiang Province, Peoples Republic of China.

Drying of loquat flowers

The fresh inflorescence was cut into pieces and dried with the following methods until the weight reached unchangeable: (1) oven drying at 60, 80 and 100°C, (2) microwave drying under high (800 W), medium (528 W) and low power (136 W), (3) natural convective drying under the ventilated and obscure room (that is room temperature), (4) freeze drying in the lyophilization machine (Edwards Vacuum, Grand Island, NY, USA). All the dried materials were then powdered in a grinder to 40-mesh size, and stored under -20°C until analysis.

Extraction of bioactive components

Plant powder of 0.2 g was extracted in TBT/C-YCL 500Tt/3P (D) ultrasonic pharmaceutical managing machine (Sinobest electronic Co. Ltd., Jining, Shangdong Province, Peoples Republic of China) with 8 ml 70% ethanol for 30 min under the selected frequency and power (47 kHz and 500 W) after 2 h soaking. The extraction was repeated again. Then the extracts of two times were mixed and filtrated, and diluted to 20 ml. The diluted solution was used to determine the content of flavonoids and phenolics. 0.5 g plant sample was solubilized in 20 ml ethanol for 2 h followed by 30 min extraction by ultrasonic machine.

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 Eppendorf tube. The crude extract was filtered though a 0.30 µm micro-filter before HPLC analysis of OA, UA and amygdalin. 80% acetone was used to extract chlorophylls and the extraction repeated several times until the materials reached almost colorless (Koca et al., 2006). Carotenoids were extracted according to the method of Zhou et al. (2007b).

Determination of bioactive components content

Total flavonoids was determined by the procedure of Zhou et al. (2007c) with some modification. Aliquots (1 ml) of loquat flower extracts were placed in two test tubes, respectively. 7 ml methanol was added to one tube. In the other tube, 1 ml 2% $ZrOCl_2 \cdot 8H_2O$ and 6 ml methanol was added. The solution was mixed well again and placed into water bath at 30°C for 1 h. The absorbance was measured at 420 nm with DU-8000 UV-Vis spectrophotometer (Beckman Coulter, USA) and ΔOD was calculated. The amount of total flavonoids was calculated as a rutin equivalent from the calibration curve of rutin standard solutions, and expressed as mg rutin g/dry plant material. All measurements were repeated three times.

Total phenolics was determined by Folin-Ciocalteu procedure (Maksimovic et al., 2005). Aliquots (0.5 ml) of loquat flower extracts were transferred into the test tubes and their volumes made up to 4.5 ml with distilled water. After addition of 0.5 ml Folin-Ciocalteu reagent and 1 ml 7% aqueous sodium carbonate solution, tubes were vortexed and placed into water bath at 30°C for 2 h. Then absorbance of mixtures was recorded at 760 nm with DU-8000 UV-Vis spectrophotometer (Beckman Coulter, USA) against a blank containing 0.5 ml of extraction solvent. The amount of total phenolics was calculated as a chlorogenic acid equivalent from the calibration curve of chlorogenic acid standard solutions, and expressed as mg chlorogenic acid g/dry plant material. All measurements were done in triplicate.

OA, UA and amygdalin were determined on a HPLC system (Beckman Coulter, USA) equipped with 125 pump, 166 UV detector and an ODS C_{18} column (250 × 4.6 mm, 5 µm) by the procedure of Zhou et al. (2007a). All these three 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, and 25:75 (A:B, v/v) for detection of amygdalin. All determinations were repeated three times. Chlorophyll was determined by DU-8000 UV-Vis spectrophotometer (Beckman Coulter, USA) Aliquots (3 ml) of pigment extracts were transferred into the cuvette and 80% acetone used as control. Then the absorbance of solutions was recorded at 663 and 645 nm. The concentrations of chlorophyll a (Ca), chlorophyll b (Cb) and total chlorophylls (Ct) were calculated by the method of Zhang (1990). All measurements were done in triplicate.

Carotenoids analysis was determined by HPLC on Waters Alliance 2695 system (Waters Corp., Milford, MA) consisting of a 2695 separation module and a 2996 PDA detector, equipped with a 250 × 4.6 mm i.d., 5 µm, YMC reverse-phase C_{30} column and a 20 × 4.6 mm i.d., YMC C_{30} guard (Waters, Milford, MA) according to the procedure reported previously (Zhou et al., 2007b). Carotenoids were identified on the basis of the same retention times and same spectral characteristics as standards. Carotenoid standards lutein, β-cryptoxanthin, and β-carotene were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Methods for carotenoid quantification have been reported previously (Xu et al., 2006).

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. Differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Effects of drying methods on flavonoids and phenolics contents of loquat flowers

The contents of flavonoids and total phenolics varied

Table 1. Effects of drying methods on flavonoids, phenolics, OA, UA and amygdalin content of loquat flowers (mg/g DW).

Drying method		Flavonoids	Phenolics	OA	UA	Amygdalin
Oven drying	60ºC	10.22±0.14^{b}	91.02±4.40^{b}	0.39±0.05^{a}	1.85±0.07^{a}	3.72±0.30^{b}
	80ºC	9.34±0.21^{cd}	72.63±3.41^{d}	0.44±0.02^{a}	1.89±0.07	2.19±0.36^{c}
	100℃	8.90±0.23^{de}	70.57±3.30^{b}	0.42±0.02^{a}	1. 98±0.07	1.01±0.04^{d}
Microwave drying	High power	11.23±0.52^{a}	104.86±4.93^{a}	0.39±0.02^{a}	1.95±0.13^{a}	2.02±0.12^{c}
	Medium power	9.70±0.30^{bc}	102.67±1.48^{a}	0.45±0.02^{a}	2.04±0.13^{a}	1.93±0.19^{c}
	Low power	8.57±0.27^{e}	69.20±2.48^{d}	0.50±0.11^{a}	1.99±0.23	0.15±0.02^{e}
Natural convective drying		9.21±0.41^{cd}	80.35±2.85^{c}	0.41±0.07^{a}	1.77±0.31^{a}	4.54±0.59^{a}
Freeze drying		11.18±0.37^{a}	106.56±2.70^{a}	0.51±0.12^{a}	1.92±0.23^{a}	4.77±0.36^{a}

Different letters within each row indicate significant differences ($p < 0.05$).

considerably with drying methods (Table 1). For oven drying, although higher temperatures resulted in shorter drying times, both components in loquat flower were damaged and therefore caused a reduction in the content. The results were in accordane with that of Harbourne et al. (2009).

On the other hand, the flavonoids and phenolics also could be destroyed by oxidation when exposed in the atmosphere for long time, though the temperature was much lower. For microwave drying, flavonoids and total phenolics contents declined with power lowing and time prolonging.

Microwave drying under low power need more time for flower drying, and it is bad for the quality of dry loquat flowers. The effects of lyophilization is better than other drying methods, but this method is not suitable for the flower processing in some rural region because of longer time, high energy-consuming and much investment. Flavonoids and total phenolics contents of high-power microwave drying and freeze drying were significantly higher than those of other drying methods. The results were consistent with that of Zong (2006), he also found that the flavonoids content of lyophilized loquat leaves was highest, while that of oven dried was lowest.

Effects of drying methods on OA and UA contents of loquat flowers

The contents of OA and UA in the loquat flowers with different drying methods had no significant differences (Table 1).

The data demonstrated that OA and UA were relatively stable components in loquat flower, and different drying methods almost had no effects on them. This phenomenon was in accordance with the results of Zong (2006), but in his research, only the effects of freeze drying, vacuum drying, natural convective drying and oven drying to OA content in loquat leaves was studied and no significant difference was found.

Effects of drying methods on amygdalin content of loquat flowers

Our results indicated that amygdalin is much stable under lower temperature (Table 1). The amygdalin contents in loquat flowers of freeze drying and natural convective drying were higher, and they were 4.77±0.36 and 4.54±0.59 mg/g DW, respectively. The amygdalin contents between these two methods had no significant difference. Effects of oven drying and microwave drying on amygdalin were similar to those of flavonoids and total phenolics. The amygdalin content significantly decreased with the drying temperature increase for oven drying. The amygdalin content of 60, 80 and 100℃ dried materials were 77.99, 45.91 and 21.17% of lyophilized samples, respectively.

Lin and Huang (2006) also found that the amygdalin content of Semen Persicae, dried at low temperature was higher. As for microwave drying, high power and medium power have no significant difference. Low power extended the heating time and therefore may greatly damage amygdalin. The amygdalin content of low-power microwave drying was only 3.14% of lyophilized samples. This result was in accordance with that of Ozkan et al. (2007) reported for ascorbic acid content in spinach. Also, low power resulted in significant increase in energy consumption (Ozkan et al., 2007).

Effects of drying methods on pigments content of loquat flowers

Effects of different drying methods on chlorophylls was illustrated in Table 2. The total chlorophyll content of freeze drying flowers was highest, the content was 282.29±16.75 μg/g DW, and the content of chlorophylls a and b were also highest accordingly. The total chlorophyll content of high-power microwave dried samples ranged second and higher than other drying methods. The results was consistent with that of Huan et al. (2007), in which the chlorophyll of green tea by microwave heating,

Table 2. Effects of drying methods on chlorophylls content of loquat flower (μg/g DW).

Drying method		Chlorophyll a	Chlorophyll b	Total chlorophylls
Oven drying	60℃	111.36±6.52^{de}	58.63±3.41^{b}	166.99±9.08^{d}
	80℃	123.61±7.56^{bc}	66.71±6.10^{b}	190.32±13.46^{bc}
	100℃	76.58±5.43^{f}	42.64±4.99^{c}	119.22±10.36^{f}
Microwave drying	High power	134.51±2.49^{b}	60.39±6.57^{b}	194.91±9.01^{b}
	Medium power	99.93±2.89^{e}	43.71±1.17^{c}	143.65±1.83^{e}
	Low power	115.44±1.38^{cd}	59.54±2.51^{b}	174.98±3.66^{cd}
Natural convective drying		100.28±8.84^{e}	42.14±3.72^{c}	142.42±12.57^{e}
Freeze drying		198.69±11.24^{a}	83.59±5.71^{a}	282.29±16.75^{a}

Different letters within each row indicate significant differences ($p < 0.05$).

Table 3. Effects of drying methods on carotenoids content of loquat flowers (μg/g DW).

Drying method		Lutein	β-Cryptoxanthin	β-Carotene	Total carotenoid
Oven drying	60ºC	13.14±1.15^{bc}	0.29±0.03^{a}	3.89±0.19^{c}	27.91±2.65^{bc}
	80ºC	11.74±1.88^{bc}	0.34±0.07^{a}	3.22±0.50^{cd}	24.17±3.96^{bc}
	100ºC	5.72±0.77^{d}	0.23±0.01^{a}	1.58±0.03^{d}	11.60±2.27^{d}
Microwave drying	High power	13.15±3.73^{bc}	0.37±0.11^{a}	4.95±1.70^{bc}	28.24±9.10^{bc}
	Medium power	15.56±1.31^{ab}	0.36±0.04^{a}	6.61±0.90^{ab}	34.15±3.26^{ab}
	Low power	11.14±2.43^{bc}	0.34±0.16^{a}	3.88±1.02^{c}	21.54±7.09^{cd}
Natural convective drying		10.47±1.95^{c}	0.26±0.08^{a}	4.42±1.02^{c}	23.41±4.67^{bc}
Freeze drying		18.36±4.19^{a}	0.37±0.10^{a}	6.89±1.51^{a}	41.31±8.72^{a}

Different letters within each row indicate significant differences ($p < 0.05$).

was higher and more stable than that by oven heating. The lowest total chlorophyll content was observed in oven-dried samples at 100℃, with the value only 42.23% of lyophilized samples.

The total carotenoids content of freeze dried loquat flowers was highest among loquat flower samples using different drying methods in this study. The contents of lutein, β-carotene and total carotenoids of lyophilized sample significantly differentiated with those of other drying materials except for β-cryptoxanthin (Table 3). Nawirska et al. (2009) also found that the highest average carotenoid content in the dried pumpkin was determined with the freeze drying method. Chen et al. (2007) reported that lyophilized mango fruit contained more carotenoids than oven dried samples, and freeze drying can maintain the carotenoids content in dried mango fruit combined with some chemical treatments. The change tendency of carotenoids content in loquat flower of oven drying was the same to flavonoids and total phenolics as mentioned earlier.

The carotenoids content decreased with the temperature rising in oven-dried samples. When the drying temperature reached 100℃, the content of lutein, β-cryptoxanthin, β-carotene and total carotenoids were 31.15, 62.16, 22.93 and 28.08% of lyophilized samples, respectively. As far as microwave drying is concerned, medium-power can maintain much high carotenoids, and the content of lutein, β-cryptoxanthin, β-carotene and total carotenoids were 84.75, 97.30, 95.94 and 82.67% of lyophilized samples, respectively. High-power microwave drying resulted in the decrease of carotenoids content, this may be caused by the damage of higher microwave frequency to carotenoids molecule (Ju et al., 2002).

Conclusions

In this paper, the effects of different drying methods on bioactive components of loquat flowers were compared. Freeze drying can effectively keep the bioactive components such as flavonoids, phenolics, OA, UA, amygdalin, chlorophylls, and carotenoids, but the drying rate is slow. Microwave drying can shorten the drying time and maintain much higher bioactive components compared to oven drying, especially the high power. For oven drying, the content of 60 and 80℃ oven drying had no significant difference. When the oven drying temperature reached 100℃, the content of all the

bioactive components except OA and UA decreased sharply. Natural convective drying had the same effect as 60 and 80°C oven drying, but the drying time was longer. All the results suggested that microwave drying is a more efficient and power-saving method for loquat flower drying. In addition, 60 to 80°C oven drying is recommended to be used under the circumstance of no microwave drying equipment.

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