Effect of boiling and drying process on chemical composition and antioxidant activity of *Chaenomeles speciosa*

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Abstract Chemical composition and antioxidant activity of fresh and boiled *Chaenomeles speciosa* (CS) slices dried by different drying methods were determined. Data were analyzed by principle component analysis and cluster analysis. All dried boiled CS from dried fresh CS slices form main cluster. The results also demonstrated that both drying methods, freeze drying and hot air drying at 60 °C had good potential in the industrial drying of fresh and boiled CS. Fresh CS dried by hot air drying at 60 °C was more suitable for the industrial production.

Keywords Rosaceae · *Chaenomeles* · Boiling · Drying method · Chemical component · Antioxidant activity

Introduction

Chaenomeles speciosa (CS), the ripe fruit of CS (Sweet Nakai (Rosaceae family) with pear shaped, is widely cultivated in China, Korea and Japan. Especially, in many parts of China, for example Sichuan, Yunnan, Chongqing, Anhui, Hubei and Zhejiang provinces, CS is cultivated as an important crop with high commercial potential as well as high total acidity and antioxidant capacity (Tarko et al. 2014). In China, CS is well-known as Mugua, and always is harvested in July and August. CS is not consumed in the form of raw fruits after harvest, but is mainly processed into dried slices, which can be used to produce fruit tea, fruit wine, fruit vinegar and materials of medicinal liquor and even seasoning. Therefore, the processing method of CS slices is of great significance. Usually, CS is harvested on sunny days, subsequently washed after cut in half, then boiled in boiling water for about 3 min until the color changed into gray, cut into slices and dried in the sun at last. In some parts of China, CS is cut into pieces without boiling process, and dried in the sun directly. In fact, there are so many rainy days in July and August, so the harvest and process of CS slices should be delayed for the weather, although all fruits should be harvested during their best picking time to obtain materials with the highest quality (Clesivan et al. 2016; Pandu et al. 2015; Simona et al. 2015; Wang et al. 2016). The best harvest time is about 10 days long, earlier or later the bioactive compounds, essential oil and antioxidant activity will be out of the best stages (Pandu et al. 2015).

Proper drying method can make the process of CS slices become easier, also can maintain the bioactive compounds at the greatest extent. Therefore, it is necessary to investigate the process methods of CS slices, including drying and boiling processes. For CS slices, drying is a

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fundamental requirement to long store or marketing, so it is important to find a technical drying method to achieve products with high quality. Sun drying is a traditional drying method with a long history. In recent years, various methods such as freeze drying, vacuum drying, hot air drying and infrared drying have gained popularity as an easy using drying method for many varieties of food products such as fruits, herbs, vegetables, snack foods and dairy products (Wang and Sheng 2006). Sun drying and hot air drying are the most widely used methods for their lower cost (Soysal 2004), and hot air is a commonly used tool as it can produce dried material in shorter times compared to sun drying. However, the antioxidants in CS slices, particularly phenolics, flavonoids, vitamin C and anthocyanins, are the most sensitive components to heat, air and light in the process of drying. In general, for vacuum drying and hot air drying, temperature always is set at the range from 40 to 80 °C for fruits (Samoticha et al. 2016; Katia et al. 2016; Sandra and Chong 2013).

Many compounds, such as flavonoids (quercetin, luteolin, catechin, epicatechin, procyanidin B1 and B2), triterpenes (oleanolic acid and ursolic acid) and phenolics (gallic acid and chlorogenic acid) (Du et al. 2013; Hamauzu et al. 2005; Huang et al. 2013; Lee et al. 2002; Ros et al. 2004; Yang et al. 2009; Zhang et al. 2010), have been reported in CS fruits, as well as activities of rheumatism and stimulating people's appetite, antioxidant, antitumor, anti-hepatitis, antimicrobial activity, immunoregulatory, anti-influenza viral and anti-Parkinson (Hamauzu et al. 2005; Sandesh et al. 2010; Yao et al. 2013; Zhao et al. 2008; Zhang et al. 2010). During the drying process, color of CS changed from green-white to red-brown, and in Pharmacopoeia of the People's Republic of China (2015 edition), pH also is an important index of CS. Thus, in order to select out the most suitable methods for the production of dried CS slices, chemical composition, color, pH and antioxidant activity are selected to evaluate quality of CS slices processed by boiling process and drying methods, including vacuum drying, sun drying, infrared drying, freeze drying and hot air drying.

Materials and methods

Materials and chemical reagents

Fresh fruits of CS were collected from Dali in Yunnan province of China at their ripening stage in July 2015, and stored in a refrigerator. All the fruits were treated in 3 days.

HPLC grade acetonitrile and glacial acetic acid were obtained from Concord Technology Co. Ltd. (Tianjin, China). DPPH (2, 2-diphenyl-1-picrylhydrazyl, purity

≥98.0%) was purchased from Sigma-Aldrich Co. LLC., while Folin–Ciocalteu reagent (FC reagent, puriss, 1 N) was purchased from Beijing Solarbio Technology Co. Ltd. (Beijing, China). Other chemical reagents including Vanillin, NaNO₂, NaOH, AlCl₃, KCl, HCl, HClO₄, methanol and so on, all were of analytical grade (purity > 90%) and purchased from Tianjin Jiangtian Chemical Technology Co., Ltd. (Tianjin, China).

Standards of gallic acid, rutin, chlorogenic acid, catechin, epicatechin, oleanolic acid and ursolic acid were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China).

Drying methods

Fresh whole CS fruits were selected, washed and cut in half, one half of them were cut into 1 cm thick slices directly and the other half were cut into 1 cm thick slices after boiled in boiling water for 1 min.

CS slices were dried by vacuum drying at 60 and 80 °C for 6 and 3 h, respectively, sun drying for 72 h, and infrared drying for 0.5 h, freeze drying for 36 h, hot air drying at 40, 60 and 80 °C for 24, 5 and 1 h, respectively.

Sample extraction preparation

Samples dried by these methods previously mentioned were cut into 1 mm wide pieces and stored in a refrigerator. Pieces of each sample (5 g) mixed with 100 mL 60% methanol in conical flasks, respectively, flasks were covered and put into an ultrasonic bath to extract for 30 min twice. The filtrate was collected separately after filtered, and then the obtained filtrates were concentrated on rotary evaporator at 60 °C under vacuum until methanol was removed completely, and the concentrated solution was moved into a volumetric flask, then distilled water was added to make up the volume to 10 mL. All these solutions (500 mg dry weight/mL) were stored at 4 °C till the following determinations.

Total phenolics content (TPc)

TPc values in extracts were determined using Folin–Ciocalteu's reagent as described by Singleton and Rossl (1965) with minor modification. Briefly, Folin–Ciocalteu reagent (1 N, 0.4 mL) was added into a test tube together with 0.4 mL sample solution, and 1.2 mL Na₂CO₃ solution (0.188 mol/L) was added 3 min later. The absorbance of each sample was measured at 765 nm with a spectrophotometer after incubating at 25 °C for 2 h. The TPc was calculated from a calibration curve, using gallic acid as a standard. The data were expressed as mg gallic acid equivalents per g dry weight.

Total flavonoids content (TFc)

TFc values were measured by aluminum chloride colorimetric method described by Alothman et al. (2009) with minor modification. Rutin was chosen as the standard. Sample solution (1 mL) was mixed with 10% NaNO₂ solution (0.3 mL) in a 10 mL volumetric flask, to which 10% Al(NO₃)₃ solution (0.3 mL) was added 6 min later, then 1 mol/L NaOH solution was added to the line. After the mixture reacted for 15 min, the absorbance was determined at 506 nm with a spectrophotometer. The data were expressed as mg rutin equivalents per g dry weight.

Total triterpenes content (TTc)

The quantitative analysis of total triterpenes was tested by the pH differential method which described by Chen et al. (2007) with minor modification. The mixture solution, which contained 0.2 mL of 5% vanillin-glacial acetic acid solution and 1 mL of HClO₄, was used as chromogenic agent, while the absorbance was measured at 550 nm with a spectrophotometer. Values of TTc were expressed as mg oleanolic acid equivalents per g dry weight.

Antioxidant activity

Total ferric reducing antioxidant power (FRAP)

Total ferric reducing antioxidant power was determined according to the method of Oyaizu (1986) with minor modification. These different extracts solution (1 mg/mL, 0.2 mL) were mixed with 0.5 mL of 0.2 M sodium phosphate buffer (pH = 6.6) and 0.5 mL of 10 mg/mL K₃[Fe(CN)₆]. The mixture was incubated at 50 °C for 20 min, followed by addition of 0.5 mL trichloroacetic acid (100 mg/mL) and centrifuged at 3500 r/min for 10 min. Then 0.5 mL of the supernatant was mixed with 0.5 mL distilled water and 0.2 mL ferric chloride (1.0 mg/mL). The absorbance of each sample was read at 700 nm using a microplate reader (Beijing Perlong New Technology Co. Ltd., DNM-9602, China) by moving 200 µL mixed liquor into a 96-well plate. Values of total ferric reducing antioxidant power were calculated according to a standard curve of vitamin C (Vc) and values were showed as mg Vc equivalent amount per g dry weight.

DPPH assay

DPPH free radical scavenging capacity was evaluated by the method described by Shimada et al. (1992) with minor modifications. CS extracts with different concentration (0.1 mL, water solution) were mixed with 0.1 mL of

0.5 mM DPPH in ethanol in a 96-well plate. The mixtures were shaken carefully and incubated at 37 °C in the dark for 30 min, and then the absorbance was measured at 517 nm by a microplate reader. The free radical scavenge ratio (%) was calculated according to the following formulation.

The free radical scavenge ratio (%) = $100 \times (1 - (A_{\text{sample}} - A_{\text{control}}) / A_{\text{blank}})$

A_{sample}: the absorbance of 0.1 mL sample solution and 0.1 mL DPPH solution.

A_{control}: the absorbance of 0.1 mL sample solution and 0.1 mL ethanol solution.

A_{blank}: the absorbance of 0.1 mL water and 0.1 mL DPPH solution.

Qualitative and quantitative analysis of nine compounds

Oleanolic acid and ursolic acid were determined as typical triterpenes, gallic acid, protocatechuic acid, chlorogenic acid, syringic acid and *p*-coumaric acid were detected as typical phenolics, while catechin and epicatechin were analyzed as typical flavonoids. Qualitative and quantitative analysis of these nine typical compounds were finished by HPLC-MS and HPLC, respectively.

Qualitative analysis of seven monomeric compounds by HPLC-MS

The HPLC-DAD-ESI/MS consisted of an Agilent 1200 HPLC coupled to a diode array detector and mass spectrometer (MSD, SL mode, Agilent, Palo Alto, CA). A reversed phase C18 column (250 mm × 4.6 mm, 5 µm, Kromasil C18), preceded by a guard column (4 mm × 3.0 mm, Kromasil) of the same stationary phase was used at a flow rate of 1.0 mL/min. A binary solvent system was employed consisting of formic acid/water (0.5/99.5, v/v) as solvent A and methanol as solvent B, and the diode array UV detector (DAD) was set at 280 nm to record the peak intensity. The gradient program was 0–5 min with 2% solvent B, 5–25 min with 2–8% B, 25–45 min with 8–12% B and 45–80 min with 12–24% B. The flow rate of the mobile phase was 1 mL/min, and the injection volume 20 µL. A split joint was used after the PDA detector, directing a flow of 0.3 mL/min to the mass spectrometer and the rest to a waste bottle.

The mass spectrometer was operated both in positive and negative ion modes. The capillary voltage was set to 4.0 kV, the cone voltage to 22 V and the extractor voltage to 3 V. The source temperature was 150 °C and the dissolution temperature 300 °C. The HPLC-ESI-MS system was operated using Bruker Daltonics software. The mass scan scopes from *m/z* 50 to *m/z* 2000.

Quantification of monomeric compounds by HPLC

The HPLC-DAD consisted of a Waters HPLC coupled to a 2996 PDA detector. A reversed phase C18 column (250 mm × 4.6 mm, 5 µm, Kromasil C18), preceded by a guard column (4 mm × 3.0 mm, Kromasil) of the same stationary phase was used at a flow rate of 1.0 mL/min. The determination of phenolics and flavonoids (gallic acid, chlorogenic acid, catechin, epicatechin) was achieved under the same gradient program with *qualitative analysis*, and the diode array UV detector (DAD) was set at 280 nm to record the peak intensity.

Oleanolic acid and ursolic acid were detected at 210 nm wave-length under the condition described in Pharmacopoeia of the People's Republic of China (2015 edition). A single mixture consisted of methanol-ultrapure water-acetic acid-triethylamine (v/v/v= 265/35/0.1/0.05). The flow rate was set at 1 mL/min, and the injection volume 20 µL.

All the nine compounds in their HPLC chromatograms were identified by retention time, respectively. Concentrations of oleanolic acid and ursolic acid were expressed as mg per g dry weight, while concentrations of gallic acid, protocatechuic acid, chlorogenic acid, syringic acid, *p*-coumaric acid, catechin and epicatechin were expressed as µg per g dry weight.

Absorbance at UV_{420 nm}

According to Kim and Lee's method (2009) with minor modification, the hot water extracts of each CS samples were diluted with distilled water to 125 mg/mL. Then, absorbances of the resulting solutions were determined by spectrophotometer at 420 nm.

pH

According to Pharmacopoeia of the People's Republic of China (2015 edition), pieces of each sample (5 g) mixed with 100 mL distilled water together in conical flasks, and keep shaking for 1 h. The filtrates were collected to determine pH values by a pH meter.

Statistical analysis

All data were carried out by three replicates and expressed as mean ± standard error of mean. The SPSS version 17.0 statistical software package was used for all statistical analysis. The significant differences were detected by LSD and S–N–K tests, correlation matrix was analyzed by Pearson correlation coefficient. Principal component analysis was used to grade samples at a comprehensive level based on the chemical composition and antioxidant

activity, while cluster analysis was finished by hierarchical cluster procedure.

Results and discussion

Chemical composition

TFc, TPc and TTc of CS samples dried by different drying methods are listed in Table 1, and TFc were the highest followed by TPc and TTc. Freeze drying method always was considered as a desired drying method, which can make all the chemical composition in dried samples remain unchanged.

According to the results, it is clear that boiled CS possessed a lower TFc than fresh CS when dried by freeze drying method, and compared with boiled CS dried by other methods, freeze dried boiled CS contained the least TFc. It is obvious that all drying methods may help to increase TFc in boiled CS except freeze drying, while these methods could decrease TFc in fresh CS other than sun drying. Especially, the highest TFc were found in sun dried fresh and boiled CS, respectively. Maybe, sun drying could improve TFc in CS. However, sun drying CS contained the highest TFC in both fresh and boiled groups, respectively. When fresh CS dried by hot air drying at 40, 60 and 80 °C, TFc decreased as temperature increased, although when dried by vacuum drying at 60 and 80 °C TFc increased as temperature increased, this result may all due to the air circulation in hot air drying process. After boiled, TFc in CS were improved when dried by vacuum drying at 60 °C, infrared drying, freeze drying and hot air drying at 40, 60 and 80 °C compared with dried fresh CS. For hot air drying, TFc in dried fresh CS decreased as the drying temperature increased, while TFc in boiled CS dried at 60 °C was higher than TFc in boiled CS dried at 40 and 80 °C. Therefore, boiling process can affect TFc in CS. All these TFc values illustrates that not only sun drying but also vacuum drying, infrared drying and hot air drying may improve TFc in boiled CS in another way, while only sun drying improved TFc in fresh CS.

However, the highest TPc existed in fresh CS dried by freeze drying, while boiled CS dried by freeze drying exhibited the lowest TPc, although all these TPc didn't show significant differences except freeze dried fresh and boiled CS. Therefore, boiling process may cause the decrease of TPc in CS, but all drying methods can help to increase TPc in boiled CS except freeze drying.

Significantly, fresh CS dried by freeze drying contained the highest total triterpenes, while boiled CS dried by freeze drying contained the lowest total triterpenes. For most CS, boiled CS always possessed higher TTc when

Table 1 TFc, TPc and TTc in different CS samples

Drying methods		Vacuum drying		Sun drying	Infrared drying	Freeze drying	Hot air drying		
		60 °C	80 °C				40 °C	60 °C	80 °C
TFc (mg rutin per g dry weight)	Fresh	12.77±0.72 ^{d,e,f}	13.74±0.32 ^f	42.14±0.48 ^g	9.71±0.89 ^{b,c}	13.99±0.32 ^f	12.28±0.12 ^{d,e,f}	11.18±0.74 ^{c,d,e}	10.5±0.31 ^{b,c,d}
	Boiled	13.17±0.19 ^{e,f}	9.09±1.97 ^b	13.77±0.44 ^f	11.30±0.39 ^{c,d,e}	4.65±0.34 ^a	11.34±1.08 ^{c,d,e}	12.25±0.81 ^{d,e,f}	11.62±2.12 ^{c,d,e,f}
TPc (mg gallic acid per g dry weight)	Fresh	2.96±0.18 ^{a,b}	3.23±0.32 ^{a,b}	2.99±0.60 ^{a,b}	2.80±0.37 ^{a,b}	3.28±0.47 ^b	2.81±0.50 ^{a,b}	3.13±0.39 ^{a,b}	2.79±0.02 ^{a,b}
	Boiled	2.80±0.34 ^{a,b}	2.96±0.32 ^{a,b}	2.79±0.34 ^{a,b}	2.83±0.66 ^{a,b}	2.14±0.10 ^a	3.00±0.34 ^{a,b}	2.63±0.06 ^{a,b}	3.11±0.33 ^{a,b}
TTTc (mg oleanolic acid per g dry weight)	Fresh	0.80±0.04 ^a	0.90±0.02 ^a	1.35±0.02 ^{b,c}	0.82±0.07 ^a	2.56±0.28 ^c	1.14±0.01 ^{a,b,c}	1.07±0.07 ^{a,b}	1.20±0.05 ^{a,b,c}
	Boiled	0.94±0.04 ^{a,b,c}	1.30±0.10 ^{a,b,c}	1.43±0.07 ^{a,b,c}	1.39±0.05 ^{a,b,c}	0.86±0.04 ^a	1.13±0.01 ^{a,b,c}	1.38±0.06 ^a	1.03±0.05 ^{a,b}

The results were given as mean values ± standard deviation (n=3)

TFc total flavonoids content, TPc total phenolics content, TTc total triterpenes content

Different small superscripts (a–g) denoted the significant difference ($P < 0.05$) of the varieties of extracts

compared with freeze dried CS. Thus vacuum drying, sun drying, infrared drying and hot air drying would play an important role to increase TTc in boiled CS, but decrease TTc in fresh CS, while boiling process can decrease TTc in fresh CS.

As known, not only drying method but also drying temperature and boiling process can affect chemical composition in CS, including TFc, TPc and TTc, and also show significant effects on TFc and TTc, which is accordance with the conclusion of Lou et al. (2015) and Samoticha et al. (2016). In this work, vacuum drying, sun drying, infrared drying and hot air drying increased values of TFc, TPc and TTc in boiled CS, while vacuum drying, infrared drying and hot air drying would decrease TFc, TPc and TTc values of fresh CS at different extent, although sun drying increased TFc value in fresh CS and increased TFc, TPc and TTc values in boiled CS, but decreased TPc and TTc in fresh CS. Boiling process undoubtedly have decreased TTc in fresh CS.

However, fresh CS slices and boiled CS slices dried by sun drying both possess the highest TFc and TTc values than slices dried by other drying methods in fresh CS group and boiled CS group, respectively, although all TPc values of dry fresh CS slices and boiled CS slices show no discrepancy in their respective groups.

Qualitative and quantitative analysis of nine compounds

Many compounds existed in CS have shown good antioxidant activity, including oleanolic acid, ursolic acid, gallic acid, protocatechuic acid, chlorogenic acid, syringic acid, *p*-coumaric acid, catechin and epicatechin, which widely existed in many kinds of fruits (Cui et al. 2006; Li et al. 2011; Wang et al. 2015), and contents of these nine selected monomeric compounds are given in Table 2.

Among these nine compounds, catechin showed the highest content in the range of 3903.64±6.02–11384.38 ±0.86 µg/g, then followed by the contents of syringic acid, protocatechuic acid, epicatechin, chlorogenic acid, *p*-coumaric acid, gallic acid, oleanolic acid and ursolic acid.

Contents of oleanolic acid and ursolic acid were higher than contents of other seven selected compounds, and contents of oleanolic acid and ursolic acid were detected to control the quality of dry CS material in Pharmacopoeia of the People's Republic of China (2015 edition). For both oleanolic acid and ursolic acid, dry fresh CS always contained more oleanolic acid and ursolic acid than dry boiled CS, and freeze dried fresh CS almost contained the least oleanolic acid and ursolic acid in the fresh CS group, while freeze dried boiled CS contained the most oleanolic acid and ursolic acid in the boiled CS group. It may demonstrate that boiling process would decrease contents of both

Table 2 Contents of nine selected monomeric compounds in different CS samples

Drying methods		Oleanolic acid (mg per g dry weight)	Ursolic acid (mg per g dry weight)	Gallic acid (μg per g dry weight)	Protocatechuic acid (μg per g dry weight)	Chlorogenic acid (μg per g dry weight)	Syringic acid (μg per g dry weight)	<i>p</i> -Coumaric acid (μg per g dry weight)	Catechin (μg per g dry weight)	Epicatechin (μg per g dry weight)
Vacuum drying	60 °C fresh	16.02±0.06 ^k	11.90±1.87 ^h	63.43±0.14 ^k	319.98±0.28 ^{b,c}	98.51±0.22 ^{b,c}	2651.92±15.75 ^c	36.51±0.73 ^{d,e}	3903.64±6.02 ^a	158.39±0.33 ^c
	Boiled	6.06±0.06 ^b	6.42±0.03 ^d	9.12±0.00 ^b	250.90±4.41 ^{b,c}	83.92±1.90 ^{a,b}	2143.15±3.02 ^b	27.10±0.21 ^c	4276.68±37.01 ^b	158.16±3.60 ^c
	80 °C Fresh	9.31±0.39 ^j	7.39±0.32 ^e	50.33±0.41 ^j	1046.51±1.59 ^f	64.48±0.05 ^a	2060.03±127.49 ^b	11.09±2.37 ^a	4420.30±9.37 ^b	78.05±0.49 ^a
	Boiled	2.37±0.01 ^c	2.52±0.03 ^a	14.84±0.09 ^c	884.16±18.77 ^e	134.93±13.47 ^d	2293.49±63.86 ^{b,c}	18.18±0.06 ^b	5352.09±69.95 ^d	205.30±8.31 ^d
Sun drying	Fresh	11.86±0.05 ⁱ	10.92±0.01 ^g	44.62±0.26 ⁱ	253.09±0.14 ^c	288.83±0.87 ^g	5794.11±7.52 ^{h,i}	47.83±0.36 ^g	11384.38±0.86 ^j	1018.56±3.19 ^m
	Boiled	7.41±0.11 ^b	8.19±0.01 ^f	9.10±0.59 ^b	580.69±0.21 ^d	315.55±2.54 ^h	3071.38±227.34 ^d	28.15±0.39 ^c	6406.08±158.26 ^e	193.30±2.39 ^d
Infrared drying	Fresh	15.23±0.04 ^e	13.33±0.06 ⁱ	26.17±0.13 ^e	2367.43±2.02 ^g	70.26±1.02 ^a	3073.75±10.15 ^d	41.38±0.35 ^{e,f}	4882.83±8.29 ^c	104.78±7.14 ^b
	Boiled	3.34±0.21 ^a	3.54±0.13 ^b	5.17±0.01 ^a	252.31±0.14 ^{b,c}	378.27±16.85 ⁱ	3302.85±9.51 ^d	40.90±9.54 ^{e,f}	7416.11±418.46 ^f	432.74±16.11 ⁱ
Freeze drying	Fresh	9.77±0.12 ^j	10.36±0.09 ^g	66.49±0.42 ^j	219.87±0.76 ^b	772.04±13.26 ^k	6032.57±24.61 ⁱ	23.51±0.89 ^c	9923.01±8.78 ⁱ	914.27±1.67 ^l
	Boiled	7.99±0.00 ^h	8.60±0.11 ^f	39.94±0.31 ^h	502.75±1.58 ^h	91.39±0.03 ^{a,b,c}	1725.96±0.65 ^a	24.70±0.04 ^c	4213.69±1.64 ^b	210.04±15.35 ^d
Hot air drying	40 °C Fresh	17.38±0.03 ^d	15.16±0.10 ^j	22.21±0.00 ^d	119.13±0.30 ^a	240.93±21.01 ^f	4902.88±13.99 ^g	45.74±0.17 ^{f,g}	9057.25±9.02 ^h	323.41±25.59 ^g
	Boiled	6.14±0.10 ^j	5.69±0.53 ^{c,d}	49.20±5.33 ^j	47.17±0.06 ^a	116.64±6.70 ^{c,d}	2623.45±351.69 ^c	38.69±0.07 ^{d,e}	5441.28±153.75 ^d	263.28±0.08 ^f
	60 °C Fresh	15.22±0.03 ^g	13.11±0.08 ⁱ	35.45±0.00 ^g	257.31±0.13 ^c	195.17±31.63 ^e	5665.69±579.76 ^h	51.03±0.13 ^g	9119.59±302.60 ^h	605.99±11.24 ^j
	Boiled	5.41±0.10 ^f	4.97±0.02 ^c	28.93±0.30 ^f	264.98±0.11 ^{b,c}	423.65±9.14 ^j	4393.11±47.41 ^f	39.88±0.01 ^{d,e}	6619.89±43.96 ^e	436.64±7.83 ⁱ
80 °C	Fresh	12.01±0.06 ⁱ	12.11±0.05 ^h	43.72±0.08 ⁱ	374.36±1.53 ^c	358.13±4.00 ^j	4018.41±22.11 ^e	47.09±1.92 ^g	8885.05±9.32 ^h	392.97±0.47 ^h
	Boiled	3.76±0.09 ^k	3.71±0.12 ^b	63.90±0.25 ^k	292.45±0.11 ^{b,c}	116.70±1.11 ^{c,d}	2647.42±19.25 ^c	34.72±0.42 ^d	5346.42±19.25 ^d	239.24±3.49 ^e

The results were given as mean values ± standard deviation (n = 3)

Different small superscripts (a–m) denoted the significant difference ($P < 0.05$) of the varieties of extracts

Fig. 1 Absorbance (a), pH values (b), DPPH scavenging capacity (c) and total ferric reducing antioxidant power (d) of fresh and boiled CS dried by different drying method

oleanolic acid and ursolic acid in fresh CS, while vacuum drying, sun drying, infrared drying and hot air drying contributed to the increase of oleanolic acid and ursolic acid in fresh CS.

For phenolics, freeze dried fresh CS showed the highest chlorogenic acid and syringic acid. Compared with fresh CS, contents of gallic acid, chlorogenic acid, syringic acid and *p*-coumaric acid decreased in boiled CS slices at different extent when they were dried by freeze drying, while protocatechuic acid increased in boiled CS. Therefore, boiling process decreased gallic acid, chlorogenic acid, syringic acid and *p*-coumaric acid except protocatechuic acid. But, unfortunately, drying methods didn't show same effects on the content of any one compound together with boiling process, and the same drying method didn't show same effects on the contents of these five selected compounds.

Catechin and epicatechin contents in CS were reduced after boiled. When dried by sun drying, fresh CS was found to contain the highest catechin and epicatechin, which were higher than freeze dried fresh CS. When dried by vacuum drying at 60 and 80 °C, fresh CS contained higher catechin and epicatechin than boiled CS. It was also found that infrared dried boiled CS owned the highest catechin and epicatechin, and freeze dried boiled CS had the lowest content of catechin. Hence, boiling process can reduce contents of catechin and epicatechin in CS, and sun drying

method was helpful to improve contents of catechin and epicatechin in fresh CS. Especially, vacuum drying, sun drying, infrared drying and hot air drying would contribute to higher catechin.

Absorbance at 420 nm

During the drying process, the color of CS changed into red and brown or even aubergine, for the brown stain. Both enzymatic browning and non-enzymatic browning appeared in CS slices during drying process, while at the early stage of drying process only enzymatic browning exists, and at the later stage of drying process non-enzymatic browning would occurred after the enzyme became inactive, for boiled CS only non-enzymatic browning occurred during the whole drying process.

Absorbance at 420 nm always was used to express the extent of color change of samples (Lou et al. 2015). As the results showed in Fig. 1a, absorbance at 420 nm of fresh CS ranged from 0.85 ± 0.02 to 1.48 ± 0.07 , while that of boiled CS ranged from 0.57 ± 0.02 to 0.99 ± 0.04 . Compared with fresh CS, color of boiled CS became more stable, and absorbances of all boiled samples became lower except boiled CS dried by hot air at 60 and 80 °C. Fresh CS dried by vacuum drying method at 80 °C showed the highest absorbance at 420 nm, and showed significant difference at $P < 0.05$ from fresh CS dried by other methods. When

dried by vacuum drying at 60 °C the absorbance of fresh CS at 420 nm was reduced, while dried by hot air fresh sample dried at 60 °C showed higher absorbance than samples dried at 40 and 80 °C.

Hot water extracts from CS dried by different drying methods showed different colors and absorbance values at 420 nm. Thus, changes in absorbance at 420 nm of hot water extracts from fresh and boiled CS after drying were investigated. In general, boiling process always was known as a good method to stop the enzymatic browning by killing enzymes. Here, boiling process only showed significant effect on the browning of CS dried by vacuum drying, infrared drying and freeze drying. Especially, colors and absorbance values at 420 nm of fresh and boiled CS slices dried by for sun drying didn't show significant difference. Thus, boiling process didn't show significant effect on sun drying CS slices. Sun drying is a clear and common but very time-consuming dry method, and it is widely used in the produce of dry CS slices by growers, while color also is an important standard to evaluate the quality of CS slices. Therefore, from this point of view, when CS slices were processed, boiling process would seem to be unnecessary.

However, in the fresh group and boiled group, freeze drying CS showed the lightest color and the lowest absorbance at 420 nm, while CS dried by hot air at 60 °C showed darker color than freeze dried CS and almost the highest absorbance at 420 nm. Therefore, both boiling process and drying methods would affect absorbance values of CS samples (Lou et al. 2015), although boiling process didn't show significant effects on their browning for CS dried by hot air drying at 60 °C. According to the report of Saliha and Leila (2016), there were many factors affected the process of enzymatic browning and non-enzymatic browning, such as temperature, pH and exist of ascorbic acid, sodium metabisulfite and cysteine.

pH values determined

As the results showed in Fig. 1b, both fresh and boiled CS dried by eight different drying methods have low pH values ranged from 3.0 to 3.5, and fresh and boiled CS dried by freeze drying showed the lowest pH values among dried fresh and boiled CS, respectively, while both fresh and boiled CS dried by hot air drying at 60 °C showed the highest pH values in their group, respectively. For fresh CS, drying methods didn't show significant effects on pH values, while drying methods showed significant difference on pH values in some extent for boiled CS. Values of pH ranged from 3.04 ± 0.02 to 3.27 ± 0.01 and from 3.05 ± 0.02 to 3.13 ± 0.00 for dried fresh and boiled CS, respectively, and pH value became lower after boiled only for freeze drying CS.

Therefore, vacuum drying, sun drying, infrared drying and hot air drying decreased pH values of CS at a large extent, although boiling process decreased pH value slightly. This might be all due to the degradation of acidic compounds in the CS slices during these drying processes (Guehi et al. 2010). This result also was more or less consistent with the result of Sandra and Chong (2013).

Antioxidant activity

The results of DPPH assay and FRAP are showed in Fig. 1c, d, respectively. DPPH assay showed a significantly difference at $P < 0.05$, while values of FRAP method didn't show a significant difference at $P < 0.05$. Generally, low DPPH EC_{50} value reveals high DPPH free radical scavenging activity and antioxidant activity, while high FRAP values would correspond to good antioxidant activity.

For all samples determined, EC_{50} values of DPPH free radical ranged from 0.92 ± 0.02 to 6.31 ± 0.70 mg dried sample/mL for fresh CS, while ranged from 2.96 ± 0.22 to 22.05 ± 4.86 mg dried sample/mL for boiled CS. However, FRAP values ranged from 3.24 ± 0.08 to 3.61 ± 0.16 mg vitamin C equivalent/mL for fresh CS, while ranged from 3.25 ± 0.13 to 3.85 ± 0.13 mg vitamin C equivalent/mL for boiled CS. The lowest EC_{50} value of DPPH was found in fresh CS, and EC_{50} values of fresh CS became lower after the boiling process only when dried by hot air at 60 and 80 °C. This is to say boiling would cause the decrease of antioxidant activity for most CS, although boiling process didn't show obvious effect on FRAP values of CS. This result, which seems same with each other, may attribute to the totally different mechanism of these two methods (Oyaizu, 1986; Shimada et al. 1992).

Correlation and principal component analysis (PCA)

Correlation analysis was used to explain the relationship among chemical composition, absorbance, pH and antioxidant activity. As the results showed, gallic acid correlated with DPPH $1/EC_{50}$ value (Pear's correlation coefficient is 0.318, $P < 0.05$), while TFC, oleanolic acid, ursolic acid, catechin, syringic acid, epicatechin, *p*-coumaric acid and pH all correlated with DPPH $1/EC_{50}$ value (Pear's correlation coefficient are 0.842, 0.413, 0.409, 0.568, 0.474, 0.622, 0.410 and 0.497, respectively, $P < 0.01$). It indicates that total flavonoids, oleanolic acid, ursolic acid, catechin, syringic acid, epicatechin, *p*-coumaric acid and pH all contribute more to DPPH free radical scavenging capacity than gallic acid, while total phenolics, total triterpenes, protocatechuic acid, chlorogenic acid and absorbance at 420 nm all didn't contribute to DPPH free radical

scavenging capacity. Chlorogenic acid was correlated with FRAP positively (Pear's correlation coefficient is 0.353, $P < 0.05$), while ursolic acid correlated with FRAP negatively (Pear's correlation coefficient is -0.304 , $P < 0.05$). This may imply that chlorogenic acid contributes to FRAP, and FRAP would increase as the increase of chlorogenic acid content.

However, TPc, gallic acid and protocatechuic acid correlated with absorbance at 420 nm significantly at $P < 0.001$ ($r = 0.396$, 0.375 and -0.386 , respectively). Therefore, TPc contributed more to absorbance at 420 nm than protocatechuic acid and gallic acid. Perhaps protocatechuic acid participates in the browning reaction of CS, and is consumed by the browning reaction. But content of protocatechuic acid in fresh CS dried by infrared drying and boiled CS dried by hot air drying at $60\text{ }^{\circ}\text{C}$ were the highest in respective group, while their absorbance values at 420 nm were not the highest. This phenomenon may due to the complex reaction in CS slices and effects of the way dealing with them.

Oleanolic acid, ursolic acid, syringic acid and *p*-coumaric acid showed significant correlation with pH values at $P < 0.01$ ($r = 0.690$, 0.598 , 0.370 and 0.564 , respectively), while TTc, catechin and absorbance at 420 nm correlated with pH significantly at $P < 0.05$ ($r = -0.333$, 0.305 and 0.335 , respectively). Low value of pH means high acidity, and it showed positive correlation with TTc, so more total triterpenes would help to the rise of acidity. This might all due to the degradation of triterpenoid acids in the CS slices during these drying processes (Guehi et al. 2010), although there are no reports about the decomposition of triterpenoid acids in details. Correlation between pH values and absorbance at 420 nm also show pH can affect absorbance at 420 nm, this may because low pH can inhibit enzymatic browning (Saliha and Leila 2016).

By PCA, all data were simplified into four principle components, they can explain 79.80% of the total variance, and the grade of dried fresh and boiled CS calculated by PCA is showed in Fig. 2a, it was obviously that sun drying fresh CS got the maximum score for the prominent distinctly advantage over others, followed by freeze drying boiled CS and fresh CS, and then fresh CS dried by hot air drying at $60\text{ }^{\circ}\text{C}$, while some fresh and boiled CS got negative scores. Thus, boiling process showed a significant effect on scores of CS. It is clear that for fresh CS, its top three drying methods are sun drying, freeze drying and hot air drying at $60\text{ }^{\circ}\text{C}$, while the best drying method of boiled CS is freeze drying, and boiled CS dried by other methods got far lower scores than freeze dried CS.

As we all know, sun drying is a traditional, environment friendly and energy saving but time-consuming drying method, while freeze drying is a costly drying method and hot air drying at $60\text{ }^{\circ}\text{C}$ is a time-saving, energy-saving and easy-operating drying method. Thus, sun drying, freeze drying and hot air drying at $60\text{ }^{\circ}\text{C}$ all are good drying methods in the industrial drying process of fresh CS, while freeze drying is good drying method in the process of boiled CS. Generally, hot air drying at $60\text{ }^{\circ}\text{C}$ can be used to dry large batches of CS with low cost. Actually, fresh CS would be more suitable for industrial drying than boiled CS, especially fresh CS dried by hot air drying at $60\text{ }^{\circ}\text{C}$ will be more suitable for the industrial production of dry CS than other drying methods, and boiling process seems to be unnecessary for now. Therefore, it will be more reasonable method for CS to cut into slices directly without boiling, order of drying methods could be used are sun drying, freeze drying and hot air drying at $60\text{ }^{\circ}\text{C}$.

Cluster analysis

In this part, all dried CS were analyzed by cluster analysis. As the results showed in Fig. 2b, all samples were divided into six clusters. All dried boiled CS were separated into two clusters, and only freeze dried boiled CS was divided into a single cluster, while other dried boiled CS composed of one cluster. All dried fresh CS were divided into four clusters, three hot air drying samples and samples dried by infrared drying and vacuum drying at $80\text{ }^{\circ}\text{C}$ composed first cluster, while other three samples dried by sun drying, vacuum drying at $60\text{ }^{\circ}\text{C}$ and freeze drying were divided into other three clusters, respectively.

Overall, all dried boiled CS were isolated from dried fresh CS as a main cluster. However, this result also demonstrated that boiling process had a significant effect on quality of CS, and for both fresh and boiled CS, freeze drying can be considered as a special and potential drying method.

Conclusion

The effects of drying methods and boiling process on chemical composition and antioxidant activity of CS were evaluated. Sun drying, freeze drying and hot air drying at $60\text{ }^{\circ}\text{C}$ were the main three drying methods of fresh CS while freeze drying was the most appropriate drying method for boiled CS. The results revealed that the boiling

Fig. 2 Scores histogram (a) and Cluster analysis of fresh and boiled CS dried by different drying methods (b). Notes BH40 for boiled CS dried by hot air drying at 40 °C, BH60 for boiled CS dried by hot air drying at 60 °C, BH80 for boiled CS dried by hot air drying at 80 °C, BV60 for boiled CS dried by vacuum drying at 60 °C, BV80 for boiled CS dried by vacuum drying at 80 °C, BSD for boiled CS dried by sun drying, BID for boiled CS dried by infrared drying, BFD for

boiled CS dried by freeze drying, FH40 for fresh CS dried by hot air drying at 40 °C, FH60 for fresh CS dried by hot air drying at 60 °C, FH80 for fresh CS dried by hot air drying at 80 °C, FV60 for fresh CS dried by vacuum drying at 60 °C, FV80 for fresh CS dried by vacuum drying at 80 °C, FSD for fresh CS dried by sun drying, FID for fresh CS dried by infrared drying, FFD for fresh CS dried by freeze drying

was not required during the drying of CS. Results of this study supported sun drying of fresh CS, which is widely used these days.

Changes in color, pH and antioxidant activity were due to change in chemical composition of CS, however detail studies on CS are limited.

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