JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Comparison of Flavonoids, Phenolic Acids, and Antioxidant Activity of Explosion-Puffed and Sun-Dried Jujubes (*Ziziphus jujuba* Mill.)

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Supporting Information

ABSTRACT: The goal of the present study was to investigate the effect of explosion puffing and sun-drying on individual phenolic acids in four forms (free, esters, glycosides, and insoluble-bound), flavonoids, total phenolic content (TPC), and their antioxidant activity on jujube samples. Phenolic compounds were identified and quantified using high-performance liquid chromatography. Antioxidant capacity of jujube samples was evaluated by 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activity and total reducing power. The results showed that all samples significantly differed in their phenolic contents, phenolic acid and flavonoid composition, and antioxidant activities. The explosion-puffed jujubes had the highest total gallic, *p*-hydroxybenzoic, vanillic, *p*-coumaric, ferulic acids, and rutin contents. Also, explosion-puffed jujubes contained a higher level of total phenolics and antioxidant activity than their counterparts. Among phenolic acid fractions in four forms, each form of phenolic acids in explosion-puffed jujubes had the most abundant content, followed by fresh and sun-dried jujubes. The glycosided and insoluble-bound phenolic acid fractions for each sample represented the highest TPC and the strongest antioxidant activity. The results indicated explosion puffing was a good choice for jujube processing.

KEYWORDS: *fresh jujube, phenolic acids, flavonoids, explosion puffing, sun-drying*

INTRODUCTION

Jujube (*Ziziphus jujuba* Mill.) which belongs to the thorny rhamnaceous plant class, is indigenous to China with a history of over 4000 years. It is widely distributed in the subtropical and tropical regions of Asia and America.[1] Jujube is known for high nutritional and functional values, as it is high in trace minerals, proteins, sugars, organic acids, vitamin C, phenolics, and polysaccharides.[2] Jujubes are consumed in various types of foods through diverse processing methods such as conventional drying, fermentation, frying, boiling, and steaming.[3] In addition to food uses, it has been commonly used traditional Chinese medicine for its analeptic, palliative, and antibechic effects.[4]

Fresh jujubes deteriorate easily within a few days after harvest, and processing its products is necessary. The most commonly and widely used process for jujube is drying, which can conserve their desirable qualities and extend their shelf life. Different drying methods, including sun-, freeze-, microwave-, and oven-drying have been reported for jujube processing.[5] Among them, sun-drying is the most common for the production of dehydrated products and can ensure proper preservation. However, since the product is exposed to direct solar irradiation, and the drying parameters cannot be controlled, the quality of the product is not excellent. Specifically, sun-drying is, therefore, not homogeneous, and the final product is caramelized and crusted. Direct exposure to the sun also destroys the color, vitamins, and flavor of the products.[6] Compared with other drying methods, freeze-dried jujubes had significant gains in the total phenolic content (TPC) but exhibited minimal effect on antioxidant activity. However, because of its high operation cost, freeze-drying is restricted to high-value production.[5]

Explosion puffing is a fast and inexpensive method for drying of fruits and vegetables, according to the long-term investigation by the Eastern Regional Research Center.[7] The explosion puffing process is carried out in a continuous explosion puffing system (CEPS) at elevated pressure and in a superheated steam, which rapidly brings the water within the dried pieces to a temperature above its atmospheric boiling point. When the pieces are immediately recovered to atmospheric pressure, the water of the pieces flashes into steam, forming a porous structure. After being puffed, the sample pieces are dried by conventional means to 3% moisture or less.[8,9] According to the literature,[10] potatoes, carrots, and apples have been successfully processed in CEPS. The favorable characteristics of the explosion-puffed product are found in flavor, color, fast rehydration, ambient temperature storage, minimal storage and transportation costs, and durability.[10] Mixing the puffed products with different flavors and marketing them in moisture-impermeable plastic film pouches provides enormous opportunities for increasing acceptance and usage of puffed products; the consumer preferences for puffing foods are mainly due to their convenience, attractive appearance, and unique flavor.[11]

Although explosion puffing is an excellent drying method, there is no literature concerning the contents of phenolic acids in free, esterified, glycosided, and insoluble-bound forms, flavonoids, and their antioxidant activity in explosion-puffed, sun-dried, and fresh fruits. To achieve a better development of the explosion puffing technology and an increased consumption

Received: April 20, 2013
Revised: September 10, 2013
Accepted: September 16, 2013
Published: September 16, 2013

of puffed products, it is critical to understand health-promoting components and their concentrations in food processing on the availability of these beneficial constituents. Therefore, the contents of the important bioactive compounds and antioxidant activity of fresh and properly sun-dried and explosion-puffed jujubes were determined and compared.

MATERIALS AND METHODS

Chemicals. The following standards were used for the quantification of phenolic compounds. Catechin, epicatechin, rutin, quercetin, gallic, vanillic, protocatechuic, *p*-hydroxybenzoic, chlorogenic, caffeic, *p*-coumaric, ferulic, cinnamic, and ellagic acids were obtained from Sigma (St. Louis, MO). Water for the mobile phase was twice distilled and purified with the Milli-Q system (Millipore, Bedford, MA). 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2-azinobis-(3-ethylbenzthiazoline-6-sulfonate) (ABTS), and Folin–Ciocalteu reagent were purchased from Sigma (Germany). The mobile phases were HPLC-grade methanol, and all other reagents used were of analytical grade.

Plant Material and Experimental Design. A local cultivar of *Z. jujube* Mill., called "pear-jujube" is grown at the loess plateau experimental orchard of Northwest A&F University, Shaanxi, China (110°17′ E, 37°36′ N and 1049 m above mean sea level). All trees were managed according to integrated cultivation protocols. The ripe jujube fruits (as judged by experienced horticulturists based on color, flavor, and structure of the fruits) were picked by hand on September 25, 2012, immediately transferred to our laboratory room, and thenstored at 0–4 °C. The uniform shape jujubes free from visible blemishes or diseases were selected for subsequent drying.

Two methods of dehydration were used: (a) explosion puffing treatment and (b) sun-drying treatment. For (a) explosion puffing, the fresh jujubes were mechanically washed, stoned, and sliced. They were immediately dipped in water containing a small amount of citric acid (0.2%) and then drained. The slices were uniformly layered in the moving wire mesh of a predrying machine (ZGW100, Chuanyang Co., Ltd., Shandong, China) to reach predetermined moisture content (approximately 30%). The samples were tightly wrapped in polyethylene films and placed into a thermostatically controlled chamber at 25 °C for 24 h to equilibrate the water and temperature profiles. First, the chamber was heated by means of superheated steam to a wall temperature of approximately 90 °C, and the pressure of chamber reached 0.2 MPa by air compressor before opening the vacuum pump. After 20 min, an abrupt pressure drop toward a vacuum was carried out by opening the snuffle valve. This sudden pressure drop caused autovaporization of superheated liquid contained in the material, and then samples were immediately puffed. Cooling water was promptly released to steam pipeline and its temperature decreased to 80 °C. Then the chamber was vacuumized for 2.5 h (about −0.098 MPa) and the cooling water was injected until desired conditions of temperature (20–25 °C) was reached. Temperature was maintained 5–10 min after the snuffle valve closed. Puffing samples were taken out of the chamber by means of opening the ventilation valve until inner pressure reached atmosphere pressure. After being puffed, the final moisture content of jujube samples approaches 6%. The process was achieved by closed explosion puffing equipment (QDPH10, Qinde New Material Co., Ltd., Tianjin, China).

For the sun-drying treatment (b), fresh jujubes were immediately exposed to the sun at a height of 1 m from the ground surface in the daytime and placed indoors at night for 18 days to reach a moisture content of 22%.[5]

Fractionation of Free and Bound Phenolic Acids. Phenolic acids were isolated from the extract according to the methods of Xu et al. and Wang et al.[12,13] A total of 10 g of fresh jujube fruits or 5 g of dried jujube fruits were extracted with 100 or 50 mL of 80% aqueous methanol in a cooled ultrasonic bath for 30 min. The supernatant was separated, and the residue was re-extracted by repeating the above steps under the same conditions. The two filtrates were combined and centrifuged at 2191*g* for 15 min. The combined supernatants were then evaporated under vacuum at 35 °C to about 25 mL and were analyzed for free phenolic acids, soluble phenolic acid glycosides, and phenolic acid esters; their residues were reserved for the determination of methanol-insoluble ester-bound phenolic acids. The aqueous suspension was acidified to pH 2 using 6 M HCl, and it was extracted five times with diethyl ether-ethyl acetate (1:1) (v/v) at a solvent to water phase ratio of 1:1 to obtain the free phenolic acids. The aqueous phase was treated by alkaline hydrolysis (4 M NaOH containing 10 mM EDTA and 1% ascorbic acid) under nitrogen for 4 h at room temperature. After acidification to pH 2 with 6 M HCl, phenolic acids released from soluble ester were extracted from the hydrolysate five times with diethyl ether-ethyl acetate (1:1) (v/v) at a solvent to water phase ratio of 1:1. Following this, the aqueous phase was hydrolyzed with 5 mL of 6 M HCl for 30 min at 85 °C under nitrogen. Phenolic acids released from soluble glycosides were separated from the hydrolysate five times as described above. The residues from the 80% methanol extractions were hydrolyzed directly with 8 mL of 4 M NaOH (containing 10 mM EDTA and 1% ascorbic acid) under the same conditions as those for the ester. After acidification to pH 2 using 6 M HCl, phenolic acids released from methanol-insoluble ester-bound phenolic acids were extracted from the hydrolysate five times as described above, and each sample was treated in triplicate. Each of the phenolic acid fractions was obtained as described above, and each was dehydrated with anhydrous sodium sulfate, filtered, and evaporated to dryness under vacuum at 35 °C. The dry residues were dissolved into 5 mL of 80% (v/v) aqueous methanol.

Characterization and Quantification of Individual Phenolic Compounds. A total of 10 g of fresh jujube fruits or 5 g of dried jujube fruits were extracted with 100 or 50 mL of 80% aqueous methanol in a cooled ultrasonic bath for 30 min. The supernatant was separated, and the residue was re-extracted by repeating the above steps under the same conditions. The two filtrates were combined and centrifuged at 2191*g* for 15 min. The combined supernatants were evaporated to dryness by rotary evaporator (R-210, Buchi Labortechnik AG, Switzerland) to obtain flavonoid crude extract. The extraction of fruit samples for the measurement of individual phenolic acid was made according to same protocol as for fractionation of free and bound phenolic acids. They were transferred to a vial prior to being injected into a HPLC system. According to Gao et al.,[5] HPLC analysis of samples was carried out on a Waters HPLC system with a UV–vis detector (2487) at 280 nm. An Atlantis T3 column (4.6 × 150 mm, 3 μm) (Waters Corp, Milford, MA) operated at 30 °C. The injection volume was 10 μL, and the flow rate was maintained at 0.8 mL/min, The elution solvents were methanol (A) and ultrapure water (pH = 2.6) (B). The gradient program was as follows: 0–15 min, 15% A; 15–25 min, 15–25% A; 25–65 min, 25–75% A; 65–70 min, 75–15% A. The identification of phenolic compounds was accomplished by comparing retention times and spectra of chromatographic peaks in the extraction solution of samples to those of the standard compounds. The concentration of each phenolic compound was expressed as microgram per gram dry weight (μg/g DW) and used for comparing the results that came from different treatments. See Supporting Information Figure 1.

Determination of the Total Phenolic Content (TPC). The TPC of each extract was determined using the method described by Gao et al.[14] Briefly, the diluted extract or control (125 μL) was mixed with 500 μL of distilled water followed by addition of Folin–Ciocalteu reagent (125 μL). After 6 min at room temperature, 7% aqueous sodium carbonate (1.25 mL) and distilled water (1 mL) were added. The extracts were mixed and allowed to be reacted for 1.5 h in the dark before the absorbance at 760 nm was measured on a spectrophotometer (UV1240, Shimadzu, Japan). A mixture of water and reagents was used as a blank. The TPC was expressed as gallic acid equivalents (GAE) in milligrams per g of DW. Absorptions were measured in three replicates.

Determination of Antioxidant Activity. *ABTS Scavenging Activity.* Free radical scavenging capacity of the extracts was assessed against $ABTS^{\bullet+}$ generated according to a previously reported protocol.[15] In brief, 5 mmol aqueous solution of ABTS and manganese dioxide (1.5 g) were incubated at ambient temperature for 30 min in the dark to generate radical cation $ABTS^{\bullet+}$ and then removing the

Table 1. Flavonoid and Phenolic Acid (μg/g DW) Compositions in Fresh and Dried Fruit of Two Drying Methods[a]

phenolic compounds	fresh	sun-drying	explosion puffing
Gallic Acid			
free	4.58 ± 0.02b	1.26 ± 0.04b	38.42 ± 1.83a
esterified	nd[b]	3.43 ± 0.53b	12.56 ± 1.89a
glycosided	8.58 ± 0.13a	nd	7.45 ± 0.30b
insoluble-bound	1.99 ± 0.34a	1.64 ± 0.42a	2.46 ± 0.43a
total	15.15 ± 0.44b	6.33 ± 0.15c	60.88 ± 0.68a
Protocatechuic Acid			
free	26.12 ± 0.13a	nd	8.78 ± 0.02b
esterified	nd	nd	nd
glycosided	nd	nd	nd
insoluble-bound	nd	14.57 ± 0.25a	3.23 ± 0.28b
total	26.12 ± 0.13a	14.57 ± 0.25b	12.01 ± 0.30c
***p*-Hydroxybenzoic Acid**			
free	2.53 ± 0.01a	nd	2.00 ± 0.04b
esterified	nd	0.52 ± 0.10b	1.22 ± 0.11a
glycosided	nd	nd	32.19 ± 1.32a
insoluble-bound	nd	nd	nd
total	2.53 ± 0.01b	0.52 ± 0.10b	35.41 ± 1.16a
Chlorogenic Acid			
free	5.17 ± 0.19a	nd	0.57 ± 0.02b
esterified	3.76 ± 0.35a	nd	nd
glycosided	nd	nd	nd
insoluble-bound	nd	nd	nd
total	8.93 ± 0.16a	nd	0.57 ± 0.02b
Vanillic Acid			
free	1.31 ± 0.34a	nd	0.43 ± 0.06a
esterified	nd	nd	nd
glycosided	22.00 ± 0.63b	nd	32.19 ± 1.32a
insoluble-bound	0.64 ± 0.09b	nd	7.33 ± 0.28a
total	23.95 ± 1.05b	nd	39.95 ± 1.04a
Caffeic Acid			
free	nd	nd	2.19 ± 0.05a
esterified	1.48 ± 0.34a	nd	2.19 ± 0.34a

phenolic compounds	fresh	sun-drying	explosion puffing
Caffeic Acid			
glycosided	nd	59.38 ± 0.34a	nd
insoluble-bound	nd	nd	1.79 ± 0.20a
total	1.48 ± 0.34c	59.38 ± 0.34a	6.31 ± 0.14b
***p*-Coumaric Acid**			
free	0.71 ± 0.07b	5.37 ± 0.07a	nd
esterified	7.92 ± 0.47a	1.84 ± 0.07b	8.18 ± 0.40a
glycosided	nd	2.58 ± 0.15a	1.57 ± 0.10b
insoluble-bound	nd	14.57 ± 0.25b	52.05 ± 1.38a
total	8.62 ± 0.46c	24.36 ± 0.11b	61.80 ± 1.08a
Ferulic Acid			
free	0.99 ± 0.00a	0.75 ± 0.02c	0.91 ± 0.01b
esterified	1.45 ± 0.14b	0.17 ± 0.09b	11.16 ± 0.77a
glycosided	2.75 ± 0.30a	0.17 ± 0.08b	nd
insoluble-bound	nd	0.59 ± 0.23a	1.48 ± 0.22a
total	5.20 ± 0.45b	1.68 ± 0.41c	13.55 ± 0.99a
Ellagic Acid			
free	1.53 ± 0.01b	6.69 ± 0.04a	nd
esterified	7.29 ± 0.34a	0.51 ± 0.04b	nd
glycosided	25.09 ± 2.35a	0.88 ± 0.12b	nd
insoluble-bound	66.31 ± 1.43a	1.67 ± 0.16b	nd
total	100.23 ± 0.59a	9.74 ± 0.07b	nd
Cinnamic Acid			
free	0.37 ± 0.02b	nd	4.62 ± 0.17a
esterified	0.16 ± 0.03b	nd	0.91 ± 0.14a
glycosided	0.80 ± 0.02b	0.70 ± 0.02c	0.94 ± 0.04a
insoluble-bound	5.90 ± 0.02a	nd	0.17 ± 0.02b
total	7.22 ± 0.05a	0.70 ± 0.02c	6.64 ± 0.26a
Flavonoid			
epicatechin	49.26 ± 1.04a	nd	5.48 ± 0.03b
catechin	10.84 ± 0.09a	nd	2.04 ± 0.03b
quercetin	0.12 ± 0.01b	nd	0.30 ± 0.00a
rutin	13.75 ± 0.10b	4.33 ± 0.12c	15.54 ± 0.08a

[a]Mean ± SD; n = 3. Values in same row marked by the different letters are significantly different at $p < 0.05$. [b]nd = not detected.

existing manganese dioxide by filtration. $ABTS^{\bullet+}$ solution was diluted with phosphate buffer solution (pH 7.4) to an absorbance of 0.70 (±0.02) at 734 nm. Next, the $ABTS^{\bullet+}$ reaction solution containing 3.0 mL of $ABTS^{\bullet+}$ and 200 μL of extracts was measured for absorbance after 1 min of reaction time at 734 nm. Trolox was used to prepare the standard curve and the results were expressed as millimoles of Trolox equivalent per 100 g of DW.

Total Reducing Power Assay. Total reducing power of the extracts was determined according to the method of Ma.[15] The appropriate dilutions of extracts (1 mL) were mixed with 2.0 mL of phosphate buffer (0.2 mol/L, pH 6.6) and 2.0 mL of potassium ferricyanide (1%) and then incubated at 50 °C for 20 min. After rapid cooling, 2 mL of trichloroacetic acid (10%, w/v) was added to stop the reaction. Then 2 mL of reaction mixture was blended with 2 mL of distilled water and 0.4 mL of 0.1% ferric chloride, followed by reaction for 30 min in the dark, and measured at 700 nm. The reducing power was expressed as milligrams of vitamin C equivalent per 100 g of DW.

Statistical Analysis. The results were expressed as mean ± standard deviation (SD) for at least three replicates for each sample. One-way analysis of variance (ANOVA) was used to identify difference among means using SPSS 18.0 software. All of the statistical differences were carried out at a significance level of $\alpha = 0.05$.

RESULTS AND DISCUSSION

Phenolic Compounds. It is well-known that flavonoids possess high health-promoting activity by virtue of their antioxidant and chelating abilities.[16] Some epidemiological studies have also found that their consumption is associated with a reduced risk of cancer[17,18] and cardiovascular disease.[19,20] Previous studies have discovered four flavonoids (catechin, epicatechin, quercetin, and rutin) in dried and fresh jujubes.[21,22] The flavonoids of jujubes are presented in Table 1. Catechin, epicatechin, and quercetin were detected only in fresh and explosion-puffed jujubes. Interestingly, the amount of quercetin increased with the explosion puffing treatment by 59% compared to the fresh jujube fruits. It is known that a thermal-induced degradation of quercetin glycosides resulted in an increase of quercetin concentration.[23] The increase in the quercetin was also observed in the steam explosion of sumac fruits, which was interpreted by hydrolyzation and deglycosylation of quercitrin.[24] The results could be related with the fact that quercitrin is unstable at high temperature, while quercetin is even stable over 200 °C.[25] This could explain that the content of quercetin was significantly increased after explosion puffing in the current research ($p < 0.05$). The fresh samples had higher contents of catechin (10.84 μg/g DW) and epicatechin (49.26 μg/g DW) compared to explosion-puffed ones, in which their contents dramatically dropped to 2.04 and 5.48 μg/g DW, respectively. This confirms the negative effect of high temperature and oxygen concentrations during the food processing.[26] Some results obtained from previous research[27] indicated that 91% and 96% of catechin and epicatechin, respectively, were lost after sun-drying in Portuguese pear. Moreover, the sun-drying treatment caused a remarkable drop

in rutin content and only 31% of the rutin was retained in comparison to fresh jujube. The quercetin and rutin of sun-dried jujubes showed different behaviors, which are due to a higher stability of rutin and the presence of sugar in the rutin molecule.[25,28] But with explosion puffing, its concentration increased by 13%. According to Gao et al.,[5] all drying treatments (sun-, freeze-, microwave-, and oven-drying) reveal a sharp fall in the rutin content. However, the rutin shows a significantly higher value in the prunes by standard high temperature treatment,[26] which is in agreement with the result as the explosion puffing treatment increased the content of rutin.

Nine free phenolic acids identified in the fresh jujubes were gallic, protocatechuic, *p*-hydroxybenzoic, chlorogenic, vanillic, *p*-coumaric, ferulic, ellagic, and cinnamic acids, while eight free phenolic acids were determined in explosion-puffed samples. Only four phenolic acids in free forms were detected in sun-dried jujubes. Protocatechuic, chlorogenic, and gallic acids were the principal free phenolic acids in fresh jujube, and their contents were higher than the reported levels.[13] This difference may be due to agronomic practices, harvesting time, post-harvest conditions, arid climatic conditions in loess plateau of China, and high altitude of local area.[14] By comparison to fresh jujubes, the contents of protocatechuic and chlorogenic acids in puffed jujubes significantly dropped to 8.78 and 0.57 μg/g DW, respectively. In the current study, loss of 66% protocatechuic acid occurred during explosion puffing. However, it was not detected in sun-dried samples. Also, according to the research of other drying methods, about 94 and 98% of protocatechuic acid were lost by freeze-drying and oven-drying treatments.[5] The results indicated that explosion-puffed jujubes could retain acceptable levels of protocatechuic acid. In the case of chlorogenic acid, it disappeared during the drying process except for its free form in puffed jujubes. These results could be attributed to elevated pressure and temperature during explosion puffing. Chlorogenic acid was reported to be sensitive to high pressure and heat.[29] For example, the content of chlorogenic acid in potatoes was reduced by 46, 60, and 100% by heating after microwave cooking, boiling, and oven baking, respectively.[30] The loss of chlorogenic acid during sun-drying may be due to polyphenoloxidase activity, it has been described in vitro as one of the best PPO substrates, and this could explain the decrease.[31] It is also possible that the compound was decomposed to caffeic acids upon heating.[32] Indeed, our results also found that there was a vast increase in the subtotal of caffeic acid in dried fruits, supporting the notion that caffeic acid could be generated by deglycosylation of chlorogenic acid. The sun-dried jujubes contained lower amounts of gallic acid than fresh fruits. Interestingly, a significant increase ($p < 0.05$) in the content of gallic acid was noticed in explosion-puffed jujubes compared to fresh counterparts. A possible reason for an increase of gallic acid in jujubes may be due to degradation of gallotannins by explosion puffing, releasing free forms of gallic acid.[33] Zadernowski et al.[34] detected that European juneberries had the highest free gallic acid content (7.2 mg/kg DW) among several small berries grown in Northeastern Poland. Noteworthy, free gallic acid concentration of explosion-puffed jujubes was nearly 5-fold higher than those of European juneberries. In addition, free cinnamic acid was present with minor concentration in fresh jujubes. The ranges of free and insoluble-bound cinnamic acids were from 0.37 and 5.90 μg/g DW, and for explosion-puffed samples were from 4.62 and 0.17 μg/g DW. There was a significant increase and reduction ($p < 0.05$) in free and insoluble-bound phenolic acids, respectively, by explosion puffing. The increase of free cinnamic acid can be justified by the fact that explosion puffing treatment could liberate cinnamic acid from bound ester mainly associated with cell walls.

With regard to esterified phenolic acid, *p*-coumaric and ferulic acids in soluble ester were detected in all samples. It is interesting to stress that the contents of *p*-coumaric and ferulic acids in soluble ester forms were much higher in puffed jujubes (8.18 and 11.16 μg/g DW) than in fresh ones (7.92 and 1.45 μg/g DW). Gong et al.[35] also observed that the yields of ferulic and *p*-coumaric acids in conjugate forms sharply increased by steam explosion. The esterified ferulic acids were found to be the major compounds among the identified ferulic acids in four forms in explosion-puffed jujubes. It has been reported that ferulic acid sugar ester shows more suppressive effect on LDL oxidation than free ferulic acid, because it has the capacity to prolong the induction period in LDL peroxidation and to decrease the maximal rate and maximal concentration of LDL oxidation system.[36] Previous study suggested naturally occurring antioxidants such as ferulic acid had important influence on neurodegenerative disorders in which oxidative stress is implicated.[37] However, 77% and 88% of the *p*-coumaric and ferulic acids, respectively, in soluble ester were lost after sun-drying treatment. Similar to ferulic and *p*-coumaric acids, explosion puffing seems to increase the esterified cinnamic acid from 0.16 to 0.91 μg/g of DW. Although their amount was low in fresh and dried jujubes, cinnamic acids were more efficient than their benzoic counterparts in antioxidant activity.[38] The explosion-puffed jujubes had higher levels (2.19 μg/g DW) of esterified caffeic acid than fresh samples (1.48 μg/g DW); it was not detected in sun-dried jujubes. The esterified phenolic acids, which are highly suitable substrates for polyphenol oxidases, may have undergone degradation reactions.[39] It is reasonable to assume that esterified caffeic acid may be degraded during sun-drying treatment. Gallic and *p*-hydroxybenzoic acids in soluble ester were only detected in dried samples.

In terms of glycosided phenolic acids, the amount of vanillic acid significantly ($p < 0.05$) increased in explosion-puffed jujubes compared to fresh fruits, while gallic acid showed a remarkable decline ($p < 0.05$) in explosion-puffed jujubes. However, they were absent in sun-dried jujubes. This is explained by the fact that some conjugated polyphenolics such as lignin were degraded at high temperature to simple phenolics. The depolymerization and repolymerization of lignin could be originated from the formation of carbonium ion under acidic conditions, which might be suppressed under very mild conditions.[40] There is much evidence to indicate that the major degradation product obtained from lignin was identified to be vanillin acid.[41,42] In the present work, our results also proved that explosion puffing could effectively increase vanillic acid yield. *p*-Coumaric acid was only identified in dried samples, its content was as high as 2.58 μg/g DW in sun-dried jujubes and as low as 1.57 μg/g DW in explosion-puffed ones. The high amount of *p*-coumaric acid in our study was discrepant with the former report of different jujube tissues (peel, pulp, and seed) where it was not detected,[13] because the linkages between *p*-coumaric acid and lignin could be broken by high temperature and maturation enzymes such as glycosidase, during the drying process.[43] Glycosided ellagic acid was the second most abundant phenolic acid present in fresh jujubes, while sun-drying treatment resulted in a significant ($p < 0.05$) decrease in its content. A similar trend was observed in the esterified and insoluble-bound

ellagic acid. Differing from this result, the content of free ellagic acid was significantly ($p < 0.05$) increased by 77% in sun-dried jujubes. Previous study[44] also revealed that the content of free ellagic acid increased by 150% in strawberries and red raspberries during jam-cooking. This increase was related to a release of hexahydroxydiphenic acid from ellagitannins, which is transformed to ellagic acid. Processing has marked effects on the different forms of ellagic acid content of fruits that might affect their health-promoting properties. Accurate knowledge about ellagic acid content of fruits and the influence of processing on it is important in assessing the effects of ellagic acid on human health and disease.[44]

In the insoluble-bound phenolic acids fraction, the study on explosion-puffed jujubes showed that as a result of drying process gallic acid in insoluble-bound forms increased by 24%, whereas 18% of insoluble-bound gallic acid was lost after sun-drying treatment. Explosion-puffed jujubes contained higher levels of insoluble-bound vanillic acid which was around 11-fold higher than that in fresh fruits. Bound phenolic acids could be released by mechanical and chemical action resulting from the high pressure and explosion.[35] However, it was not identified in sun-dried jujubes. The results suggested that the distribution and contents of phenolic acids were changed after drying treatments, so it was necessary to adopt some proper processing method to maintain functional values. On the basis of former studies,[45,46] it is evident that processing methods have notable effects on the content of phenolic acid. *p*-Coumaric acid in insoluble-bound form was found only in dried samples. Statistically significant differences in the content of insoluble-bound *p*-coumaric acid between sun-drying and explosion puffing treatments were measured in the study. The minor caffeic acid in insoluble-bound form was detected only in explosion-puffed jujubes. Many analytical procedures have been employed for the determination of phenolic compounds for fresh and dried jujubes,[4,5] but those currently used for the determination of phenolics in extracts from jujubes only target major flavonoids and soluble phenolic acids. This makes the comparison of our results with those reported in the literature difficult. Moreover, the behavior of phenolic compound during drying treatment is not very clear, because its content can increase or decrease. This needs to be investigated further.

In all, explosion-puffed jujube had the highest total (free, esters, glycosides, and insoluble-bound forms) gallic, *p*-hydroxybenzoic, vanillic, caffeic, *p*-coumaric, ferulic acid, and rutin contents. It is considered to possess abundant functional values because of its high level of phenolic compounds, which exhibited antioxidant, anti-inflammatory, antiallergic, and anticarcinogenic activity.[47−49]

TPC and Antioxidant Activity. Table 2 summarizes the results of free, esterified, glycosided, and insoluble-bound forms TPC (mg of GAE/g of DW). With explosion puffing, the free, esterified, glycosided, and insoluble-bound forms TPC of jujubes were 6.93, 6.74, 18.91, and 21.35 mg of GAE/g of DW with significant increases ($p < 0.05$) of 279, 170, 380, and 402%, respectively. Whereas the contents of sun-dried jujubes were 1.26, 0.89, 2.74, and 2.89 mg of GAE/g of DW with decreases ($p < 0.05$) of 31, 64, 30, and 32%, respectively. One likely reason for this result is that the effect of sudden pressure drop causes part of the water to vaporize, inducing an expanded and porous structure, which is beneficial to solvent access and resulted in higher extraction contents in explosion-puffed jujubes. Indeed, it has been confirmed that puffed red ginseng showed higher extraction yields (41.1−48.2 g/100 g) than nonpuffed ones (29.7−45.7 g/100 g).[50] Furthermore, explosion puffing could inactivate polyphenol oxidases present in jujube fruits and avoid degradation of some polyphenols. In addition, it seems that part of the lignin was dissolved as low molecular phenolic compounds by the hydrolysis reaction with high temperature and pressure steam.[35] It is thought that puffing treatment is an effective pretreatment for extraction and separation of antioxidant compounds.[51] Gong et al.[35] also reported that the total free phenolics and total soluble conjugates increased from 98.9 to 664.1 and 73.2 to 208.2 GAE mg/g DW, respectively, after steam explosion, and similar results have been observed by Conde et al.[40] Moreover, Dewanto et al.[52] also found thermal processing may release more bound phenolic acids from the breakdown of cellular constituents, although disruption of cell walls may also lead to the release of the oxidative and hydrolytic enzymes that would destroy the antioxidants in fruits and vegetables; thermal treatment would deactivate these enzymes and prevent the loss of phenolic compounds. There could be a few explanations for the decrease in TPC of sun-dried jujubes. The loss of TPC after sun-drying may be caused by the enzymatic processes that occurred during the process. Some degradative enzymes such as polyphenol oxidases were not immediately deactivated, therefore, they are able to degrade phenolic compounds before the jujube fruits are completely dry. On the other hand, the climatic factors also resulted in an uneven loss of the TPC.[53] Many studies have also reported that TPC of plant samples was reduced after drying treatments.[5,53,54] Notably, about 85%, 87%, and 84% of the total phenolics presented in fresh, explosion-puffed, and sun-dried jujubes, respectively, were in bound forms. It is difficult to digest and may survive gastrointestinal digestion to reach the colon, providing site-specific health benefits.[52,55]

The contents of esterified phenolics were significantly higher than those of free phenolics in fresh jujubes ($p < 0.05$). In contrast, the levels of esterified phenolics were lower than free phenolics in dried samples. Free phenolic acids comprised 13% and 16% of the total phenolic acids present in explosion-puffed and sun-dried jujubes, while the corresponding esterified phenolic acids constituted 12% and 11%, respectively. The results showed that drying treatments could increase the proportion of free phenolic acids in jujubes due to the release of esterified phenolic acids. It has been described that steam explosion may effectively hydrolyze the ester and ether bonds among phenolic compounds, lignin, and carbohydrate.[35,51] A significant increase in the content of total free phenolics was also reported for sweet corn following thermal treatment with both increased heating time and temperature, which probably is attributed to the release of esterified and insoluble-bound phenolics.[52] In this sense, the results also demonstrated that the percentage of free phenolic acids in total phenolics sharply

Table 2. Total Phenolic Contents of Fresh Fruit and Dried Fruit of Two Drying Methods[a]

	total phenolic contents (mg of GAE/g of DW)		
phenolic acid fraction	fresh	sun-drying	explosion puffing
free	1.83 ± 0.07b	1.26 ± 0.09b	6.93 ± 0.36a
esterified	2.50 ± 0.08b	0.89 ± 0.04c	6.74 ± 0.03a
glycosided	3.94 ± 0.05b	2.74 ± 0.05c	18.91 ± 0.03a
insoluble-bound	4.25 ± 0.09b	2.89 ± 0.10c	21.35 ± 0.39a
total phenol content	12.52 ± 0.12b	7.79 ± 0.10c	53.66 ± 0.68a

[a]Mean ± SD; $n = 3$. Values in same row marked by the different letters are significantly different at $p < 0.05$.

Table 3. Antioxidant Activity of Fresh and Dried Fruit of Two Drying Methods[a]

	reducing power (mg of VC equiv/100g of DW)			ABTS (mmol of Trolox equiv/100g of DW)		
phenolic acid fraction	fresh	sun-drying	explosion puffing	fresh	sun-drying	explosion puffing
free	63.31 ± 0.25b	63.25 ± 1.53b	133.01 ± 3.48a	2.13 ± 0.02b	0.93 ± 0.05c	2.88 ± 0.05a
esterified	73.71 ± 5.77a	52.81 ± 2.64b	81.00 ± 0.82a	2.00 ± 0.08a	0.33 ± 0.01b	2.10 ± 0.09a
glycosided	144.25 ± 1.49a	125.35 ± 7.24b	81.00 ± 0.82a	2.35 ± 0.09b	1.08 ± 0.01c	3.23 ± 0.02a
insoluble-bound	156.72 ± 8.69b	143.56 ± 10.16b	677.38 ± 6.24a	3.91 ± 0.05b	1.11 ± 0.04c	4.08 ± 0.07a
total phenol content	437.99 ± 4.65b	1054.33 ± 11.00a	384.97 ± 1.81c	10.39 ± 0.33b	3.45 ± 0.16c	12.29 ± 0.33a

[a]Mean ± SD; $n = 3$. Values in same row marked by the different letters are significantly different at $p < 0.05$.

increased after explosion puffing, consistent with the literature.[51,52] It has been reported that insoluble-bound phenolics are abundant in cell walls and linked by hydrogen bonding, hydrophobic interactions, and covalent bonds such as ester bonds between phenolic acids and polysaccharides.[35,56] It was extrapolated that explosion puffing technique possibly contributed to the release of bound phenolic acid. Likewise, the similar effect of steam explosion on bound phenolic acid was reported by other authors.[35]

Antioxidant activity is shown in Table 3. ABTS and reducing power assays were developed to evaluate the antioxidant activity of all samples. Both ABTS and reducing power assays appear to have similar trend. The glycosided and insoluble-bound phenolic acids have high antioxidant activity in all samples, while low antioxidant activity of esterified phenolic acids and free phenolic acids was observed in dried and fresh jujubes. The antioxidant activity of phenolic acids in the four forms in explosion-puffed jujubes was significantly higher than that of the fresh and sun-dried counterparts. This may be explained by the presence of more phenolic compounds in the puffed samples, such as vanillic, *p*-coumaric, ferulic acids, and rutin, which have strong influence on the antioxidant activity. Stojceska et al.[57] reported that extrusion treatment increased the level of total antioxidant capacity and total phenolic compounds in puffed products. This is probably attributed to high temperature and wounding of vegetables during extrusion processing.[58] These treatments could increase the activity of phenolic metabolism enzymes which lead to accumulation of phenolic compounds.[59] The high antioxidant properties of explosion-puffed samples also could be due to some non-enzymatic browning reactions, such as Maillard reaction, which have been associated with the formation of compounds with strong antioxidant capacity.[60] Nevertheless, sun-dried jujubes have the lowest TPC, which resulted in a corresponding decrease in the antioxidant activity exhibited by $ABTS^{\bullet+}$ scavenging and reducing power activity. Previous research[43] also revealed that drying, generally speaking, is regarded as detrimental because of the possibility of inducing oxidative decomposition either enzymatically by polyphenol oxidase and glycosidase or by thermal degradation of phenolic compounds.

To further investigate the relationship between phenols in different forms of different samples and their antioxidant activities, the correlation between the antioxidant activity measured by ABTS and reducing power assays and TPC of all samples is established. A significant positive correlation ($r = 0.99$, $p < 0.01$) was found between reducing power and TPC. Additionally, $ABTS^{\bullet+}$ scavenging activity also exhibited positive correlation with TPC ($r = 0.74$, $p < 0.01$). The positive correlation between phenolic content and antioxidant activity has been found by many authors.[61] The result reflected that total phenolics seem to be a good indicator of the antioxidant potential in fruit.

In summary, to our best knowledge, this is the first study comparing the contents of flavonoids, individual phenolic acid in free, esterified, glycosided, and insoluble-bound forms, total phenolics, and antioxidant activity in jujube subjected to sun-drying and explosion puffing methods with those of fresh fruits. The identification of the quantitative analysis of the phenolic compounds present in fruits is considered to be of great importance for evaluation of food drying technology in terms of nutritional and functional compounds content. The results revealed that explosion-puffed samples were richer in phenolic compounds, with high amounts of total gallic, *p*-hydroxybenzoic, vanillic, *p*-coumaric, ferulic acids, and rutin. Traditional sun-drying treatment resulted in significant declines in the TPC and antioxidant activity. Explosion-puffed jujubes had significant gains in the TPC, which leads to a corresponding increase in antioxidant properties. This result is of interest to nutritionists and consumers, as they can expect a beneficial effect from the consumption of puffed products. Consequently, the industrial processing of dried jujubes may be improved by using the explosion puffing technique, as drying time is considerably reduced and the obtained fruit has higher phenolic content and antioxidant activity. Explosion puffing as an environment-friendly and a commercially feasible technology may be developed to enhance the health functionality of jujubes.

■ ASSOCIATED CONTENT

Supporting Information

Figure 1. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

Funding

We are grateful to the National Technology Support Project with federal grants 2007BAD88B05 and 2011BAD29B04, People's Republic of China.

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