Food Sci Biotechnol (2017) 26(3):563–571
DOI 10.1007/s10068-017-0099-x

Evolution of the antioxidant capacity and phenolic contents of persimmon during fermentation

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Received: 17 October 2016 / Revised: 22 January 2017 / Accepted: 7 February 2017 / Published online: 29 May 2017

Abstract The changes in antioxidant capacity and phenolics of persimmon during alcoholic fermentation, acetification, and short aging were investigated. An increase in the antioxidant activity was observed when persimmon was transformed from puree to vinegar. The total content of phenolics remained stable, in contrast to the concentration of condensed tannin, which significantly ($p < 0.05$) increased during alcoholic and acetic fermentations, although followed by a decrease after aging. The phenolic compounds were characterized and quantitated. Gallic acid was the main phenolic compound, and its content increased by 14.4% during alcoholic fermentation and reduced by 53.5% during acetic fermentation. Additionally, the flavan-3-ol compounds increased during alcoholic fermentation and acetification. Vanillyl alcohol, (−)-epigallocatechin, and *p*-coumaric acid were not observed in persimmon puree but detected in persimmon wine and vinegar. These results indicate that alcoholic and acetic fermentation can improve the antioxidant capacity of persimmon fruit.

Keywords Persimmon · Vinegar · Antioxidant activity · Fermentation · Phenolic compounds

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Introduction

The persimmon (*Diospyros kaki* Thunb.) tree originated in China and is now widely cultivated in Japan, Korea, and Brazil [1]. According to the data of Food and Agriculture Organization of the United Nations Statistics Division, the world production of persimmons reached 4.6 million metric tons in 2013, for which China's production accounted for 43% as the main producer. The fruit is characterized by a thin skin and fragile succulent parts, making its storage difficult at room temperature, and therefore, limiting its consumption duration to only a few days after being harvested at maturation [2]. Consequently, only a few fruits are actually consumed fresh, while most of them need to be processed for economic and food-safety reasons [3]. The fruit is commonly dried to obtain a persimmon cake. Fermenting the fruit is another possibility of commercializing the product.

Persimmon is traditionally used for medicinal purposes against coughs, paralysis, bleeding, and hypertension [4]. A scientific study conducted on hyperlipidemic rats revealed the beneficial effect of the supplementation of persimmon fruits on diets [5–7]. The results indicated that persimmon consumption can reduce the lipid levels and atherosclerotic aorta lesions of rats. Additionally, several in vitro and in vivo studies have highlighted the potential antioxidant property of the persimmon fruit and its extracts [8, 9]. More specifically, persimmon vinegar can effectively inhibit lipid oxidation in fatty tuna homogenates [10] and has anti-obesity properties [11]. These biological activities are mainly attributed to the phenolic compounds.

Fermentation is a process that often increases the antioxidant activity of a plant-based food, including fruits and vegetables [12]. For instance, a beverage manufactured from fermented *yakju* grape exhibits a higher antioxidant

activity and total phenolic content than unfermented beverage [13]. It is generally approved that a moderate consumption of fermented beverages prevents metabolic disorders due to the antioxidant properties of phenolic compounds produced during the fermentation process [14–16]. Therefore, fermentation of persimmon to produce vinegar presents a solution for long-term storage at room temperature with a high potential for the healthy-food market. Nowadays, the demand for vinegar is growing in several countries such as the USA and China. As mentioned above, persimmon vinegar is a highly beneficial product. However, the effect of alcoholic and acetous fermentations and aging on individual phenolic compounds in the production and the variations in the antioxidant capacity of persimmon vinegar remain largely unknown. We, therefore, examined the changes in the antioxidant activity, total content of phenolic compounds, and condensed tannin levels during fermentation and short aging. Our study could provide useful antioxidant nutritional information on persimmon vinegar and showed its huge market potential.

Materials and methods

Materials and strains

Full mature persimmons (*Diospyros kaki* Thunb. cv. *Jixin*) were harvested in September from an orchard located in the Guangdong Province, China. The fruits were selected, washed, and then stored at −20 °C until further manipulation. Active wine dry yeast (*Saccharomyces cerevisiae*) was purchased from Angel yeast Co., Ltd. (Yichang, China). Acetic acid bacteria (*Acetobacter pasteurianus*, AS1.41) was obtained from Guangdong Microbiology Culture Center (Guangzhou, China).

Chemical reagents and standards

The standard compounds of gallic acid, (−)-gallocatechin (GC), (−)-epicatechin (EC), (−)-epigallocatechin (EGC), (−)-epigallocatechin-3-gallate (EGCG), vanillyl alcohol, vanillic acid, *p*-coumaric acid, and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), and the reagents 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{+}$) and 2,2-diphenyl-1-picrylhydrazyl radical (DPPH·) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The mass spectrometry (MS)-grade solvents, acetonitrile, methanol, and formic acid, were supplied by Merck (Darmstadt, Germany). Water for high-performance liquid chromatography (HPLC) and high-performance liquid chromatography quadrupole time of flight mass spectrometry (HPLC–QTOF MS/MS) was purified using a Milli-Q system (Millipore, Bedford, MA, USA). The Folin–Ciocalteu reagent was purchased from Yuanye Bio-Technology Co., Ltd. (Shanghai, China). All other chemicals were of analytical grade and obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

Persimmon vinegar production

After thawing the frozen persimmons at room temperature, the seeds were removed. Ten kilograms of the fruit were mashed and mixed with 4 L of water. The mixture was incubated at 85 °C for 15 min and then cooled to room temperature. Next, the pulps were inoculated with 1 g/L active wine dry yeast (*S. cerevisiae*) and sulfur dioxide was added to obtain a final concentration of 60 mg/L. The cultures were then incubated at 28 °C for one week. The resulting wine was poured into a 30 L fermentor and acetified by inoculation with 1% (v/v) acetic acid bacteria (*Acetobacter pasteurianus*, AS1.41). The cultivation was performed at 120 r/m and 0.12 vvm (vol/vol/min) of aeration at 30 °C for 1 week. After fermentation, the resulting vinegar was filtered and further incubated at 85 °C for 10 min (to avoid oxidation of acetic acid to carbon dioxide and water), and finally, aged for 3 months at room temperature. At each fermentation and aging stage, samples of puree, wine, and vinegar were taken and stored at −80 °C until analysis.

Determination of physicochemical properties

Brix values were measured by an Abbe refractometer (RFM3400, Bellingham + Stanley, UK). Total acidity was determined by automatic potentiometric titration and the values were expressed as acetic acid equivalent. The contents of alcohol were measured by distillation [17].

Extraction of the phenolic compounds

The phenolic compounds were extracted with a solution of 1% (v/v) hydrochloric acid in methanol. The samples of puree, wine, and vinegar were homogenized with the same volume of the extraction solvent and treated with ultrasounds (10 min). The solution was centrifuged at 10,000×*g* for 10 min at 4 °C. The extraction process was repeated three times, and then, the combined supernatants were filtered through a 0.22 μm membrane filter for analysis.

Determination of total content of phenolic compounds

The total content of the phenolic compounds was analyzed by applying the Folin–Ciocalteu method [18] with some modifications and using gallic acid as the standard. In brief, the appropriate dilution of extracts (1 mL) was combined

with the Folin–Ciocalteu reagent (2 mL). After incubating for 5 min, 2 mL of 10% (m/v) sodium carbonate was added and the solution was allowed to stand at room temperature in the dark for 1 h. The value of absorbance at 760 nm (UV-1800 spectrophotometer, Shimadzu, Kyoto, Japan) was converted to that of the total content of phenolic compounds, and the result was expressed in mg of gallic acid equivalent (GAE) per liter.

Determination of the concentration of condensed tannin

The concentration of condensed tannin was determined by the vanillin method [18]. Briefly, the vanillin reagent [2.5 mL, 1% (m/v)] and sulfuric acid in methanol (2.5 mL, 5 mol/L) were added to a diluted sample of phenolics extract (1 mL, 0.5 mg/mL). The mixture was kept at room temperature for 30 min, and then, absorbance was recorded at 760 nm by using a spectrophotometer (UV-1800 spectrophotometer, Shimadzu). Catechin was used as the standard, and the results were expressed in mg of catechin equivalent (CE) per liter.

Chromatographic separation of individual phenolic compounds

The content of phenolic compounds was determined by HPLC with photodiode array detection (LC-20AT, Shimadzu, Kyoto, Japan). The separation of the individual phenolic compounds was carried out with an XBridge Shield RP18 column (4.6 × 250 mm, 5 µm, Waters, Wexford, Ireland). The binary gradient consisted of formic acid [0.2% (v/v)] in water (A) and acetonitrile (B). The gradient was applied as follows: 0–60 min, 5–15% B; 60–65 min, 15% B; 65–66 min, 15–20% B; 66–73 min, 20% B; 73–80 min, 20–60% B; 80–89 min, 60% B; 89–90 min, 5% B. The injection volume of the phenolics extract was 10 µL. The column temperature was set to 35 °C and the flow rate was 1 mL/min.

HPLC–QTOF–MS/MS analysis of the phenolic compounds

The phenolic compounds were identified on an HPLC system equipped with a quadrupole-time of flight (QTOF) MS instrument (ekspert^{TM}, SCIEX, Concord, Ontario, Canada). The separation of the phenolic compounds was carried out following the same procedure as described above. The MS spectra were acquired in the negative mode and scanned from *m/z* 100 to 1200. The cone voltage was 40 V, the source temperature was stabilized at 130 °C, the capillary voltage was 4.5 kV, the desolvation gas (nitrogen) flow rate was 600 L/h, and the desolvation temperature was 350 °C.

Determination of DPPH radical scavenging activity

The DPPH radical scavenging activity was assayed by the method proposed in the literature [19] with some modifications. Briefly, the phenolics extract (50 µL) was added to a freshly prepared solution of DPPH (150 µL, 200 µM in ethanol). After shaking, the solution was allowed to stand at room temperature in the dark for 20 min and absorbance was measured at 517 nm on a multidetection microplate (Multiskan GO, Thermo Scientific, Vantaa, Finland). The scavenging activity was reported as µM Trolox equivalents (TEs) per liter.

Determination of the ABTS radical cation ($ABTS^{\cdot+}$) scavenging activity

The ABTS radical cation scavenging activity was determined using the method described by Re et al. [20]. The reagent of ABTS was dissolved in water and the radical cation was produced by reaction with a solution of potassium persulfate (2.45 mM). The mixture was kept in the dark at 25 °C for 12–16 h. The radical remained stable for more than 2 days under this condition. The ABTS radical cation solution was diluted beforehand with ethanol until an absorbance of 0.70 ± 0.02 at 734 nm was obtained. After addition of the diluted ABTS radical cation solution (4.0 mL) to the phenolics extract (40 µL), the reaction mixture was kept at room temperature for 6 min, and then, the absorbance was recorded at 734 nm. The scavenging activity was expressed in µM TEs per liter.

Statistical analysis

The reported results are mean values ± standard deviation (mean ± SD) of triplicates. The data were evaluated by the software one-way ANOVA from SPSS version 18.0 (Chicago, IL, USA) with Tukey's multiple-range test. In the assessment of the relationship between antioxidant activity and phenolics, Pearson's correlation coefficient was used. Differences were considered to be statistically significant at $p < 0.05$.

Results and discussion

Evolution of physicochemical properties during persimmon vinegar fermentation and short aging

The contents of Brix, total acidity, and alcohol are shown in Table 1. Brix values of persimmon puree decreased significantly ($p < 0.05$) during the alcoholic fermentation. At the same time, the alcoholic contents increased from 0 to 5.19%. Total acidity increased from 0.83 to 32.5 g/L during the fermentation. No significant ($p > 0.05$) changes

Table 1 Physicochemical properties of persimmon vinegar during fermentation and aging

	Brix	Total acidity (g/L)	Alcoholic content (%)
Puree	11.36 ± 0.25[b,1]	0.83 ± 0.03[a]	ND
Wine	3.71 ± 0.09[a]	3.2 ± 0.17[b]	5.19 ± 0.13
Vinegar	3.51 ± 0.11[a]	32.5 ± 1.06[c]	ND
One month	3.37 ± 0.15[a]	31.9 ± 1.13[c]	ND
Two month	3.23 ± 0.16[a]	32.0 ± 2.11[c]	ND
Three month	3.18 ± 0.21[a]	31.3 ± 2.08[c]	ND

ND not detected

[1] All data are expressed as means (±SD) of three analyses. Different letters in the same row indicate significant differences at $p < 0.05$

in Brix, total acidity, and alcohol were observed during the aging period. These results indicated that the fermented process was completed.

Evolution of antioxidant capacity during persimmon vinegar fermentation and short aging

The antioxidant capacity was determined by DPPH assay and the ABTS test [Fig. 1(A), (B)]. The determined values by the DPPH test increased during the alcoholic fermentation and acetification, and then decreased after the three-month aging period, resulting in an overall increase of 18.0% from the fresh puree stage to the aged product after 3 months. Regarding the ABTS assay, the antioxidant activity increased significantly ($p < 0.05$) during fermentation and decreased during the aging process. However, an overall increase of 11.4% was observed. Therefore, the results indicated that fermentation improved the antioxidant activity of persimmon. A similar behavior has been reported in a study of the antioxidant activity in persimmon alcoholic fermentation [16]. Both free and bound forms of phenolic compounds were present in the fruit. Bound phenolic compounds were mainly formed via ether and ester covalent bonds and could be released during food processing [21]. The improvement in the antioxidant activity could be attributed to the breakdown of the cell walls liberating or triggering the synthesis of bioactive compounds [12]. Furthermore, the solubility of polymerized polyphenols in water was lower than that in ethanol; therefore, their contents increased as the content of ethanol increased during the alcoholic fermentation.

Evolution of the total content of phenolic compounds and condensed tannin concentration during persimmon vinegar fermentation and short aging

During the alcoholic fermentation process, the total content of phenolic compounds remained stable [Fig. 1(C)], while the amount of condensed tannin increased significantly ($p < 0.05$) [Fig. 1(D)]. The trend of the content of condensed tannin during fermentation correlated with the antioxidant capacity determined previously by the ABTS assays, as supported by other studies on the antioxidant activity of wines and vinegars [22]. It has been reported that during alcoholic and acetic fermentations, the metabolic activity of yeast and acetic acid bacteria modifies the texture of the persimmon puree and may consequently impact the chemical composition of persimmon. In persimmon puree, condensed tannin usually existed in cells. The fermentation process-induced breakdown of persimmon cell wall could release the condensed tannin [12], which resulted in the improvement in condensed tannin content during fermentation.

At the aging stage, the concentration of condensed tannin decreased by 60.8% from the vinegar stage to the 3-month aging stage. The decrease in the content of condensed tannin during the aging stage may be due to its oxidation. It was reported that tannin can be oxidized to form polymeric compounds, which have a large-molecular-weight and limited solubility in water. Furthermore, the oxidized tannin can be incorporated within the protein and results in haze formation [23]. In fact, the haze was observed in persimmon vinegar after aging for one month. The discrepancy of the results of total phenolics and condensed tannin may stem from the different reaction mechanisms of the applied methods. The vanillin assay is based on the reaction between the electrophilic protonated vanillin carbocation with flavanol under strong acidic conditions [18]. It is widely used for detecting procyanidins and related monomers. In contrast, Folin–Ciocalteu is a non-specific reagent used to determine the total content of phenolic compounds. This method is based on a redox reaction that can also occur with other reducing compounds such as sugars, carotenoids, ascorbic acid, or amino acids present in the reaction mixture, thus leading to over-estimated values [24]. Despite the possible over-estimated values obtained by this method, it is commonly applied for the determination of the total content of phenolic compounds.

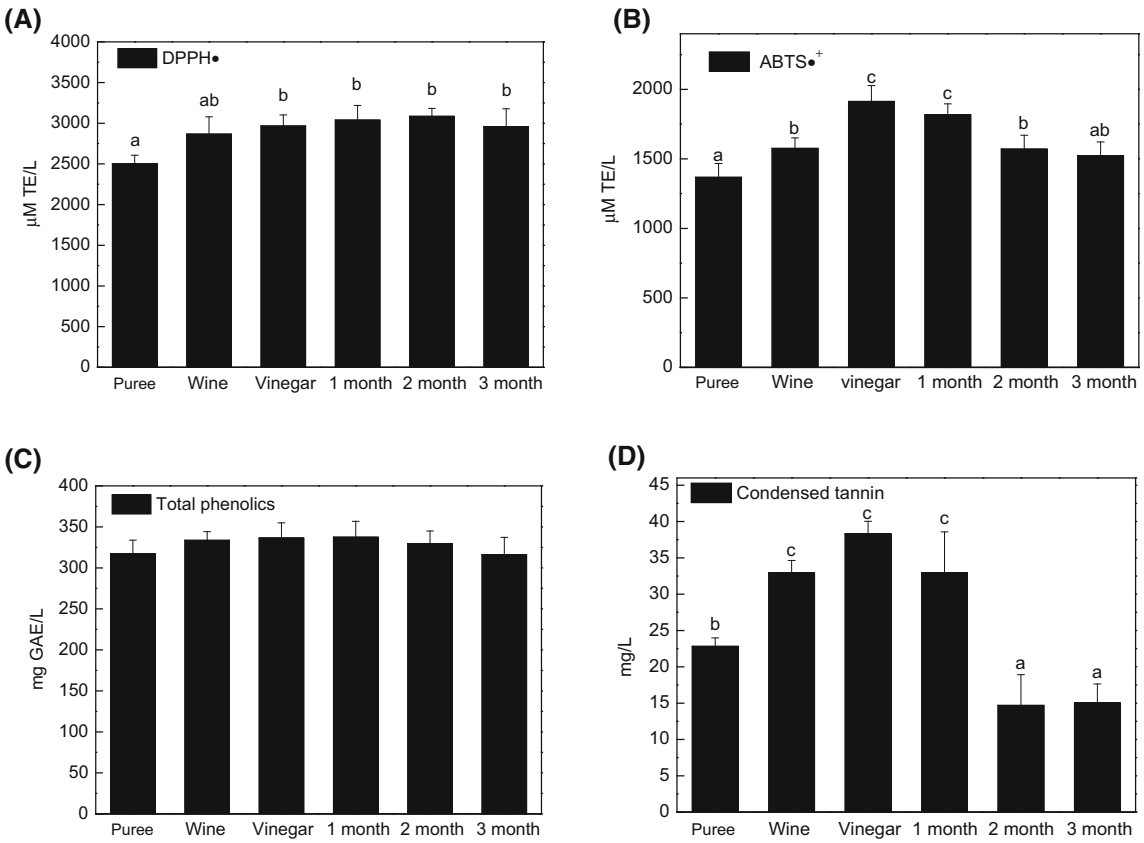
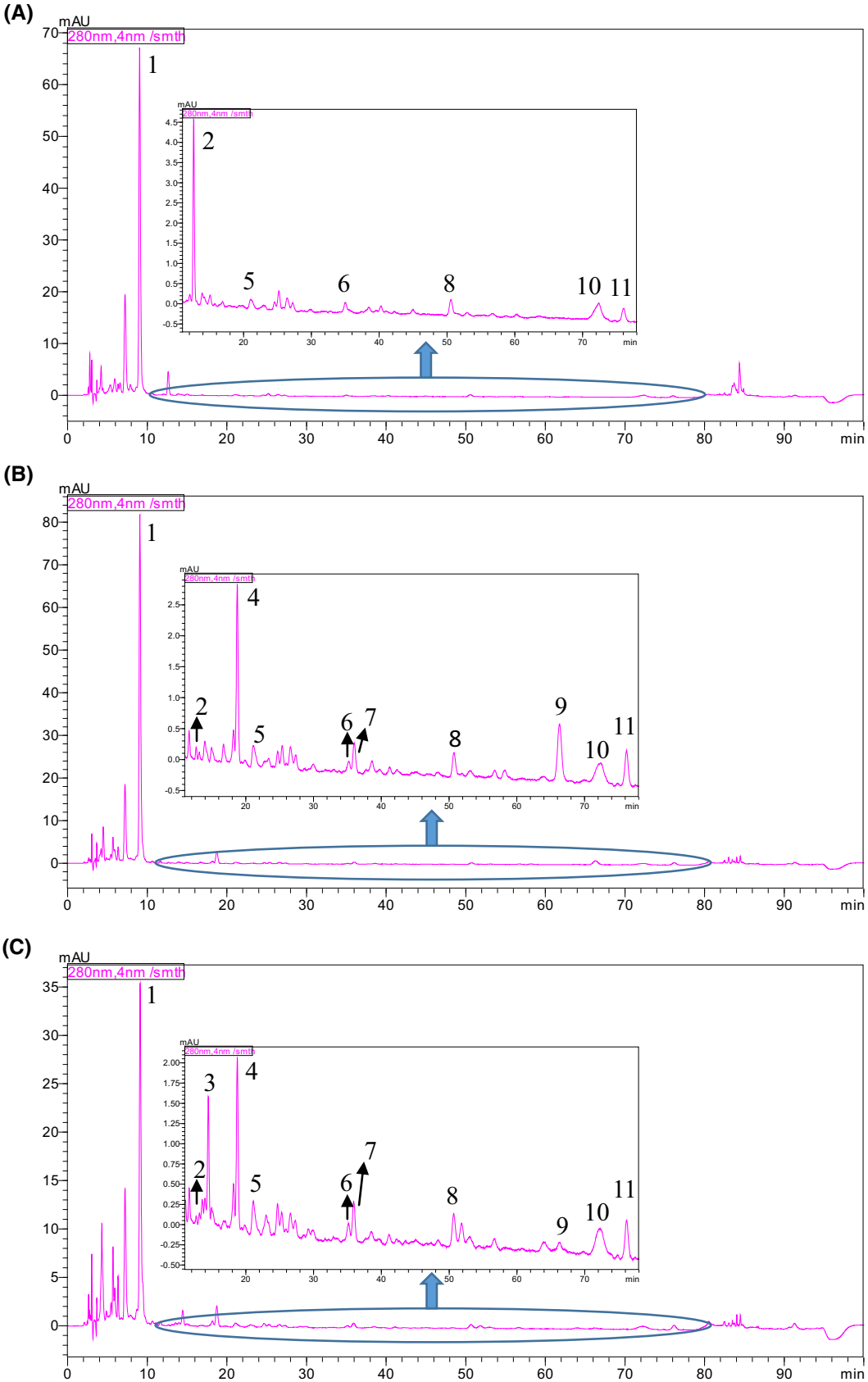


Fig. 1 Evolution of the DPPH radical (**A**), ABTS radical cation (**B**) scavenging activities, total content of phenolic compounds (**C**) and concentration of condensed tannin (**D**) of persimmon vinegar during fermentation and aging. Different letters indicate significant differences at $p < 0.05$

Identification of phenolic compounds

The antioxidant capacity was closely related to the chemical structures of the phenolic compounds. To determine the effect of fermentation and aging on the phenolic composition of persimmon puree, the individual phenolic compounds were first identified by HPLC–QTOF–MS/MS. The HPLC profiles of persimmon puree, wine, and vinegar detected at 280 nm are illustrated in Fig. 2. The identification was based on matching the peaks observed by mass spectrometry, retention time (t_R), and UV–vis spectral profiles with those of commercially available standards.

Among the different compounds, two hydroxybenzoic acids were identified. The HPLC profile exhibited the main Peak 1 (Fig. 2) attributed to gallic acid, and was assigned to pseudomolecular ion peak at *m/z* 169, which fragmented into *m/z* 125 (dihydroxy phenol moiety). Furthermore, the identified compound presented maximum absorbance at 270 nm and a retention time similar to that of the gallic acid standard. Peak 6 was attributed to vanillic acid because of the assigned ion peak at *m/z* 167.0355 matching the molecular formula $C_8H_8O_4$, in accordance with existing data [25]. Additionally, the corresponding UV–vis spectra and retention time corresponded to those of the standard. Only one type of gallotannin was identified in persimmon vinegar and was characterized by Peak 3. This peak was tentatively identified as galloyl hexoside due to the $[M–H]^-$ ion at *m/z* 331.0668, which fragmented into ions at *m/z* 169 (gallic acid, loss of hexoside from the peak appearing at *m/z* 331). This assignment has been previously reported in persimmon [26]. Only one compound belonging to the subclass of hydroxycinnamic acids was present. This compound was characterized as *p*-coumaric acid (Peak 9). Four flavanol compounds were detected (Peaks 5, 7, 8, and 11). Peaks 5 and 7 showed the same $[M–H]^-$ ion at *m/z* 305.0669 with identical mass fragments at *m/z* 261, 219, 179, 165, 137, and 125, corresponding to the loss of one moiety of C_2H_4O, $C_4H_6O_2$, $C_6H_6O_3$, $C_6H_4O_4$ or $C_7H_8O_3$, $C_8H_8O_4$, and $C_9H_8O_4$, respectively. By comparing the retention times of the phenolics with those of the standards, these compounds were confirmed as gallocatechin (Peak 5) and epigallocatechin (Peak 7), respectively (Table 2; Fig. 2). Peak 8 was identified as epicatechin on the basis of the pseudomolecular ion detected at *m/z* 289.0365, related fragmented ions, retention time, and absorbance maximum with those of the standard. Peak 11 was identified as


(A)
mAU
280nm,4nm /smth
1
2
5
6
8
10 11
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(B)
mAU
280nm,4nm /smth
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(C)
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◀**Fig. 2** Representative HPLC chromatograms at 280 nm of persimmon puree (**A**), wine (**B**), and vinegar (**C**). Peak number identities are displayed in Table 2

epigallocatechin gallate due to the corresponding retention time, and the ion $[M–H]^-$ was detected at *m/z* 457.0752 with related fragment ions at *m/z* 305 (gallocatechin or epigallocatechin moiety), 287 (loss of H_2O from *m/z* 305), 169 (gallic acid), and 125 (dihydroxy phenol moiety). Peak 4 presented an ion $[M–H]^-$ detected at *m/z* 153.0558 with a fragment ion appearing at *m/z* 123, and was thus identified as vanillyl alcohol. Peaks corresponding to three unknown compounds could not be assigned to specific groups because the information provided by the mass spectra was not sufficient to determine their chemical structures.

Evolution of the content of individual phenolic compounds

The amounts of individual phenolic compounds were determined and the results are shown in Table 3. Gallic acid was the main phenolic compound found in persimmon. Its concentration increased from puree to wine, reduced after acetic fermentation, and remained stable during the short aging stage. The amount of three flavanol compounds (GC, EC, and EGCG) increased during the fermentation process. Interestingly, vanillyl alcohol, EGC, and *p*-coumaric acid were not observed in the persimmon puree but detected in its wine form. The contents of these phenolics increased by alcoholic fermentation, which could be due to the yeast action. It was reported that microbial enzymes such as tannase, glucosidase, and cellulase produced during fermentation could facilitate the liberation of phenolic compounds by disintegrating the cell walls of the plant [12]. Li et al. [27] found that the probiotic bacteria could decompose flavan-3-ol molecules into simple phenolic compounds such as *p*-coumaric acid. High-molecular-weight phenolic compounds could also be depolymerized under the presence of lactic acid bacteria [28]. Galloyl hexoside was only observed in persimmon vinegar after the acetification step and in the persimmon aged during one month. This compound could be the hydrolytic product of gallotannin, which consisted of a hexoside core esterified with gallic acid [25]. Additionally, the contents of vanillyl alcohol, *p*-coumaric acid, and EGCG reduced significantly ($p < 0.05$) at the aging stage. A gradual reduction was observed in the levels of GC and EGC during aging, while the content of vanillic acid remained constant during this process. No specific change was observed in the content of EC. The degradation of phenolics is determined by their chemical structures. It has been reported that the galloyl moiety at the C–3 position of EGCG possesses a higher DPPH radical scavenging ability than that of the ortho-trihydroxyl group in the B ring of EGC [29]. On the other hand, EGCG is more susceptible to oxidation when compared to EGC and GC, explaining the more pronounced decline in the EGCG level.

Correlations between antioxidant activity and phenolics

The relationship between antioxidant capacity and phenolics was elucidated by Pearson's correlation coefficient. As shown in Table 4, the ABTS radical cation scavenging activity was positively and significantly ($p < 0.05$)

Table 2 Identification of phenolic compounds in persimmon puree, wine, and vinegar

Peak	t_R	λmax (nm)	[M–H] (*m/z*)	MS/MS (*m/z*)	Molecular formula	Identification
1	9.05	270	169.0141	125	$C_7H_6O_5$	Gallic acid
2	12.66	278	205.0358	143, 111, 87	$C_7H_{10}O_7$	Unknown 1
3	14.44	270	331.0668	169	$C_{13}H_{16}O_{10}$	Galloyl hexoside
4	18.72	275	153.0558	123	$C_8H_{10}O_3$	Vanillyl alcohol
5	21.04	270	305.0669	261, 219, 179, 165, 137, 125	$C_{15}H_{14}O_7$	Gallocatechin
6	35.11	259, 291	167.0355	167, 152 (151), 123	$C_8H_8O_4$	Vanillic acid
7	35.90	270	305.0664	261, 219, 179, 165, 137, 125	$C_{15}H_{14}O_7$	Epigallocatechin
8	50.65	278	289.0365	247, 245, 179, 137, 125	$C_{15}H_{14}O_6$	Epicatechin
9	66.32	309	163.0346	119	$C_9H_8O_3$	*p*-Coumaric acid
10	72.46	275	327.2179	229, 211, 171	$C_{18}H_{32}O_5$	Unknown 2
11	76.20	274	457.0752	331, 305, 287, 169, 125	$C_{22}H_{18}O_{11}$	Epigallocatechin gallate

Table 3 Changes in individual phenolics of persimmon vinegar during fermentation and aging

Compound	Contents (mg/L)					
	Puree	Wine	Vinegar	1 month	2 months	3 months
Gallic acid	29.44 ± 0.26^{b,1}	34.41 ± 0.44^{c}	16.00 ± 0.80^{a}	15.80 ± 2.33^{a}	17.56 ± 1.31^{a}	17.64 ± 1.59^{a}
Unknown 1	1.82 ± 0.01^{c}	0.070 ± 0.003^{b}	0.036 ± 0.001^{c}	0.035 ± 0.001^{c}	ND	ND
Galloyl hexoside	ND	ND	0.65 ± 0.009^{a}	0.58 ± 0.045^{a}	ND	ND
Vanillyl alcohol	ND	3.05 ± 0.016^{c}	2.14 ± 0.013^{b}	2.01 ± 0.094^{b}	1.56 ± 0.093^{a}	1.50 ± 0.065^{a}
GC[2]	5.22 ± 0.48^{a}	7.95 ± 0.71^{bc}	9.13 ± 0.28^{d}	8.26 ± 0.21^{c}	8.42 ± 0.34^{c}	7.54 ± 0.38^{b}
vanillic acid	0.31 ± 0.019^{b}	0.21 ± 0.003^{a}	0.23 ± 0.014^{a}	0.21 ± 0.006^{a}	0.21 ± 0.005^{a}	0.21 ± 0.001^{a}
EGC	ND	7.67 ± 0.50^{bc}	7.99 ± 0.08^{c}	7.12 ± 0.49^{b}	6.35 ± 0.07^{a}	6.59 ± 0.53^{ab}
EC	1.31 ± 0.01^{ab}	1.48 ± 0.09^{ab}	1.71 ± 0.06^{b}	1.25 ± 0.11^{a}	1.85 ± 0.12^{b}	1.68 ± 0.11^{b}
p-Coumaric acid	ND	0.59 ± 0.002^{b}	0.06 ± 0.002^{a}	0.04 ± 0.001^{a}	ND	ND
Unknown 2	0.81 ± 0.03^{c}	0.71 ± 0.02^{bc}	0.78 ± 0.02^{c}	0.69 ± 0.05^{ab}	0.60 ± 0.03^{a}	0.57 ± 0.04^{a}
EGCG	1.41 ± 0.06^{a}	2.10 ± 0.04^{b}	2.08 ± 0.11^{b}	1.45 ± 0.08^{a}	ND	ND

ND not detected

[1] All data are expressed as means (±SD) of three analyses. Different letters in the same row indicate significant differences at $p < 0.05$

[2] GC, (−)-Gallocatechin; EGC, (−)-Epigallocatechin; EC, (−)-Epicatechin; EGCG, (−)-Epigallocatechin gallate

Table 4 Pearson's correlation coefficients between antioxidant activity and phenolics

	DPPH	ABTS
Total phenolics	0.549	0.823*
Condensed tannin	0.004	0.701
Gallic acid	−0.678	−0.589
Unknown 1	−0.941**	−0.626
Galloyl hexoside	0.357	0.922**
Vanillyl alcohol	0.627	0.579
GC	0.898*	0.828*
Vanillic acid	−0.922**	−0.505
EGC	0.866*	0.725
EC	0.520	0.103
p-Coumaric acid	−0.052	−0.025
Unknown 2	−0.565	0.151
EGCG	−0.314	0.408

GC, (−)-Gallocatechin; EGC, (−)-Epigallocatechin; EC, (−)-Epicatechin; EGCG, (−)-Epigallocatechin gallate

* Significant at $p < 0.05$; ** significant at $p < 0.01$

correlated with total phenolics, galloyl hexoside, and GC. The DPPH radical scavenging activity was positively associated with the contents of GC and EGC. However, no significant ($p > 0.05$) correlation was observed between DPPH radical scavenging activity and total phenolics. Further studies are required to reveal the possible mechanism of differences between DPPH radical and ABTS radical cation scavenging activity.

In conclusion, alcoholic and acetic fermentation significantly ($p < 0.05$) increased the antioxidant activity of persimmon and its tannin concentration. Among the different phenolic compounds present in persimmon, gallic acid represented the major compound, and its content increased during the alcoholic fermentation but reduced during acetic fermentation. In particular, three phenolic compounds, vanillyl alcohol, EGC, and *p*-coumaric acid, were produced by the fermentation processes. Yeast and acetic bacteria possess the ability to enhance the levels of flavan-3-ol compounds, although some of them decreased when the vinegar was aged for three months. Finally, our results suggested that the antioxidant capacity was increased and the concentrations of the phenolic compounds were elevated during fermentation. The antioxidant activity was positively correlated with total phenolics and GC content. Thereby, persimmon vinegar possesses health-promoting benefits and presents high potentials for product development and food market.

Acknowledgements The authors are grateful for the financial support from the Science and Technology Program of Guangzhou (201510010063), the National Natural Science Foundation of China (31501541), and Natural Science Fund of Guangdong Province (2015A030312001, 2014A030310208). The authors thank Enago (www.enago.co.kr) for the English language review.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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