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Original Article

Physicochemical Components and Antioxidant Activities of Daebong Persimmon (*Diospyros kaki cv. Hachiya*) Peel Vinegars

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Abstract

To evaluate utilization of persimmon peels as novel bio-materials, the general composition and antioxidant activities of Daebong persimmon vinegar (DPV), Daebong persimmon peel vinegar (DPPV) and commercial persimmon vinegar (CPV) were investigated. The pH of DPPV had slightly higher than that of other vinegars. The titratable acidities of vinegars were 3.24% (DPV), 2.77% (DPPV) and 7.78% (CPV), respectively. The reducing sugar contents showed that DPV had slightly higher than that of other vinegars. The browning degree of DPV was lower than CPV in contrast to the turbidity. The results of Hunter's color value have showed that overall values of CPV had significantly higher than DPPV and DPV. The total phenolic contents of DPV and CPV were 19.49 and 17.13 mg/100g GAE, respectively. The total flavonoid contents of DPPV (8.04 mg/100g CE) were two fold higher than that of DPV (3.85 mg/100g CE). The antioxidant activities, by DPPH and FRAP assays, of DPV showed stronger than those of other vinegars. Free sugars were mainly composed of fructose and glucose. Organic acids were presented in the order acetic acid, succinic acid, malic acid, tartaric acid, citric acid and oxalic acid. These results suggest that the Daebong persimmon peels could be utilized for vinegar production as a health-benefit material.

Keywords : *persimmon peel vinegar, Viscozyme L, organic acids, antioxidant*

Introduction

Persimmon trees (*Diospyros kaki*) are widely cultivated in the southern area of S. Korea. It is one of the alkaline foods containing vitamin A, C and carbohydrates such as glucose and fructose. The persimmon is one of the very popular fruits with apple and grape in S. Korea (Kim et al. 2003). Moreover, it has anti-cancer effect due to the abundant vitamin A, B1, C, D, chlorophyll, folic acid and pantothenic acid (Choi et al. 2006). The persimmon can be classified as sweet (*D. kaki T*) and astringent (*D. kaki L*) types. The sweet-type of persimmon is consumed as fresh fruits, whereas, the astringent-type of persimmon is consumed as products of dried type, because it has astringent taste due to the tannin (Moon et al. 1995). Daebong persimmon (*D. kaki cv. Hachiya*), one of the astringent-type variety in S. Korea, is somewhat bigger than other varieties, and has outstanding flavor and taste (Joo et al. 2011). Recently, they are consumed as dried products. Consequently, large amount of persimmon peel, caused an environmental contamination, is wasted in the production area. Despite of this problem, persimmon peel is useful resource as a novel biomaterial due to the abundant dietary fiber and polyphenolic compounds such as crotenoid and tannin (Kim et al. 2005, Kim et al. 1998). Furthermore, anti-diabetic effects in the rats fed with persimmon peel have been reported (Teisedre et al. 2000).In previous studies, to utilize of persimmon peel as novel food materials, we investigated the physicochemical properties of persimmon peel powders with different particle sizes (Hwang et al. 2011) and optimized persimmon peel vinegar fermentation by enzymatic hydrolysis (Hwang et al. 2013). In this research, to improve the value of wasted persimmon peel, we evaluated the physicochemical properties and antioxidant capacities of commercial persimmon vinegar, brewed vinegars from Daebong persimmon and peel.

Materials and Methods

Materials

Persimmon (*Diospyros kaki* L. cv. Daebong) and their peels were collected at the production area (Gwangyang, Korea) of the dried persimmon. Then, persimmon peels were dried to 5% of moisture in the shade and stored at 4℃ until use. The samples for the brewing vinegar were pulverized and screened to powder (>250 μm) (Hwang et al. 2011). The commercial persimmon vinegar was purchased from Yedamcho co., S.

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Korea. Gallic acid, glucose, fructose, sucrose, oxalic acid, citric acid, tartaric acid, malic acid, succinic acid, acetic acid, (±)-Catechin, Folin-Ciocalteau's phenol reagent, 1,1-dipheny1-2-picrylhydrazyl (DPPH), 2,4,6-tris(2-pyridyl)-1,3,5-triazine (TPTZ), trolox, Sodium acetate ($C_2H_3NaO_2$), acetic acid ($C_2H_4O_2$), HCl, $FeCl_3 \cdot 6H_2O$ were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

Alcoholic and acetic acid fermentations

To improve the fermentable carbohydrate contents, the persimmon peel powder was enzymatic hydrolyzed by a diastatic enzyme. The persimmon peel powder and distilled water were mixed in a ratio of 1 : 4 and added 2.3% of Viscozyme L. They incubated in a shaking incubator at 50℃ for 6 hr. The resultant hydrolyzed solution was filtered and the supernatant was used for brewing vinegar. In alcoholic fermentation, after pectinase (Pectinex 5XL, Novozymes AS, Denmark) treatment to decrease viscosity, the hydrolyzed solution was inoculated by 0.02% of *Saccharomyces cerevisiae* and incubated at 25℃. After 20 days, alcoholic fermented solution was filtered to remove the residual pulp, and supernatant containing 7% of alcohol was used for acetic acid fermentation. The filtered alcoholic fermented solution was inoculated by 10% (v/v) of vinegar starter and incubated in a shaking incubator at 110 rpm, 30℃ for 14 days. In case of Daebong persimmon vinegar, to remove the seeds, persimmon was divided into four pieces and crushed. The alcoholic and acetic acid fermentations were same as described above.

Physicochemical analyses

pH was measured using pH meter (Mettler-Toledo GmbH Schwerzenbach, Switzerland). Titratable acidity was measured according to AOAC methods (2000). Reducing sugar content was determined by the dinitrosalicylic acid method (Miller et al. 1959), and the contents were expressed as mg of glucose equivalents. Browning degree and turbidity were detected using UV-Spectrophotometer (UV 1601 PC, Shimadzu Co., Kyoto, Japan) at 420 and 660 nm. The Hunter's color values were measured with a colorimeter Minolta (Model CR 200; Minolta corp., Ramsey, NY). Free sugar content was determined using a high performance liquid chromatography (HPLC, LC-10A Shimadzu Co., Japan) with a RI detector and a zorbax Carbohydrate Analysis column (4.6 × 150 mm, i.d. 5 μm). The mobile phase was 75% of acetonitrile, and operating conditions were flow rate of 1.4 mL/min and injection volume of 25 μL at room temperature. Organic acids were determined using a high performance liquid chromatography (HPLC, LC-10A Shimadzu Co., Japan) with a UV detector (214 nm) and an AminexHPX-87H column (7.5 × 300 mm, i.d. 9 μm, Bio-RAD Laboratories, USA). The mobile phase was 5 mM of sulfuric acid, and operating conditions were flow rate of 0.6 mL/min and injection volume of 25 μL at room temperature. The contents of free sugars and organic acids were expressed as mg of 100 g of vinegar.

Determination of antioxidants and antioxidant activities

Total phenolic content was measured using a spectrophotometer (UV 1601 PC, Shimadzu Co., Kyoto, Japan) according to the Folin-Ciocalteu's method (Sato et al. 1996), and was expressed as gallic acid equivalents (GAE) in mg/100 g. Total flavonoid content was determined according to the colorimetric assay of Jia et al. 1999, and expressed as (±)-catechin equivalents (CE) in mg/100 g. DPPH radical-scavenging activities were examined by the method of Blois et al. 1958 with minor modifications, absorbance was read at 517 nm. The activities were calibrated and expressed as gallic acid equivalents (GAE) in μM. The ferric-reducing antioxidant power (FRAP) assay was executed according to the method of Benzie et al. 1996 with slight modifications. The values were expressed as trolox equivalents (TE) in μM.

Statistical analysis

Data was expressed as means±standard deviations (SD) of triplicate experiments and analyzed by SAS program version 9.3 (Statistical analysis system, SAS Institute, Inc., Cary, NC). One way analysis of variance (ANOVA) and the Duncan's New Multiple-range test were used to determine the differences among these means. *p*-Values<0.05 were regarded as significant.

Results and discussion

pH, titratable acidity and reducing sugar contents

The pH, titratable acidity and reducing sugar contents of vinegars are shown in Table 1. The pH values of Daebong persimmon vinegar (DPV), Daebong persimmon peel vinegar (DPPV) and commercial persimmon vinegar (CPV) were 3.43, 3.51, 3.16, respectively. The pH of DPPV had slightly higher than that of other vinegars. The titratable acidities of vinegars were 3.24% (DPV), 2.77% (DPPV) and 7.78% (CPV), respectively. In contrast with pH result, CPV had significantly higher than that of other vinegars. The reducing sugar contents showed that DPV had slightly higher than that of other vinegars. The Korean quality standards of fruit vinegars require that fruit vinegars consisting of acetic acid have to be made from fruit wines, usually without any additional flavoring. Total acidity of vinegars have to be reached at 4-29% (persimmon vinegar; more than 2.6%). Therefore, these results indicate that vinegars produced in this study are suitable for the food standards.

Table 1. Titratable acidity, pH and reducing sugar of Daebong persimmon vinegar, Daebong persimmon peel vinegar and commercial persimmon vinegars

Vinegar[1)]	pH	Titratableacidity (%)	Reducing sugarcontent (mg/100 g)
DPV	3.43±0.01[2)b]	3.24±0.08[b]	1132.70±2.18[a]
DPPV	3.51±0.01[a]	2.77±0.11[b]	717.30±1.96[c]
CPV	3.16±0.01[c]	7.78±0.47[a]	930.19±46.01[b]

[1)]DPV: Daebong persimmon vinegar, DPPV: Daebong persimmon peel vinegar, CPV: Chungdobansi persimmon vinegar.
[2)]Means±SD (n=3) with different letters are significantly different at $p<0.05$.

Browning degree, turbidity and Hunter's color value

The browning degree, turbidity and Hunter's color values were shown in Table 2. These values of related to color reflect the quality of appearance. The browning degree of vinegars had showed that the value of CPV had slightly higher than that of other vinegars. In contrast with browning degree, the turbidity had showed that the value of DPV had higher than that of others. The results of Hunter's color value have showed that overall values of CPV had significantly higher than DPPV and DPV. Seo et al. (2000) reported that the tannic acid contained persimmon vinegars affect the quality of appearance, and persimmon have different tannic acid contents according to varieties and degree of de-astringency.

Table 2. Browning, turbidity, Hunter's color value in Daebong persimmon vinegar, Daebong persimmon peel vinegar and commercial persimmon vinegars

Vinegar[1)]	Browning (O.D.at 420 nm)	Turbidity (O.D. at 660 nm)	Hunter's color value[2)]			
			L	a	b	ΔE
DPV	0.469±0.001[3)c]	0.219±0.001[a]	94.58±0.01[a]	-0.57±0.01[c]	9.83±0.01[c]	11.24±0.00[c]
DPPV	0.923±0.006[b]	0.046±0.001[c]	88.30±0.03[b]	0.97±0.02[b]	41.05±0.08[b]	42.70±0.08[b]
CPV	1.113±0.014[a]	0.079±0.003[b]	83.60±0.11[c]	4.23±0.05[a]	47.39±0.14[a]	50.32±0.17[a]

[1)]Same as Table 1
[2)]L : Degree of whiteness (white +100 ↔0 black).
a : Degree of redness (red +100 ↔-80 green).
b : Degree of yellowness (yellow +70 ↔-80 blue).
ΔE : Overall color difference ($\sqrt{\triangle L^2+\triangle a^2+\triangle b^2}$) .
[3)]Means±SD (n=3) with different letters are significantly different at $p<0.05$.

Free sugars and organic acids contents

The free sugars and organic acids contents were analyzed to confirm the acetic acid fermentation. In all of vinegar samples, fructose and glucose were detected. The CPV had significantly higher than that of others. Furthermore, the glucose contents were higher than fructose in DPV and CPV except DPPV. No sucrose contents were determined in all of vinegar samples. This result reveals that sucrose of persimmon and peel was completely consumed for fermentation. In organic acid composition, the acetic acid which is major quality factor of vinegars had showed the highest contents in all samples. Succinic acid is also the quality factor of vinegar fermentation for good taste and flavor to food. The DPV and DPPV had higher succinic acid contents than that of CPV. These results suggested that the Daebong persimmon and their peel are also useful resource for the vinegar fermentation.

Antioxidant activities

The antioxidant activities of vinegars were measured by DPPH radical scavenging and FRAP assay. The FRAP assay is widely used in the evaluation of the antioxidant activity, like as DPPH assay. In the both of assay, antioxidant activities of vinegars indicated the same order of activities. In particular, the DPPH and FRAP assay of DPV showed significant antioxidant activity, with respective values of 75.15 µM GAE and 16196.25 µM

TE. In contrast, CPV exhibited weaker antioxidant activity, with respective values of 39.34 μM GAE and 3323.13 μM TE, than that of other vinegars. Although persimmon and their peel have already been known to act as a radical scavenger against reactive oxygen species, these results have demonstrated that the vinegar from Daebong persimmon and peel could function as a hydroxyl radical scavenger. Further study is required to evaluate the antioxidant activities *in vivo.*

Table 3. Free sugar contents of Daebong persimmon vinegar, Daebong persimmon peel vinegar and commercial persimmon vinegars

Vinegar[1)]	Free sugar content (mg/100 g)			
	Fructose	Glucose	Sucrose	Total
DPV	292.70[2)]	2556.37	-[3)]	2849.07
DPPV	276.86	29.97	-	306.83
CPV	2224.01	3599.27	-	5823.28

[1)]Same as Table 1
[2)]Mean (n=3).
[3)]Not detected.

3.85~8.04 mg/100 g CE, respectively. The DPV has the highest total phenolics with value of 19.49 mg/100 g GAE, whereas, the DPPV has the highest total flavonoids with value of 8.04 mg/100 g CE. These results reveal that persimmon fruit and their peel have different antioxidant composition, and persimmon peel could be also utilized as novel health functional materials.

Table 6. Total phenolic and total flavonoid contents of Daebong persimmon vinegar, Daebong persimmon peel vinegar and commercial persimmon vinegars

Vinegar[1)]	Total phenolic content[3)]	Total flavonoid content[4)]
DPV	19.49±0.69[2)a]	3.85±0.15[c]
DPPV	17.54±0.94[a]	8.04±0.25[a]
CPV	17.13±2.00[a]	6.30±0.42[b]

[1)]Same as Table 1
[2)]Means±SD (n=3) with different letters are significantly different at $p<0.05$.
[3)]Unit: mg/100 g GAE
[4)]Unit: mg/100 g CE

Table 4. Organic acid contents of Daebong persimmon vinegar, Daebong persimmon peel vinegar and commercial persimmon vinegars

Vinegar[1)]	Organic acid content (ppm)						
	Oxalic acid	Citric acid	Tartaric acid	Malic acid	Succinic acid	Acetic acid	Total
DPV	22.04[2)]	45.30	293.16	172.33	987.16	8301.74	9821.74
DPPV	38.99	30.34	668.60	307.31	659.56	7606.86	9331.65
CPV	7.06	84.83	278.38	537.38	472.88	18505.03	19885.56

[1)]Same as Table 1
[2)]Mean (n=3).

Table 5. DPPH radical scavenging activites and ferric reducing antioxidant power of Daebong persimmon vinegar, Daebong persimmon peel vinegar and commercial persimmon vinegars

Vinegar[1)]	DPPH (μM GAE)	FRAP (μM TE)
DPV	75.15±0.16[a 2)]	16196.25±132.29[a]
DPPV	53.91±1.59[b]	4431.46±53.16[b]
CPV	39.34±1.25[c]	3323.13±37.50[c]

[1)]Same as Table 1
[2)]Means±SD(n=3) with different letters are significantly different at $p<0.05$.

Total phenolic and flavonoid contents

The total phenolic and flavonoid contents of the vinegars were shown in Table 6. The contents of the total phenolics and flavonoids of vinegars ranged 17.13~19.49 mg/100 g GAE,

Conclusions

In this research, in order to evaluate utilization of Daebong persimmon peel as novel health functional materials, we produced the Daebong persimmon peel vinegar by enzymatic hydrolysis using a Viscozyme L as pretreatment, and also produced the Daebong persimmon vinegar. The DPPV, DPV produced in laboratory and CPV were analyzed to compare the physicochemical properties and antioxidant activities. The pH, titratable acidity and reducing sugar contents of vinegars ranged 3.16-3.51, 2.77-7.78% and 717.30-1132.70 mg/100 g, respectively. The browning degree and overall color difference were CPV>DPPV>DPV, whereas, the turbidity was DPV> CPV>DPPV. The total free sugars and total organic acids contents of vinegars ranged 306.83-5823.28 mg/100 g and 9331.65-19885.56 ppm, respectively. The antioxidant activities by DPPH and FRAP assay were DPV>DPPV>CPV. The total phenolics and flavonoids contents of vinegars ranged 17.13-19.49 mg/100 g GAE and 3.85-8.04 mg/100 g CE, respectively. The overall antioxidant contents of DPV and DPPV were higher than that of CPV. Taken together these results, the DPPV produced in this research is suitable for the food standards, and it has strong antioxidant activities. Thus, the Daebong persimmon peel could be developed as a health-benefit material through further research such as efficacy *in vivo*.

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