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Changes in the Antioxidant Activity of Onion (*Allium cepa*) Extracts with Heat Treatment

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Abstract We evaluated the effects of heat treatment on various properties of onion extracts (*Allium cepa*). Onion was heated at various temperatures (110-150°C) for various times (1-5 hr), and the total polyphenol, flavonoid, and free sugar contents, and antioxidant activity were investigated. With increased heating temperatures and exposure times, the total contents of polyphenols and flavonoids, as well as antioxidant activity increased. The highest total polyphenol content (189.80 mg/100 g) occurred after heating for 2 hr at 140°C. The highest total flavonoid content (252.51 mg/100 g) occurred after heating for 3 hr at 150°C. The antioxidant activity assessed using the DPPH method was highest, at 2.19 mg/mL (IC_{50} value), after heating for 2 hr at 150°C. Correlations between antioxidant activity and the total polyphenol, total flavonoid, and fructose contents were highly significant (all p<0.01). The optimal heating time and temperature were 2 hr and 130°C.

Keywords: onion (*Allium cepa*), heat treatment, antioxidant activity, flavonoid, polyphenol

Introduction

Onion (*Allium cepa*) is one of the most widely used medicinal and edible plants in the world (1). Onion contains very high levels of flavonoids, especially quercetin and its glycosides, and is one of the major sources of flavonoids in Western diets. The flavonoids, quercetin, and its glycosides are often present in vegetables, and glycosides of kaempferol, luteolin, and apigenin are also found in onions (1, 2). In several clinical studies, onion and onion extracts decreased blood lipid levels, increased fibrinolysis, decreased platelet aggregation, and lowered blood pressure (3).

The fresh weight of onions is 60-70% water, and the other most significant components are organosulfur-containing compounds. Onions mainly contain cysteine sulfoxides. When the tissues are chopped, the enzyme allinase is released and converts the cysteine sulfoxides into thiosulfinates. These compounds are reactive, volatile, odor producing, and lachrymatory (4). In addition to the nutritional compounds of onion, its antioxidant, anti-bacterial, and antifungal activities toward a variety of organisms have been investigated extensively (5).

Browning of onion due to thermal treatment is the result of several reactions. These include Maillard condensation between reducing sugars and amino acids, caramelization, and ascorbic acid browning processes (6). Many studies of onion have been conducted, but most were limited to physiological examinations. There are few studies of the chemical and physical properties of onion in response to high temperature and pressure treatment (HTPT). Recent studies have shown that thermally processed foods, especially fruits and vegetables, have higher biological activity because of various chemical changes during heat treatment (7, 8). For example, polyphenol and flavonoid contents and antioxidant activity increase with an increase in HTPT in plants such as ginseng (9), licorice (10), garlic (11), pear (12), and *shiitake* mushroom (13). However, no studies have examined the effects of heat treatment on onion.

Thus, our objective was to investigate changes in the polyphenol, flavonoid, and free sugar contents, and the antioxidant activity of onion with heat treatment, as well as to determine the optimal heating regimen to increase antioxidant activity.

Materials and Methods

Samples and treatments Onion (*Allium cepa*) purchased from Hamyang, Gyeongnam, Korea, was harvested on June 2005 and stored at -20°C. The onion was put into a sample bottle and sealed tightly. The sample bottle was subjected to high-pressure steam generated by a temperature and pressure controlling apparatus (Jisico, Seoul, Korea). The samples were heated to temperatures of 110, 120, 130, 140, or 150°C for 1, 2, 3, 4, or 5 hr (9-12). The heated samples were juiced and centrifuged at 1,800×g for 10 min, and the supernatant was filtered through a 0.45 μm syringe filter (Millipore, Billerica, MA, USA). The onion juice was kept at -20°C until chemical analysis.

Total polyphenol content The total polyphenol content of heated onion juice (HOJ) was determined using the Folin-Ciocalteu method (14). In a 10 mL test tube, 2 mL of 2% Na_2CO_3, 0.1 mL of HOJ appropriately diluted, and 0.1 mL of 50% Folin-Ciocalteu phenol reagent (Sigma-Aldrich, St. Louis, MO, USA) was added and mixed.

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After exactly 30 min, the absorbance was read at 750 nm, and the total polyphenol concentration was calculated from a calibration curve (R^2=0.9969) using tannic acid (Sigma-Aldrich) as a standard (20-200 mg/L). All extracts were analyzed in triplicate.

Total flavonoid content The total flavonoid content of HOJ was determined using the colorimetric method described by Jia *et al.* (15), with some modifications. Standard solution or HOJ (250 μL) was mixed with 1.25 mL of distilled water and 75 μL of 5% $NaNO_2$ solution. After 5 min, 150 μL of 10% $AlCl_3 \cdot H_2O$ was added. After 6 min, 500 μL of 1 M NaOH and 275 μL of distilled water were added to the mixture. The solution was mixed well and the intensity of the pink color was measured at 510 nm. All extracts were analyzed in triplicate. The absorbance was read at 510 nm, and the total flavonoid concentration was calculated from a calibration curve (R^2= 0.9991) using (+)-catechin hydrate (Sigma-Aldrich) as a standard (20-200 mg/L). All extracts were analyzed in triplicate.

Free sugar content The free sugar content was measured according to a modification of the method described by Bae *et al.* (16) using fructose, glucose, and sucrose (Sigma-Aldrich) as standards for the calibration curve. Samples of HOJ were filtered through a 0.45 μm syringe filter (Millipore) and analyzed using high-performance liquid chromatography (HPLC, Waters 2695; Waters, New Castle, DE, USA). Free sugar was separated on the carbohydrate column (4.6×150 mm, Waters) and the mobile phase was water-acetonitrile (25:75%, v/v) at a flow rate of 1 mL/min. The injection volume was 20 μL and the detector used the refractive index (Waters 2414; Waters). All extracts were analyzed in triplicate.

Antioxidant activity (IC_{50}) using the DPPH assay The scavenging activity of HOJ on the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical was measured according to the method of Tepe *et al.* (17), with some modifications. Aliquots (0.8 mL) of 0.2 mM DPPH (Sigma-Aldrich) methanolic solution were mixed with 0.2 mL of HOJ. The mixture was shaken vigorously and then left to stand for 30 min under low light. The absorbance was measured at 520 nm. The DPPH radical-scavenging activity (%) was calculated as:

$$\text{Radical scavenging activity (\%)} = (1 - A_{sample}/A_{control}) \times 100$$

where A_{sample} is the absorbance in the presence of the sample and $A_{control}$ is the absorbance in the absence of the sample. The sample concentration providing 50% inhibition (IC_{50}) was calculated from a plot of percent inhibition against sample concentration. All extracts were analyzed in triplicate.

Statistical analysis The results were reported as the mean ± standard deviation (SD). The significance of differences among treatment means was determined by one-way analysis of variance using SAS version 9.1 (SAS Institute, Cary, NC, USA) with a significance level of 0.05. Correlations between the 4 parameters measured were also examined.

Results and Discussion

Total polyphenol and flavonoid contents Phenolic compounds are secondary metabolic products that occur throughout the plant kingdom. They contain the phenolic hydroxyl group, which has an antioxidative effect via interactions with the phenol ring and its resonance stabilization effect (16-18). Highly significant differences in the total polyphenol content of HOJ were observed with various heating temperatures and times (p<0.001; Fig. 1), with heating temperature having the greater effect (Table 1). The total polyphenol content of HOJ increased significantly with increased heating temperature (110 to 150°C) and time (1 to 5 hr; p<0.001). The total polyphenol content in raw onion extracts was 21.31±1.53 mg/100 g. The total polyphenol content of HOJ increased gradually with heating from 110 to 140°C for 2 hr and then decreased thereafter. Heating at 140°C for 2 hr increased the total polyphenol content of HOJ to 189.80±11.20 mg/100 g.

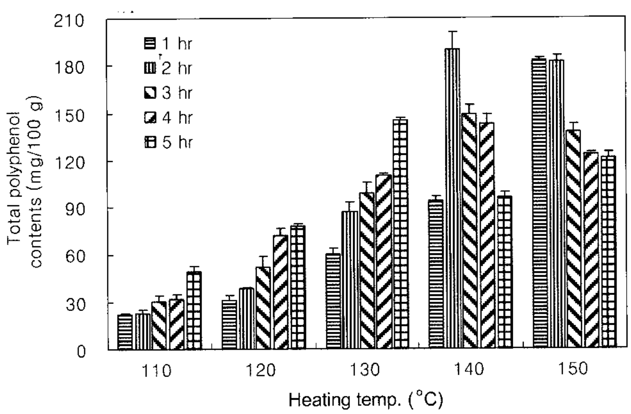

Fig. 1. Changes in the total polyphenol content of onion juice with heating temperature and time. (Raw onion: 21.31±1.53 mg/100 g)

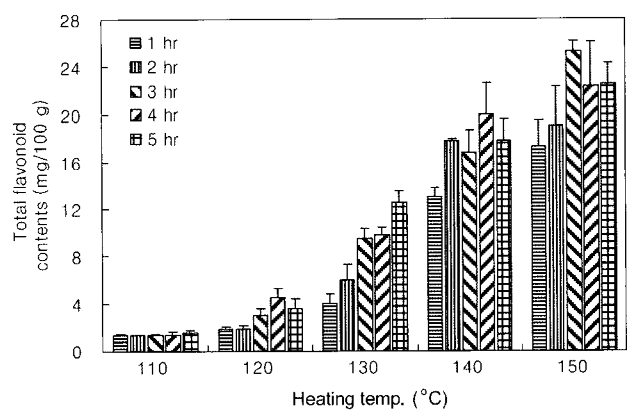

Fig. 2. Changes in the total flavonoid content of onion juice with heating temperature and time. (Raw onion: 11.72±1.34 mg/100 g)

Table 1. Analysis of variance for the polyphenol, flavonoid, and fructose contents, and the IC_{50} of the electron-donating ability (EDA, %) of heated onion juice

	Variable[1)]	df	Sum of square	Mean of square	F-value
Polyphenol	X_1	4	15.92	3.98	53.40***[2)]
	X_2	4	0.68	0.17	2.30***
Flavonoid	X_1	4	458,691	114,573	244.44***
	X_2	4	20,521	5,130	10.94***
Fructose	X_1	4	27.21	6.80	152.07***
	X_2	4	5.42	1.36	30.31***
IC_{50} of EDA(%)	X_1	4	2,558	640	47.19***
	X_2	4	488	122	9.01***

1)X_1, temperature (°C); X_2, time (hr).
2)***p<0.001.

The total flavonoid content was affected by heating temperature and time, similar to the total polyphenol content (Fig. 2, Table 1). The total flavonoid content in HOJ increased significantly with increased heat (110 to 150°C) and time (1 to 5 hr; p<0.001). The total flavonoid content in raw onion extracts was 11.72±1.34 mg/100 g. With heating, the total flavonoid content of HOJ increased to 13.25±1.46 mg/100 g and then to 252.51±9.32 mg/100 g. The highest total flavonoid content of 252.51±9.32 mg/100 g occurred with heating at 150°C for 3 hr. The total flavonoid content of HOJ gradually increased with increasing heating temperature and time to 150°C for 3 hr and then decreased thereafter. The total polyphenol and flavonoid contents of HOJ increased significantly with increased heating temperatures and times. Dewanto *et al.* (19) suggested that there is a significant increase in soluble phenolic compounds in sweetcorn after heating. Stewart *et al.* (20) reported that heat treatment increased the level of free flavonols because heating released bound phenolic compounds through the breakdown of cellular constituents (13). Our results are also consistent with those of Jeong *et al.* (21), who found that phenolic compounds in citrus peels can be liberated by heating.

Fructose content The fructose content of HOJ was affected more by heating temperature than by heating time (Fig. 3, Table 1). The fructose content of raw onion extracts was 2.65±0.36%. The fructose content did not differ significantly among conditions of 110 to 130°C for 2 hr, and was between 0.56±0.02 and 2.86±0.12%. However, the fructose content gradually decreased after heating at 130°C for 3 hr and further decreased on heating at 150°C for 5 hr (0.56±0.02%).

Heating causes non-enzymatic browning, nutrient loss, and the formation of undesirable products such as 5-hydroxymethyl-2-furaldehyde (22). However, novel compounds with antioxidant activities and pro-oxidative activities (i.e., Maillard reaction products), and interactions among different compounds (e.g., lipids and natural antioxidants, lipids and Maillard reaction products) can occur as a result of heating.

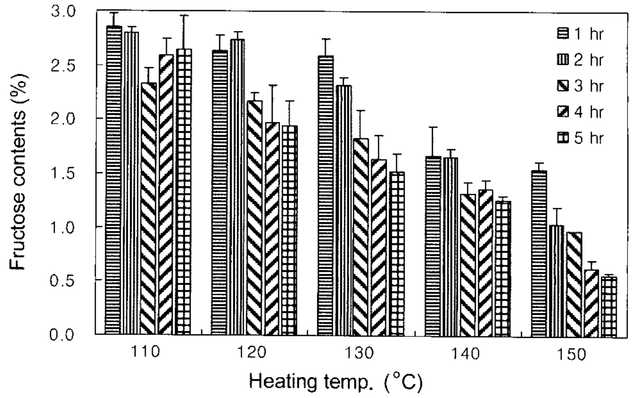

Fig. 3. Changes in the fructose content of onion juice with heating temperature and time. (Raw onion: 2.65±0.36%)

Radical scavenging The radical scavenging activity toward the stable free radical DPPH was evaluated for the onion extracts. The antioxidant activity of HOJ was expressed as the IC_{50} value, i.e., the concentration necessary for a 50% reduction in the DPPH radical (Table 2). The antioxidant activity of HOJ was significantly affected by heating temperature and time (Table 1), with temperature having a greater effect than exposure time. The antioxidant activity of HOJ increased significantly with increased heating temperature (110 to 150°C) and time (1 to 5 hr; p< 0.001). The IC_{50} of raw onion extract was 60.80±3.15 mg/mL. After heating, the IC_{50} decreased to between 35.15±1.72 and 2.19±0.23 mg/mL. The lowest IC_{50} was 2.19±0.23 mg/mL, which occurred with heating at 150°C for 2 hr. The IC_{50} of HOJ decreased gradually with heating at 110 to 150°C for 2 hr and then increased with time.

Several studies have reported the effects of heating on the antioxidant activity of various foods. For example, the antioxidant activities of tomato (7), *shiitake* mushroom (*Lentinus edodes*) (13), sweet corn (19), and citrus peels (21) increased, depending on the heating temperature and time. There were significant correlations between the antioxidant activity of HOJ and the total phenolic and total flavonoid content (r = -0.74 and -0.64, respectively; Table 3). Velioglu *et al.* (23) reported strong relationships between antioxidant activity and total phenolic content in several fruits, vegetables, and grain products. Other research

Table 2. IC_{50} values (mg/mL) indicating the electron-donating ability (EDA, %) of onion juice in relation to heating temperature[1)] (Raw onion: 60.80±3.15 mg/mL)

Time (hr)	Temperature (°C)				
	110	120	130	140	150
1	35.15±1.72	13.23±0.43	6.81±0.47	3.14±0.05	2.50±0.33
2	19.48±2.35	10.27±0.29	3.64±0.43	2.41±0.11	2.19±0.23
3	13.06±0.98	7.95±0.61	3.85±0.51	3.13±0.26	2.60±0.03
4	11.21±0.65	5.59±0.63	3.68±0.44	2.99±0.03	2.27±0.01
5	11.70±0.53	7.02±1.13	3.20±0.16	3.21±0.24	2.90±0.20

1)Values are means of triplicate±SD.

Table 3. Correlation coefficients for total polyphenol, flavonoid, and fructose contents, and the IC_{50} of the electron-donating ability (EDA, %) of heated onion juice

Factor	Polyphenol	Flavonoid	Fructose	IC_{50}
Polyphenol	1.00	$0.89^{***1)}$	-0.85^{**}	-0.74^{***}
Flavonoid	-	1.00	-0.94^{*}	-0.64^{***}
Fructose	-	-	1.00	0.71^{**}
IC_{50}	-	-	-	1.00

$^{1)*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

indicates that heating causes enhanced antioxidant activity in fruits and vegetables because of the enhancement of the antioxidant properties of naturally occurring compounds or the formation of novel compounds such as Maillard reaction products that have antioxidant activity (24, 25). Therefore, it is possible that the release of phenolic compounds from onion after heat treatment could increase its antioxidant activity. Further studies will be conducted to identify new antioxidant compounds in onions.

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