J. of Korean Oil Chemists' Soc.,
Vol. 32, No. 3. September, 2015. 418~426
ISSN 1225-9098 (Print)
ISSN 2288-1069 (Online)
http://dx.doi.org/10.12925/jkocs.2015.32.3.418

Analysis of Total Phenol, Flavonoid content and Antioxidant Activity of Various Extraction Solvents Extracts from Onion (*Allium cepa* L.) Peels

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(Received August 20, 2015; Revised September 1, 2015; Accepted September 3, 2015)

Abstract : Total phenol contents, total flavonoid contents and antioxidant activity of 70% methanol, 70% ethanol and chloroform-methanol (CM, 2:1, v/v) extracts from onion (*Allium cepa* L.) peels were studied. The IC_{50} values of DPPH (2,2-diphenyl-1-picrylhydrazyl) radical and ABTS [2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt] radical scavenging activity in 70% ethanol extract were remained to be lowest followed by 70% methanol extract and CM extract. And the total phenol content (113.56±0.86 mg CAE/g), total flavonoid content (49.63 mg QE/g) and ferric reducing antioxidant power value were also found to be the highest. In contrast, 70% methanol extract possessed the strongest antioxidant activity by β-carotene bleaching assay. CM extract displayed the lowest antioxidant activity compared with other extracts. Onion peels exhibited strong antioxidant activity and abundant phytochemicals, which could be used in a various food products to add phytochemicals and promote good health.

Keywords : onion (Allium cepa, L.) peels, total phenol, flavonoid, antioxidant activity

1. Introduction

The overproduction of oxidative radicals such as reactive oxygen species (ROS) or reactive nitrogen species (RNS) results in oxidative stress, which can cause many human diseases, including cancer, aging, atherosclerosis, coronary heart diseases and neurodegenerative diseases [11,12,17]. The using of antioxidant system such as glutathione (GSH), Se, vitamin C, vitamin E, superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) can help to eliminate the excessive oxidative radicals [5]. Fruit and vegetables contain not only essential nutrients needed for daily life but also a wide variety of bioactive compounds (antioxidant phytochemicals) for health promotion and disease prevention [15,19].

Onions (*Allium cepa* L.) have served as an important dietary resource and provide essential nutrients to human. Recently, the consumption of onion has attracted people's interest due to its special flavor and health

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benefits [20]. The biological effects are summarized as antioxidant activity, antimicrobial, antibacterial and ant-inflammatory activities [31]. In several clinical studies, onion and onion extracts decreased blood lipid levels, increased fibrinolysis, decreased platelet aggregation, and lowered blood pressure [25]. Previous studies have found that outer layer of red onion had the highest antioxidants and antioxidant activities compare with the purple, white and green varieties of onion, as determined by *in vitro* antioxidant and free radical scavenging activities [27]. Onion peels contain significantly higher levels of flavonoids than the edible portion of the vegetables. The primary flavonoids include quercetin diglucoside, quercetin 4'-glucoside and quercetin aglycone, and in some cases, isorhamnetin monoglycoside or kaempferol monoglycoside [26]. Quercetin was conducted to show antioxidant activity, antibacterial, anti-inflammatory activity, antihistamine effect, allergy medication and anticancer and antivirus activities [3,20].

The significance of onions intake as a good dietary source of natural antioxidants for preventing some diseases have already been confirmed. Although onion peels are a waste product of daily diet, their value in antioxidant activity has attracted our attention. In the current study, the total phenol contents, total flavonoid contents and antioxidant activity of 70% methanol, 70% ethanol and chloroform-methanol (CM, 2:1, v/v) extracts from onion peels were evaluated. The antioxidant activity was assayed by DPPH radical, ABTS radical scavenging activity, ferric reducing antioxidant power and β-carotene bleaching assays.

2. Materials and Methods

2.1. Materials

Onions (*Allium cepa* L.) were purchased from Eomgung market (Busan, Korea). Onions were peeled and ground with a blender (HMF-3250S, Hanil Electric Co., Seoul, Korea). The onion peel flours were stored at -80℃ (SW-UF-400, Sam-Won Co., Busan, Korea) for further analyses.

2.2. Preparation of onion peel extracts

Onion peel flours were mixed with various extraction solvents including 70% methanol, 70% ethanol and chloroform-methanol mixture (CM, 2:1, v/v) in a ratio of 1:10 and kept in the dark about 24 h, and then used the Advantec No. 1 filter paper (Tokyo, Japan) to filter. The process of extraction was repeated 3 times. The filtrate was evaporated by rotary vacuum evaporator (EYELA, N-N series, Tokyo, Japan) until the solvents were completely removed. The extracts were collected and sealed in brown reagent bottles and frozen at -80℃ until required for further analyses.

2.3. Measurement of total phenol contents (TPC)

The total phenol contents were determined by using Folin-Denis' phenol reagent and caffeic acid was used as a standard for the calibration curve [7,14]. In brief, samples (0.5 mL) were mixed with 3.0 mL of distilled water and 0.5 mL of Folin-Denis' phenol reagent in the test tubes. After incubated for 3 min, 0.5 mL of 10% sodium carbonate (w/v) was added. Then the mixture was incubated in the temperature for 60 min. The absorbance of the reaction mixture was measured at 700 nm using uv/vis-spectrophotometer (Specord 200, Analytikjena, Jena, Germany). Total phenol contents were expressed as mg of caffeic acid equivalents per g of extract (mg CAE/ g extract).

2.4. Measurement of total flavonoids content (TFC)

Total flavonoid content was determined by aluminum chloride colorimetric method with some modifications [6,8]. Samples (0.5 ml)

were mixed with 0.5 ml of 10% aluminum nitrate enneahydrate, 0.5 ml of 1 M sodium acetate and 2.0 ml of 80% ethanol (v/v). After this step, reaction mixture was incubated at room temperature for 40 min, and then the absorbance was read at 415 nm. Quercetin was used as a standard. Total flavonoids contents were expressed as mg of quercetin equivalents per g of extract (mg QE/g extract).

2.5. DPPH radical scavenging activity assay

DPPH radical scavenging activity was measured according to the method of Blois and Duan et al [1,8]. Samples (2.0 mL) were mixed with 0.4 mM DPPH (2.0 mL) and then vigorously shaken. The mixture solution was stood in the dark for 30 min at 37℃ water bath. Ascorbic acid was used as positive control. Then the absorbance of the reaction mixture was read with spectrophotometer at 517 nm. The percentage inhibition of DPPH radical scavenging activity was calculated based on the control reading using the following calculation:

DPPH radical scavenging activity (%)

$$= (1-\frac{A_s}{A_c}) \times 100$$

where A_s is the absorbance in the presence of sample or BHA, and A_c is the absorbance of control reaction.

2.6. ABTS radical scavenging activity assay

ABTS radical scavenging activity was evaluated according to the method of Sun et al [28]. The mixture of 15 mL of 7 mM ABTS and 15 mL of 2.45 mM potassium persulfate was stored at room temperature in the dark for 16 h to get the green-blue free radical $ABTS^{\bullet+}$. Then the solution was diluted with ethanol until the absorbance was 0.7±0.02 at 734 nm. Samples (0.1 mL) were mixed with 2.9 mL of ABTS working solution. After 10 min of reaction, the absorbance was taken at 734 nm. Ascorbic acid was used as positive control. The percentage of ABTS radical scavenging effect was calculated as follow:

ABTS radical scavenging effect (%)

$$= (1-\frac{A_s}{A_c}) \times 100$$

where A_s is the absorbance in the presence of sample or BHA, and A_c is the absorbance of control reaction.

2.7. Ferric reducing antioxidant power (FRAP) assay

The working FRAP reagent was prepared by mixing 10 mL of 0.3 M sodium acetate buffer (pH 3.6), 1 mL of 10 mM TPTZ in 40 mM hydrochloride acid and 1 mL of 20 mM ferric chloride. The freshly prepared FRAP reagent (3.0 mL) was mixed with 0.2 mL of sample solution. After incubated at 37℃ water bath for 30 min, the absorbance was read at 593 nm. Ascorbic acid was used as the positive control. The FRAP values were expressed as the absorbance of samples [4,9].

2.8. β-carotene bleaching assay

The antioxidant activity of different extract was evaluated according to the β-carotene bleaching method following the method of Elzaawely et al [10]. In brief, a solution of β-carotene was prepared by dissolving 1 mg of β-carotene in 10 mL of chloroform. One milliliter of this solution was then added to a round-bottomed flask containing a mixture of 20 mg linoleic acid and 200 mg Tween 40. After the chloroform was removed under vacuum using a rotary evaporator at 40℃, 100 mL of aerated distilled water were added to the flask with vigorous shaking. The emulsion obtained was freshly prepared before experiment. An aliquot (3.0 mL) of the β-carotene-linoleic acid emulsion was mixed with 0.3 mL of sample extracts, positive control standards (ascorbic acid). Then the mixture was incubated at 50℃ for 120 min. Absorbance readings were performed

immediately (t=0 min) and after 120 min of incubation at 470 nm with. Antioxidant activity (AOA) was calculated using the following formula:

$$= (1-\frac{A_0 - A_{120}}{A'_0 - A'_{120}}) \times 100$$

A_0 and A'_0 are the initial absorbance of sample and control, whereas A_{120} and A'_{120} are the absorbance of sample and control after 120 min.

2.9. Statistical analysis

The experimental data in triplicate were subjected to analysis of variance (ANOVA) and expressed as mean±SD (n=3). ANOVA was performed by using the one-way analysis of variance procedures. Duncan's multiple-range test was used to analysis the significant difference of means, and $p<0.05$ was considered to be statistically significant for all statistic procedures. IBM SPSS statistic 21 program was used for data analysis.

3. Results and Discussion

3.1. Yields

The various extraction yields of onion peels by 70% methanol, 70% ethanol and chloroform-methanol (CM, 2:1, v/v) were shown in Table 1. The extraction yield by 70% methanol was found to be the highest (11.16%), followed by 70% ethanol (9.39%) and CM (4.43%).

3.2. Total phenol contents (TPC)

Phenolic compounds known to be antioxidants play the very important role of protecting organisms against harmful effects of oxygen radicals and other highly reactive oxygen species [29]. The scavenging ability of phenols is mainly due to the phenolic structure of hydroxyl substituent on the aromatic ring [22]. Furthermore, a positively and highly significant relationship between total phenolics and antioxidant activity was documented by Velioglu et al [30], which implied a compound with higher content of phenol possessed higher antioxidant activity. Total phenol contents were determined by according to the colorimetric Folin-Denis' method with caffeic acid as a stand compound ($y=0.1141x+0.0076$, $R^2=0.9941$). The total phenol contents of

Table 1. Extraction yields, total phenol contents, total flavonoid contents and IC_{50} values in the antioxidant activity evaluation assays of onion (*Allium cepa* L.) peels

Assays	70% methanol	70% ethanol	CM[2)]
Extraction yields (%)	11.16	9.39	4.43
Total phenol content (mg GAE/g)	$94.24\pm0.59^{b3)}$	113.56 ± 0.86^{c}	85.95 ± 1.57^{a}
Total flavonoid content (mg QE/g)	43.33 ± 0.41^{b}	49.63 ± 0.55^{c}	27.57 ± 0.35^{a}
DPPH[1)] (IC_{50}, mg/mL)	0.08 ± 0.00^{b}	0.05 ± 0.00^{a}	0.12 ± 0.00^{c}
ABTS (IC_{50}, mg/mL)	0.46 ± 0.01^{b}	0.43 ± 0.00^{a}	0.62 ± 0.01^{c}
β-carotene bleaching (IC_{50}, mg/mL)	0.12 ± 0.02^{a}	0.65 ± 0.06^{b}	0.89 ± 0.14^{c}

[1)] DPPH radical scavenging activity (DPPH), ABTS radical scavenging activity (ABTS) and β-carotene bleaching assays.
[2)] CM: chloroform-methanol mixture (2:1, v/v).
[3)] The values are means±SD (n=3). Values with the different letters in the same row are significantly different ($p<0.05$) by Duncan's multiple range tests.

onion peels by different extraction solvents (70% methanol, 70% ethanol and CM) were showed in Table 1. Total phenol contents of various extracts decreased in the order: 70% ethanol extract (113.56±0.86 mg CAE/g), 70% methanol extract (94.24±0.59 mg CAE/g) and CM extract (85.95±1.57 mg CAE/g), respectively. And the total phenol contents of various extracts from red onion peel showed wide variation from 23.1±0.9 to 384.7±5.0 mg GAE/g extract [27].

3.3. Total flavonoid contents (TFC)

Flavonoids have long been suggested to show antioxidant capacity and antiallergic, antiinflammatory, antiviral, antiproliferative and anticarcinogenic activities [13,19]. The content of flavonoid in extracts from various extraction solvents were calculated in accordance with the calibration curve of quercetin (y=0.1092x+0.0034, R^2 =9996). As can be seen in Table 1, total flavonoid contents of various extracts increased in the order: CM extract (27.57±0.11 mg QE/g), 70% methanol extract (43.33±0.41 mg QE/g) and 70% ethanol extract (49.63±0.55 mg QE/g), respectively. Total flavonoid content was determined to range from 165.2±3.2 to 1.3±0.21 mg QE/g extract in the five fractions of red onion peel [27].

3.4. DPPH radical scavenging activity

DPPH is a stable free radical and can be scavenged by antioxidants through donating hydrogen. The discoloration from purple to yellow induces the absorbance of reaction mixture decreases at 517 nm [16]. Fig. 1 described the DPPH radical scavenging abilities of various extracts appeared to be associated with increasing concentrations (0.2 mg/mL, 0.4 mg/mL and 0.6 mg/mL). All extracts exhibited excellent DPPH radical scavenging ability even if their effects were lower than that of ascorbic acid. Results showed that the antioxidant activity of various extracts decreased in the following order: 70% ethanol extract (IC_{50}=0.05±0.00 mg/mL), 70% methanol extract (IC_{50}=0.08±0.00 mg/mL) and CM extract (IC_{50}=0.12±0.00 mg/mL) (Table 1). The DPPH radical scavenging activity of methanolic extract from interior and outer leaves onion was found to be 92.00±1.99% and 93.45±0.42%, respectively. Correlation between total phenol content, total flavonoid content and DPPH radical scavenging activity in all extracts was could be observed. This founding was in keeping with the reports of Do et al [6].

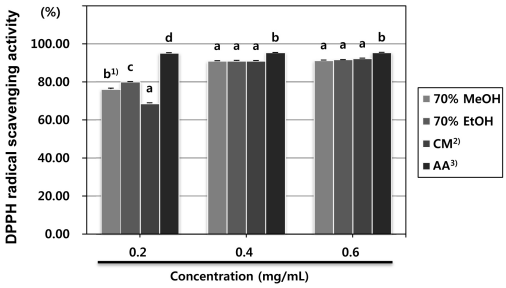

Fig. 1. DPPH radical scavenging activity of various extracts from onion (*Allium cepa* L.) peels.

1) The values are means±SD (n=3). Bars with the different letters are significantly different ($p<0.05$) by Duncan's multiple range tests.

2) CM: chloroform-methanol mixture (2:1, v/v) extract.

3) AA: ascorbic acid.

3.5. ABTS radical scavenging activity

The radical-cation $ABTS^{\bullet+}$ is produced by the oxidation of ABTS. In the absence of antioxidants, ABTS is rather stable, but it reacts actively with an H-atom donor (i.e. phenolics). Therefore, the blue/green chromophore would discolor gradually or be converted into a non-colored form of ABTS up to the antioxidant capacity of antioxidants [21]. Fig. 2 and Table 1 showed the inhibitory effect of various extracts on ABTS radical. As can be seen from results, ABTS radical scavenging activity was marked and

concentration-related. The IC_{50} values of different fractions increased in the following order: 70% ethanol extract (IC_{50}=0.43±0.00 mg/mL), 70% methanol extract (IC_{50}=0.46±0.01 mg/mL) and CM extract (IC_{50}=0.62±0.01 mg/mL), respectively. Obviously, the 70% ethanol extract exhibited the strongest scavenging activity against ABTS radical. Our results showed that the ABTS radical scavenging activity of onion peel extracts depended on their total phenol and flavonoid composition in a quantitative way, which was in consistent with the results of Velioglu et al [30]. It was reported that quercetin and kaempferol and their glycosides were the most abundant flavonoids in the acid hydrolysed samples of onion [18]. Earlier studies have shown quercetin is the most effective $ABTS^{\cdot+}$ radical scavenger, followed by myricetin, rutin, luteolin, apigenin, and kaempferol [23].

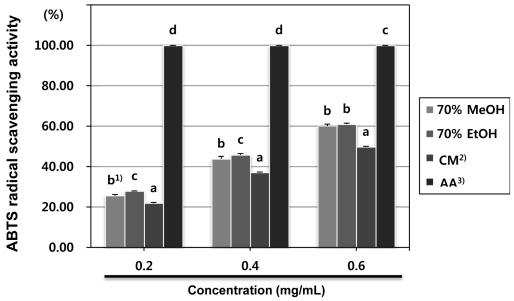

Fig. 2. ABTS radical scavenging activity of various extracts from onion (*Allium cepa* L.) peels.

1) The values are means±SD (n=3). Bars with the different letters are significantly different ($p<0.05$) by Duncan's multiple range tests.

2) CM: chloroform-methanol mixture (2:1, v/v) extract.

3) AA: ascorbic acid.

3.6. Ferric reducing antioxidant power (FRAP)

Antioxidant potential of different fractions was estimated from their ability to reduce the ferric-tripyridyltriazine ($Fe^{Ⅲ}$-TPTZ) complex to ferrous-tripyridyltriazine ($Fe^{Ⅱ}$-TPTZ) at low pH, forming an intense blue color with an absorption maximum at 593 nm develops [4]. The antioxidant activities through the ferric reducing antioxidant power model system of onion peels extracts at 0.2 to 0.6 mg/mL concentrations compared with ascorbic acid were presented in the Fig. 3. The results revealed concentration-dependent ferric reducing antioxidant activities in all the tested concentrations of various extracts. Ascorbic acid showed significantly higher FRAP values than other extracts. At a concentration of 0.6 mg/mL, the highest FRAP value was found in 70% ethanol extract (2.12±0.10), followed by 70% methanol extract (1.75±0.13) and CM extract (1.73±0.03). There was no significant difference between 70% methanol extract and CM extract. Bahorun et al [2] has reported the antioxidant activity of onion (8.47±1.86 μmol Fe^{2+}/ g fresh weight) assessed by FRAP assay was stronger than that of mugwort, tomato and carrot. A correlation between FRAP value and total phenol content could

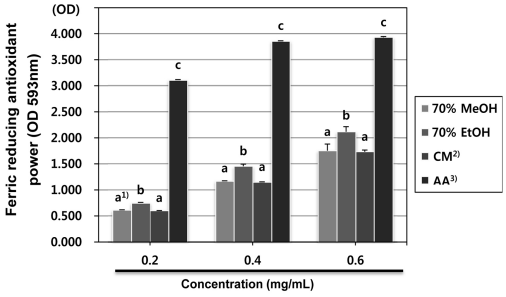

Fig. 3. Ferric reducing antioxidant power of various extracts from onion (*Allium cepa* L.) peels.

1) The values are means±SD (n=3). Bars with the different letters are significantly different ($p<0.05$) by Duncan's multiple range tests.

2) CM: chloroform-methanol mixture (2:1, v/v) extract.

3) AA: ascorbic acid.

also be observed from 70% ethanol extract. This significant correlation was in accordance with the reports of the Santas et al [24], who found total phenol contents of onion extracts strongly correlated with their antioxidant activities evaluated by FRAP assay.

3.7. β-carotene bleaching assay

The antioxidant activities of various extracts at 0.2 mg/mL to 0.6 mg/mL concentrations compared with ascorbic acid measured by the bleaching of β-carotene were presented in Fig. 4. The highly unsaturated β-carotene molecules in this system can be attacked by free radicals generating from the oxidation of linoleic acid, and as a consequence, the characteristic orange color disappears. The presence of antioxidant can avoid the destruction of the β-carotene by neutralizing the free radicals formed in the system to keep the orange color [10]. As depicted in Fig. 4, the results exhibited concentration-dependent antioxidant activity by β-carotene bleaching method in all the tested concentrations of various extracts. The antioxidant activity of various extracts was found to decrease in the following order: 70% methanol extract (IC_{50}=0.12±0.02 mg/mL), 70% ethanol extract (IC_{50}=0.65±0.06 mg/mL) and CM extract (IC_{50}=0.89±0.14 mg/mL) (Table 1). Although ascorbic acid always showed the most effective antioxidant activity, 70% methanol extract displayed stronger antioxidant activity compared with other extracts. It was probable that the antioxidative components in extracts can reduce the extent of β-carotene destruction by neutralizing the linoleate free radical and other free radicals in this system. Correlation between total phenol content of onion extracts and antioxidant activity was not found. This result was in agreement with the founding of Ismail et al [14], who also found although the amounts of total phenolic in shallot bulb were lower than other vegetables, they had the highest antioxidant activity.

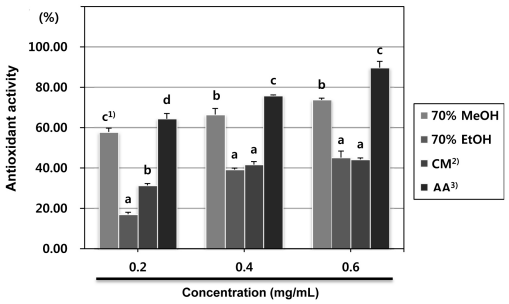

Fig. 4. Antioxidant activity of various extracts from onion (*Allium cepa* L.) peels by using β-carotene bleaching method.

1) The values are means±SD (n=3). Bars with the different letters are significantly different ($p<0.05$) by Duncan's multiple range tests.

2) CM: chloroform-methanol mixture (2:1, v/v) extract.

3) AA: ascorbic acid.

4. Conclusion

There is currently an upsurge of interest in phytochemicals as new sources of natural antioxidants. The aim of this study is to investigate the antioxidant activity of 70% methanol, 70% ethanol and chloroform-methanol (CM, 2:1, v/v) extracts from onion (*Allium cepa* L.) peels. The correlation between total phenol contents, total flavonoid contents and their antioxidant activity measured in DPPH radical scavenging activity, ABTS DPPH radical scavenging activity and ferric reducing antioxidant power (FRAP) could be found clearly. In these assays, total phenol contents, total flavonoid contents and antioxidant activities of various extracts decreased in the following order: 70% ethanol extract, 70% methanol extract and CM extract. However, 70% methanol extract possessed the strongest antioxidant activity followed by 70% ethanol extract and CM extract in the β-carotene bleaching assay. In conclusion, onion peels possessed abundant

total phenols, flavonoids and strong antioxidant activity. From the nutritional point of view, the results of this study indicated that regular consumption of onion peel could be recommended to use to promote health benefits for consumers such as reduced risk of cardiovascular diseases and cancer.

Acknowledgement

This work was supported by a 2-Year Research Grant of Pusan National University.

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