

ANTIOXIDANT ACTIVITY AND HPLC ANALYSIS OF LYCOPENE, B-CAROTENE AND A-TOCOPHEROL FROM GEUK (*MOMORDICA COCHINCHINENSIS* SPRENG) FRUIT

Miran Jang and Gun-Hee Kim*

Plant Resources Research Institute, Duksung Women's University, Seoul 132-714, Korea

Abstract

Geuk (Momordica cochinchinensis spreng) tropical fruits, which are produced in Jeju Island, Korea, well known for containing high levels of carotenoids and α -tocopherol. In this study, geuk fruits were separated two parts (pulp; yellow-orange and seed aril; red). The extracts of geuk fruits were simultaneously analyzed for carotenoids and α -tocopherol contents using HPLC. The results showed that lycopene contained as the highest content in both seed aril and pulp and seed aril (846.84 $\mu\text{g/g}$) had lycopene two times more than pulp (390.15 $\mu\text{g/g}$). On the other hand, β -carotene of pulp (29.43 $\mu\text{g/g}$) was seven times more than seed aril (211.32 $\mu\text{g/g}$). α -Tocopherol was contained higher in seed aril (252.15 $\mu\text{g/g}$) than pulp (201.60 $\mu\text{g/g}$). The extracts and major components of geuk fruits (β -carotene, lycopene and α -tocopherol) were evaluated antioxidant activity using DPPH and ABTS assay. In both tested antioxidant assay, the seed aril extract showed stronger activity than the pulp extract. Lycopene, was more efficient than β -carotene, was active similar with α -tocopherol. Owing to the geuk fruits have powerful antioxidants, such as lycopene, β -carotene and α -tocopherol, which suggest that geuk fruits in the diet or in functional food products might provide greater health beneficial effects.

Key words: *Geuk (Gac, Momordica cochinchinensis spreng)*, carotenoids, α -tocopherol (vitamin E), antioxidant activity

INTRODUCTION

In Asia, tropical areas are restricted mainly to the Southeast region, however, because of continuous climate change, tropical areas are expanding northward. Therefore, many scientists of Korea are studying for cultivation techniques and utilizations of tropical fruits the preparation against global warming. Several tropical fruits and vegetables were introduced to Korea and recently, the cultivation of tropical fruit, geuk had been to succeed in Jeju Island, Korea.

Geuk (*Gac, Momordica cochinchinensis Spreng*) is called a fruit from heaven and belongs to the Cucurbitaceae (melon) family and is an indigenous fruit of Vietnam. The red seed arils (membrane) of the ripe geuk fruits are mainly used in cooking 'Xoi gac' (traditional dish when ceremonial occasions), as a colorant and many parts of geuk are being used, such as the seeds, and the oil or root as a component of traditional medicine in Vietnam (Dang et al., 2010).

Geuk fruits are extraordinary sources of carotenoids (β -carotene and lycopene), mainly in the red seed aril. Moreover, Geuk fruits comparatively contain high concentrations of vitamin E (Vuong et al., 2006). Carotenoids and tocopherols are very well known to be beneficial to the human health. Several carotenoids (α -carotene, β -carotene and lycopene) and tocopherols (vitamin E) are two important groups of a natural source of antioxidants (Kim et al., 2007). Epidemiological studies have shown that high intakes of fruits and vegetables are associated with a lower incidence of chronic diseases, including cardiovascular disease (Bazzano et al., 2002). Also, α -carotene, β -carotene, and β -cryptoxanthin have been suggested as precursor vitamin A, which is important to maintain healthy skin, bone, gastrointestinal, and respiratory systems (de Pee and West, 1996). Tocopherols are vitamin E analogues, they function as the main antioxidants in biological lipid cell membranes, protecting against various free radicals and reactive oxide species, as well as preventing oxidative damage to tissues (Hosomi et al., 1997).

There has been limited research has been carried out on the geuk fruits produced in the native tropical regions, even there is no study on geuk fruits introduced and cultivated in Korea. Biosynthesis of carotenoids is affected by the result of photosynthesis, because, even if same botanical, The contents of carotenoids may also differ depending on local environment (Crozier et al., 2006). Thus, in the food science field, many researches on nutrient contents and biological effects of nonnative tropical geuk fruits of Korea are necessary. Many previous

studies have reported that major compounds from geuk fruit which is lycopene, α -carotene, β -carotene and tocopherols. However, there is not many health benefits in geuk fruits, in particular, the information on the contribution of carotenoids and tocopherols in ripe fruits of geuk fruit to antioxidant effect is limited. In the present study, fresh ripe geuk fruits were separated into two parts (pulp; yellow-orange and seed aril; red) and we simultaneously determined nutrient contents (carotenoids and tocopherol) of different parts of the geuk fruit, using HPLC and evaluated the antioxidant effect by using ABTS, DPPH and FRAP assay.

MATERIALS AND METHODS

Geuk Fruits Collection and Preparation

Geuk fruits were harvested in Agricultural Research Center for Climate Change (National Institute of Horticultural and Herbal Science, Rural Development Administration, Jeju, Korea) in February, 2011 and were transported to the laboratory within 24 h. Among the fully ripe fruits, three fruits were selected to be used in this experiment, which were randomly selected. All fruits were separated into two parts (Fig. 1, pulp and seed aril) and then lyophilized. All dry geuk fruits were ground into powder and stored at -20°C until analysis.

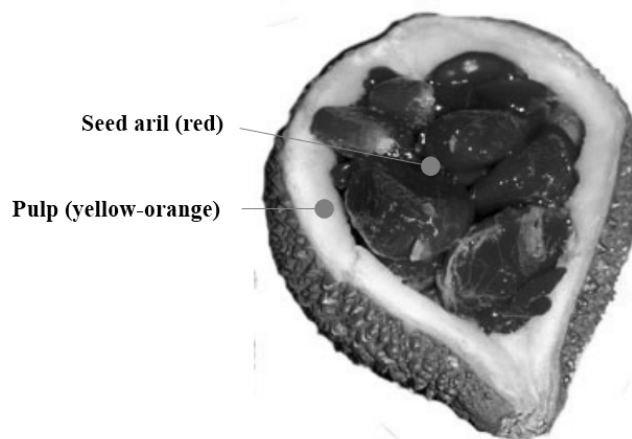


Figure 1. Ripe geuk fruit

Chemicals and Standards

Acetonitrile (ACN), tetra hydro furan (THF), methanol (MeOH) and Hexane (all HPLC grad) were purchased from Millipore (Bedford, MA, USA). Butylated hydroxyl toluene (BHT), Vitamin C (ascorbic acids), carotenoids (α -carotene, β -carotene, β -cryptoxanthin and lycopene) and tocopherols (α -tocopherol and γ -tocopherol) standards, pyrogallol, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), ferric chloride and 4,6-tripryridyls-triazine (TPTZ) were obtained from Sigma Aldrich Co. (St. Louis, MO). Stock solutions of all carotenoids and tocopherols standards were prepared in HPLC-grade water, at a concentration of $1000\text{ }\mu\text{g/mL}$ and stored at -70°C . The working solutions that were used to establish calibration curves were prepared by diluting the stock solutions with HPLC-grade water to the concentrations of 0, 50, 125, 250 and $500\text{ }\mu\text{g/mL}$. These curves, constructed with five different concentrations for each carotenoid, each concentration in triplicate should pass through or very near the origin, and be linear with a correlation coefficient ≥ 0.99 .

Extraction of lipophilic fractions

The extraction of carotenoids must be carried out very quickly, avoiding exposure to light, oxygen and high temperatures. Moreover, to prevent carotenoid losses, during the extraction procedure, the addition of antioxidants, such as ascorbic acid, pyrogallol and BHT, has been recommended (van Breemen et al. 2002, Rodríguez-Bernaldo de Quirós and Costa 2006). In this study, carotenoids and tocopherol were extracted using a method modified (van Breemen et al., 2002; Teow et al., 2007). A total of 200 milligrams of lyophilized samples

were homogenized in 2 mL of 80% ethanol containing 2% pyrogallol. Hexane (2 mL) containing BHT (100 mg/L) was added to the mixture, followed by vortex mixing for 1 min, until the carotenoids upper hexane layer was visibly yellow. Each sample was centrifuged at 2000 rpm for 5 min. The upper hexane layer and lower ethanol layer were separated to each different glass tube. The extraction was repeated five times until the last fraction of hexane was devoid of color. To confirm the complete extraction, carotenoids and tocopherols contents of the lower ethanol layer were analyzed and were not detected. The sample evaporation to dryness was carried out using evaporator (EYELA, Tokyo Rikakikai Co., Japan) under 40°C. Then the lipophilic extract of pulp and seed aril were dissolved in methanol and used for analysis antioxidant property. The lipophilic extracts were reconstituted with 2 mL of methanol:acetonitril:tetrahydrofuran (50:45:5, v/v/v) and this mixture of solvents was used as a mobile phase in an isocratic high-performance liquid chromatography (HPLC) system to separate the carotenoids. The reconstituted samples were placed in autosampler vials, and then analyzed by HPLC.

HPLC Simultaneous Determination of Carotenoids and Tocopherol

Identification and quantification of carotenoids and tocopherols in Gauk fruits in and out part extracts were carried out using an HPLC (Dionex, Sunnyvale, CA, USA) on a Nova-Pak C18 (3.9 x 150 mm, 4 µm) column (Waters Corp., Milford, MA). Wavelengths of 450 and 290 nm were used for the determination of carotenoids and tocopherols, respectively. The mobile phase for separations of both carotenoids and tocopherols consisted of methanol:acetonitril:tetrahydrofuran (50:45:5, v/v/v) and a 20 µL volume was injected and eluted with the mobile phase described above at a flow rate of 1 mL/min.

MEASUREMENT OF ANTIOXIDANT ACTIVITY

DPPH assay

Antioxidant activities of the samples were also analyzed by investigating their ability to scavenge the DPPH[•] free radical (modified from Kilani et al., 2005; Chen et al., 2007). DPPH[•] has an intense violet color with a maximum absorbance at 517 nm, but turns colorless as unpaired electrons are scavenged by antioxidants. Using 96-well micro plate, reaction mixtures containing 100 µL of sample and 100 µL of 0.2 mM DPPH[•] (prepared in methanol) were placed for 30 min at room temperature. The reacted mixtures were recorded at 540 nm. When geuk fruit extract and carotenoids were mixed with DPPH solution, a dark brown color was produced, which interfered with the absorbance readings at 515 nm (optimum wavelength using DPPH assay). To reduce this interference, the wavelength was changed to 540nm where DPPH still had significant absorbance but the brown color did not show up. The scavenging activity was calculated, according to following formula: $(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}} \times 100$.

ABTS assay

Antioxidant activity was measured by improved Azinobis (ethylbenzothiazoline 6-sulphonic acid) radical scavenging (ABTS) method (Re et al., 1996), with minor modifications. Briefly, 7.0 mM ABTS (in 20 mM sodium acetate buffer, pH 4.5) and 2.45 mM potassium persulphate mixed for the production of ABTS cation (ABTS^{•+}) and kept in dark for 16-24 h at room temperature. ABTS^{•+} solution (dark blue-green radical) was diluted to an absorbance of 0.7 ± 0.01 at 734 nm with PBS. For sample analysis of 950 µL of the diluted ABTS^{•+} solution was added to 50 µL of ethanolic extracts and was mixed thoroughly. Reaction mixture was allowed to stand (10 min) in a dark at room temperature, after reaction, the mixture was then transferred to a 96-well micro plate and then absorbance was recorded at 734 nm. As unpaired electrons are sequestered by antioxidants in the sample, the test solution turns colorless and the absorbance at 734 nm is reduced. As DPPH assay, the scavenging activity was calculated, according to following formula: $(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}} \times 100$.

Statistical analyses

Statistical analyses about HPLC data were conducted using t-tests and about Antioxidant properties data were conducted, using ANOVA, followed by Duncan's test with SPSS ver. 19.0 statistical analysis software (SPSS, Chicago, IL, USA). All results are expressed as the mean of triplicate determinations $\pm$ standard deviation (SD). The confidence limits used in this study were based on 95% ($p < 0.05$).

RESULTS AND DISCUSSION

Carotenoids and Tocopherol Contents

A representation of the carotenoids and tocopherols profiles of geuk fruit were shown in Fig. 2 and Fig. 3. Under the HPLC conditions adopted, carotenoids and tocopherols were well separated and the peak eluted in the following order; lycopene, α -carotene and β -carotene at 450 nm and α -tocopherol at 290 nm. Two different parts of geuk fruits were analyzed, and the quantitative results are shown in Table 1. The major carotenoids were lycopene and β -carotene. Seed aril contained the highest concentration of lycopene was 846.84 $\mu\text{g/g}$. α -Carotene and β -carotene concentrations were 30.93 $\mu\text{g/g}$ and 29.43 $\mu\text{g/g}$, respectively. Among tocopherols, α -tocopherol was only detected, with the mean concentration of 252.15 $\mu\text{g/g}$. Pulp also was found to contain the highest amount of lycopene (390.15 $\mu\text{g/g}$) and contained relative amounts β -carotene (211.32 $\mu\text{g/g}$) and α -Carotene (71.58 $\mu\text{g/g}$). α -Tocopherol value of pulp was 204.60 $\mu\text{g/g}$.

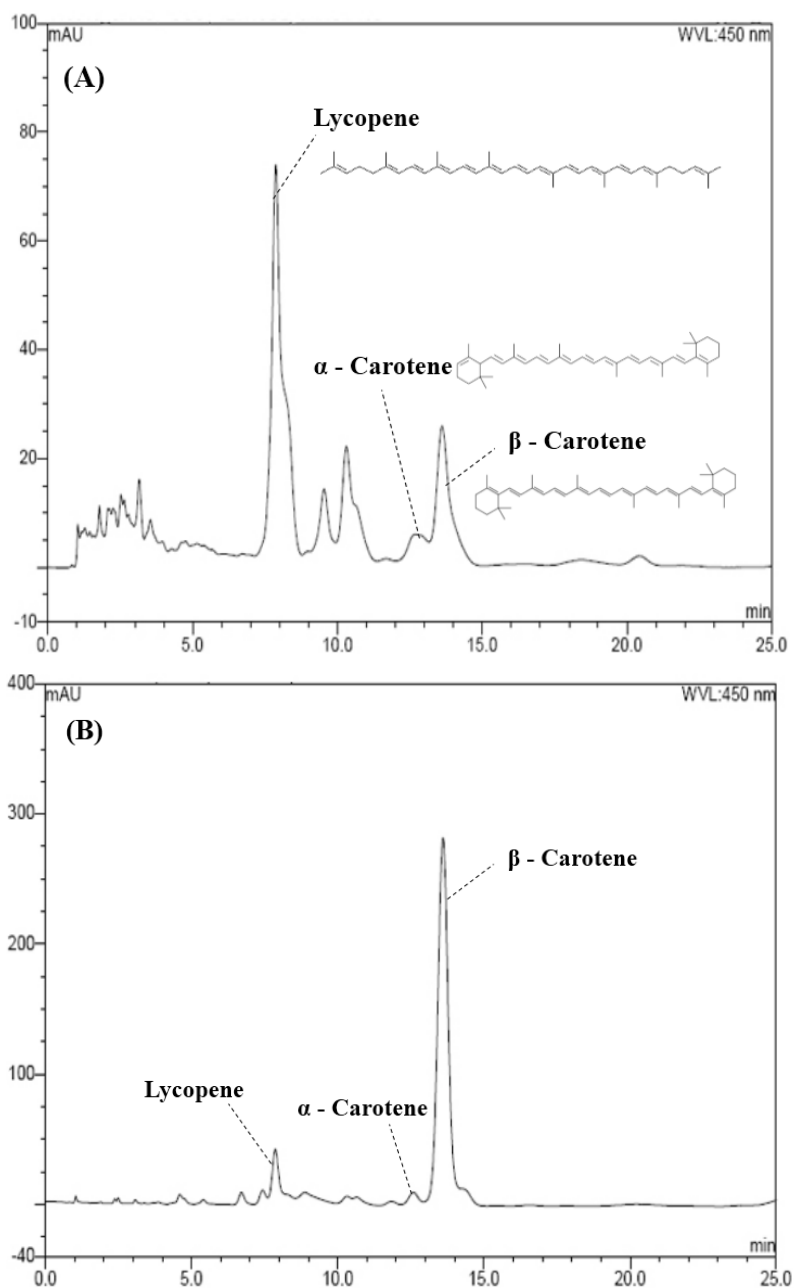
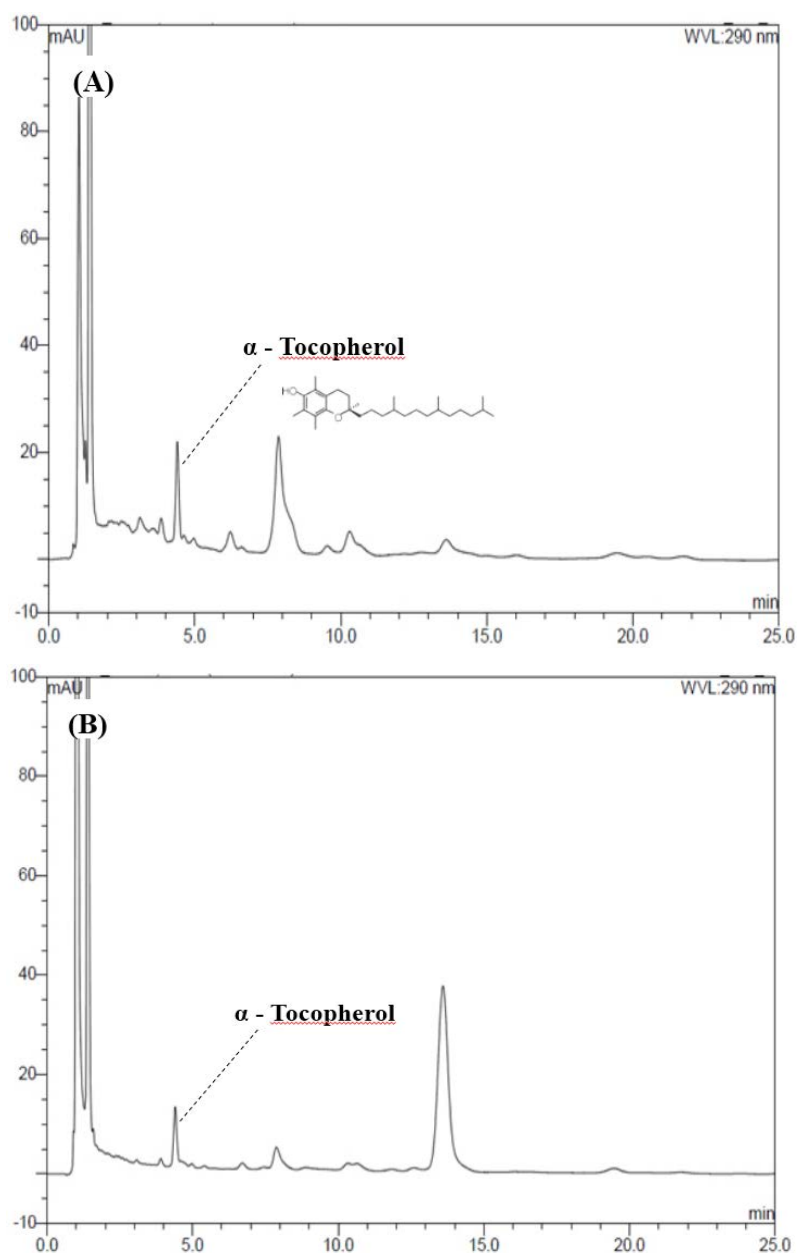


Figure 2. HPLC profile of carotenoids extracted from geuk fruits (A; Seed aril and B; Pulp)**Figure 3.** HPLC profile of α -tocopherol extracted from geuk fruits (A; Seed aril and B; Pulp)**Table 1.** Carotenoids and tocopherol contents of geuk ($\mu\text{g/g}$ of dry weight)

Carotenoids and tocopherol	Geuk fruits	
	Seed aril	Pulp
α -Carotene	$30.93 \pm 0.09^{\text{D}}$	71.58 ± 3.87
β -Carotene	29.43 ± 0.30	211.32 ± 60.94
Lycopene	846.84 ± 2.61	390.15 ± 7.08
α -Tocopherol (vitamin E)	252.15 ± 0.12	204.60 ± 1.02

1) Data are presented as the mean $\pm$ SD of triplicate determinations

Carotenoids of pulp, which contained significantly lower than seed aril and the α -tocopherol concentration was also significantly lower in the pulp than in the seed aril (Aoki et al., 2002; Ishida et al., 2004). Consistent with these observations, lycopene that was contained in the seed aril showed two folds more than that of the pulp. However, contrastively, other carotenoids more were contained in the pulp than in the seed aril. In particular, β -carotene was contained sevenfold more than the seed aril. Carotenoid content in fruits and vegetables depends on several factors, such as genetic variety, maturity, post-harvest storage, processing and preparation. The difference of carotenoids contents from geuk fruits could also be due to the ripening stage, since lycopene appears earlier than that of the β -carotene in the carotenogenesis pathway (Dang et al., 2010; Rodríguez-Bernaldo de Quirós A and Costa, 2006; Kubolola and Siriamornpun, 2011). Therefore, it is conceivable that less mature fruits contain higher lycopene and lower β -carotene concentrations than the more mature fruits. In addition, the discrepancies carotenoid levels might be due to a degradation of carotenoids, during extraction, analysis, transport, and storage as most are sensitive to light and heat (Bauernfield, 1971).

Several assays have been presented for the measurement of the antioxidant activity of single compound and/or complex mixture. The antioxidant effect of geuk fruits demonstrated by Kubola and Siriamornpun (2011) and Kha et al. (2010). And the major components of geuk fruits; lycopene, β -carotene, α -tocopherol and their mixture play a crucial role in determining the antioxidant properties (Liu et al., 2008; Chen et al., 2009). The carotenoids and α -tocopherol are important for human health because of their high pharmacological activities as radical scavengers.

Antioxidant activity

DPPH[•] radical scavenging activities

The DPPH[•] radical has been used as a stable free radical to determine the antioxidant capacity of natural compounds (Tayade et al., 2013). Antioxidant activities of geuk fruits extract and their single compounds were evaluated based on scavenging of DPPH[•] radical (Fig. 4). DPPH[•] radical scavenging activity was quantified in terms of percentage inhibition of a free radical by antioxidants in each sample. There was a significant variation in the percentage of inhibition of geuk fruits and seed aril extracts (38.14-69.35% inhibition) have stronger antioxidant activity than pulp extract (34.47-60.33% inhibition). Lycopene (92.05-93.09%) and tocopherol (92.57-92.59%) were shown more potent activity than β -carotene (70.66-85.15%), significantly. Geuk fruits and β -carotene extract obtained from each part effectively extinguished DPPH[•] radical in a dose-dependent manner, significantly. But the reaction of lycopene and tocopherol does not appear to be dose-dependant. Lycopene (92.05%) and tocopherol (92.57%) at the lowest concentration (0.5 μ g/mL) reached a scavenging capacity of maximum. These findings support the result previously reported in a study where the antioxidant activity was dependent on the actual composition of seed and pulp extracts (Kubolola and Siriamornpun, 2011). Seed extract have shown more potent antioxidant effect than pulp because seed aril have more lycopene and α -tocopherol which are very strong antioxidants than pulp.

In this free radical scavenging assay, it was previously recognized that the scavenging of DPPH[•] depended in past on the number of available hydroxyl groups on the antioxidant (Mensor et al., 2001). α -Tocopherol contains one hydroxyl group in its structure and where a hydrogen atom can be donated to the DPPH[•] free radical are useful antioxidants; whereas, lycopene and β -carotene not contain any hydroxyl groups. Liu et al. (2008) and Muller et al. (2011) could detect very low antioxidant activity of carotenoid compared with α -tocopherol. However, Chen et al. (2009) found higher antioxidant effect of lycopene than α -tocopherol. The activity of carotenoids increased with increasing the number of conjugated double bonds. Lycopene and β -carotene have eleven conjugated double bonds (Muller et al., 2011). Above all, lycopene has unique structural and chemical features that will contribute to its specific biological properties (Clinton, 1998; Liu et al., 2008). Lycopene is an acyclic hydrocarbon carotene with eleven double bonds in its structure, and is considered to be higher efficient quencher of singlet oxygen ($O_2^{\bullet}$) with β -carotene (Paiva and Russell 1999; Chen et al., 2009).

ABTS^{•+} radical scavenging activities

Using ABTS assay, geuk fruits extract obtained from each part effectively extinguished radical in a dose-dependent manner, significantly (Fig. 5). ABTS^{•+} radical scavenging activity was quantified in terms of percentage inhibition of the ABTS^{•+} radical cation by antioxidants in each sample. In the ABTS result, in common with the DPPH result, there was a significant variation in the percentage of inhibition of geuk fruits and seed aril extracts (64.22-92.90% inhibition) have stronger antioxidant activity than pulp extract (50.39-91.64%

inhibition). The extracts of two parts from geuk at 5 mg/mL have shown similar and/or higher activity than three single bioactive compounds, significantly. For ABTS^{•+} radical scavenging activity, the rank order of extracts and single bioactive compounds were similar to the DPPH results. In the antioxidant activity of major component, α -tocopherol (93.75-95.41%) have shown the best activity, and followed by lycopene (89.21-93.11%) and β -carotene (76.06-90.49%). Teow et al. (2007) reported that there is a poor correlation between β -carotene content and the antioxidant activities. B-Carotene content in the lipophilic extracts of geuk fruits correlated poorly with ABTS and DPPH assays and these results are similar.

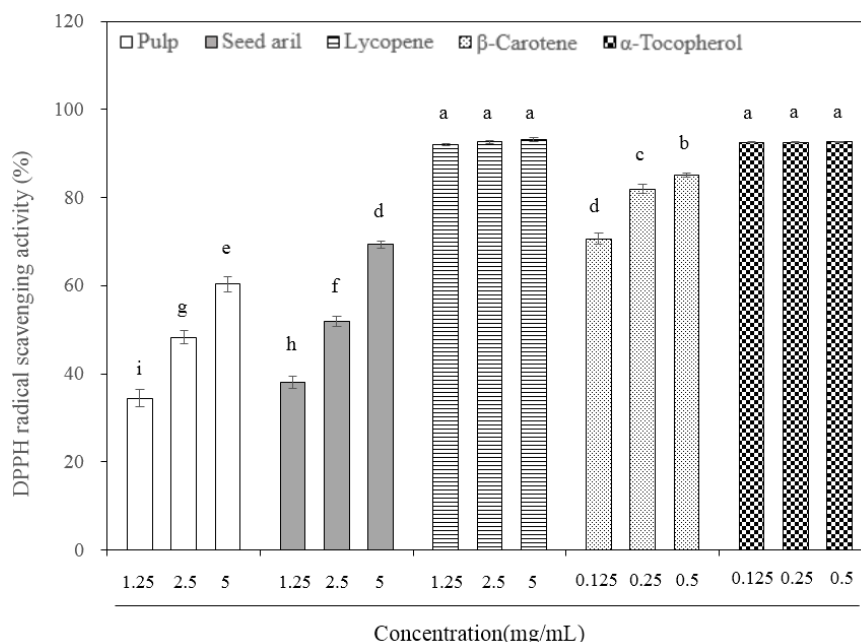


Figure 4. DPPH radical scavenging activities. The result are presented as mean $\pm$ S.D. of three determinations. Small letters a to i: values denoted by different capital letters are significantly different at $P < 0.05$.

The ABTS activities of all the clones studied were significantly higher than their DPPH values. The ABTS method can be used over a wide pH range (Prior et al., 2005), whereas the DPPH method is limited to neutral and higher pH applications. Additionally, the ABTS assay is much faster than the DPPH assay and was not affected by color interference, as mentioned above.

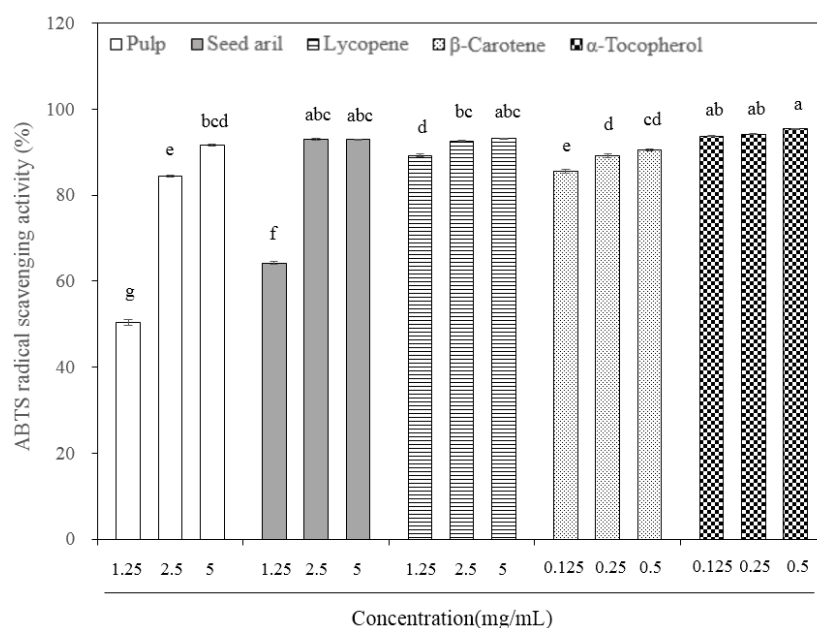


Figure 5. ABTS radical scavenging activities. The result are presented as mean $\pm$ S.D. of three determinations. Small letters a to g: values denoted by different capital letters are significantly different at $P < 0.05$.

CONCLUSION

In Korea, against global warming, tropical fruits of geuk cultivation techniques have been studied. Geuks are nutritious fruits which have high concentration of fat-soluble antioxidative compounds such as carotenoids and tocopherol. In particular, a lot of α -tocopherol, lycopene and β -carotene are found in seed aril and pulp of geuk fruits. Geuk fruits have shown strong antioxidant activities from DPPH and ABTS methods. The seed aril extract have more potent antioxidant activities than pulp extract because seed aril have more lycopene and α -tocopherol, strong antioxidants. Pulp extract was contained more β -carotene but, the activity of β -carotene was more weak than lycopene and α -tocopherol. These properties and the potential use of geuk fruits as health functional food will be explored in future studies.

ACKNOWLEDGMENTS

This work was supported by Priority Research Centers Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2009-0094017) and we gratefully acknowledge Dr. Ki-cheol Seong of Agricultural Research Center for Climate Change (Rural Development Administraion, Jeju, Korea) for donating geuk fruits.

REFERENCES

- Aoki, H., Kieu, N.T., Kuze, N., Tomisaka, K. & Chuyen, V.N. 2002. Carotenoid pigments in gac fruit (*Momordica cochinchinensis* Spreng). *Biosci Biotech Biochem* 20: 456–473.
- Bauernfield, J.C. 1971. Carotenoid vitamin A precursors and analogs in foods and feeds. *J Agric Food Chem* 20: 456–473.
- Bazzano, L.A., He, J., Ogden, L.G., Loria, C.M., Vupputuri, S., Myers, L. & Whelton, P.K. 2002. Fruit and vegetable intake and risk of cardiovascular disease in US adults: the first national health and nutrition examination survey epidemiologic follow-up study1–3. *Am J Clin Nutr* 76: 93–99.
- Chen, J., Shi, J., Macnughton, L., Kakuda, Y., Xue, S.J., Ma, Y. & Zhang, Y. 2007. The scavenging capacity of combinations of lycopene, β -carotene, vitamin E, and vitamin C on the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). *J Food Biochem* 33: 232–245.
- Clinton, S.K., 1998. Lycopene: chemistry, biology, and implications for human health and disease. *Nutr Rev* 56: 35–51.
- Crozier, A., Clifford, M.N. & Ashihara, H., 2006. Plant secondary metabolites. Chennai, Blackwell publishing. India, pp. 47–49.
- Dang, T.T.N., Pham, N.B., Nguyen, T.H. & Thai, K.P., 2010. Changes in lycopene and beta carotene contents in aril and oil of gac fruit during storage. *Food Chem* 121: 326–331.
- De Pee, S. & West, C.E., 1996. Dietary carotenoids and their role in combating vitamin A deficiency: a review of the literature. *Eur J Clin Nutr* 50: 38–53.
- Hosomi, A., Arita, M., Sato, Y., Kiyose, C., Ueda, T., Igarashi, O., Arai, H. & Inoue, K. 1997. Affinity for α -tocopherol transfer protein as a determinant of the biological activities of vitamin E analogs. *FEBS Lett* 409: 105–108.
- Ishida, B.K., Turner, C., Chapman, M.H. & McKeon, T.A. 2004. Fatty acid and carotenoid composition of Gac (*Momordica cochinchinensis* Spreng) fruit. *J Agric Food Chem* 52: 274–279.
- Kha TC, Nguyen & Roach PD. Effects of spray drying conditions on the physicochemical and antioxidant properties of the Gac (*Momordica cochinchinensis*) fruit aril powder. *J Food Eng* 98:385–392 (2010).
- Kilani S, Ammar RB, Bouhlef I, Abdelwahed A, Hayder N, Mahmoud A, Ghedira K, & Chekir-Ghedira L. Investigation of extracts from (Tunisian) *Cyperus rotundus* as antimutagens and radical scavengers. *Environ Toxicol Phar* 20:478–484 (2005).

- Kim YN, Giraud DW & Driskell JA. Tocopherol and carotenoid contents of selected Korean fruits and vegetables. *J Food Compos Anal* 20:458–465 (2007).
- Kubola J & Siriamornpun S. Phytochemicals and antioxidant activity of different fruit fractions (peel, pulp, aril and seed) of Thai gac (*Momordica cochinchinensis* Spreng) *Food Chem* [127](#):1138–1145 (2011).
- Liu D, Shi J, Jbarra AC, Kakuda Y & Xue SJ. The scavenging capacity and synergistic effects of lycopene, vitamin E, vitamin C, and β -carotene mixtures on the DPPH free radical. *LWT–Food Sci Technol* 1344–1349 (2008).
- Mensor LL, Menezes FS, Leitao GG, Reis AS, dos Santos TC & Coube CS. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytother Res* 15:127–130 (2001).
- Mortensen A & Skibsted LH. Importance of carotenoid structure in radical-scavenging reactions. *J Agric Food Chem* 45:2970–2977 (1997).
- Muller L, Theile K & Bohm V. In vitro antioxidant activity of tocopherols and tocotrienols and comparison of vitamin E concentration and lipophilic antioxidant capacity in human plasma. *Mol Nutr Food Res* 54:731–742 (2010).
- Mullar L, Frohlich K & Bohm V. Comparative antioxidant activities of carotenoids measured by ferric reducing antioxidant power (FRAP), ABTS bleaching assay (α TEAC), DPPH assay and peroxy radical scavenging assay. *Food Chem* 129:139–148 (2011).
- Paiva SA & Russell RM. Beta-carotene and other carotenoids as antioxidants. *J Am Coll Nutr* 18:426–433 (1999).
- Prior RL, Wu X & Schaich K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *J Agric Food Chem* 53: 4290–4302 (2005).
- Rawat S, Bhatt ID & Rawal RS. Total phenolic compounds and antioxidant potential of *Hedychium spicatum* Buch. Ham. ex D. Don in west Himalaya, India. *J Food Compos Anal* 24:574–579 (2011).
- Re R, Pellegrini N, Proteggente A, Pannala A, Yang M & Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med* 26:1231–1237 (1999).
- Rodríguez-Bernaldo de Quirós A & Costa HS. Analysis of carotenoids in vegetable and plasma samples. *J Food Compos Anal* 19:97–111 (2006).
- Tayade AB, Sharma PD, Chauhan RS, Chaurasia OP & Srivastava RB. Antioxidant capacities, phenolic contents, and GC/MS analysis of *Rhodiola imbricata* Edgew. root extracts from trans-himalaya. *J Food Sci* 78:402–410 (2013).
- Teow CC, Truong VD, McFeeters RF, Thompson RL, Pecota KV & Yencho GC. Antioxidant activities, phenolic and β -carotene contents of sweet potato genotypes with varying flesh colours. *Food Chem* 103:829–838 (2007).
- Van Breemen RB, Xu X, Viana MA, Chen L, Stacewicz-sapuntzakis M, Duncan C, Bowen PE & Sharifi R. Liquid chromatography–mass spectrometry of cis- and all-trans-lycopene in human serum and prostate tissue after dietary supplementation with tomato sauce. *J Agric Food Chem* 50:2214–2219 (2002).
- Vuong LT, Franke AA, Custer LJ & Murphy SP. Short communication *Momordica cochinchinensis* Spreng. (gac) fruit carotenoids reevaluated. *J Compos Anal* 19: 664–668 (2006).
- Wong CC, Li HB, Cheng KW & Chen F. A systematic survey of antioxidant activity of 30 Chinese medicinal plants using the ferric reducing antioxidant power assay. *Food Chem* 97:705–711 (2006).
- Wootton-Beard PC, Moran A & Ryan L. Stability of the total antioxidant capacity and total polyphenol content of 23 commercially available vegetable juices before and after *in vitro* digestion measured by FRAP, DPPH, ABTS and Folin–Ciocalteu methods. *Food Res Int* 44:217–224 (2011).
- Young AJ & Lowe GM. Antioxidant and prooxidant properties of carotenoids. *Arch Biochem Biophys* 385:20–27 (2001).