Effects of gardenia seed, green tea, and cactus pear in rice batter on the chemical quality of lotus root *bugak* and frying oil

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Abstract This study investigated the effects of the addition of gardenia seed, green tea, or cactus pear (*Opuntia ficus-indica*) to rice batter at 10% on the lipid oxidation, pigments, antioxidants, and antioxidant activity of lotus root *bugak* and frying oil. Lipid oxidation was evaluated based on the conjugated dienoic acid and *p*-anisidine values. Lipid oxidation and tocopherol degradation were significantly reduced in the gardenia seed-added *bugak* and frying oil, whereas the cactus pear-added *bugak* and frying oil showed an increase. The addition of green tea had no significant effects on the lipid oxidation of *bugak* and frying oil. The *in vitro* antioxidant activity of lotus root *bugak* significantly increased with the addition of gardenia seed, green tea, or cactus pear. The results suggested that green tea and gardenia seed could improve the health and food functionality of antioxidation for lotus root *bugak*, respectively.

Keywords: lipid oxidation, lotus root *bugak*, gardenia seed, green tea, cactus pear (*Opuntia ficus-indica*)

Introduction

Bugak, a fried Korean traditional dish, is rich in phytochemicals such as antioxidants and fibers. Pan frying with a small volume of oil after drying of battered vegetables and seaweeds is necessary to prepare *bugak*. Although frying gives a crispy texture to *bugak*, it can degrade beneficial components in vegetables because of oil oxidation at high temperatures, resulting in deteriorated flavor quality and storage stability (1).

Studies have reported the improvement in lipid oxidative stability of *bugak* by the addition of water extract of green tea (2) and *dasima* (*Laminaria japonica*) (3) or mulberry leaf (4). Lotus root reduces blood pressure (5) and prevents diabetes (6), possibly through the actions of alkaloids and polyphenols (7). A study recently reported that lotus root *bugak* with fermented rice batter significantly decreases the levels of plasma triacylglycerols, total cholesterol, and LDL cholesterol in the blood (8). However, antioxidant contents and *in vitro* antioxidant activity of lotus root *bugak* are significantly lower compared with those of lotus root itself (9); this difference suggests that a process is required to restore such levels to improve the chemical and physiological quality of *bugak*. Lotus root is white; therefore, it can be easily colored, resulting in increased consumer preference. Examples of food materials that provide colors are gardenia seed, green tea, and cactus pear (*Opuntia ficus-indica*).

Given that these food coloring materials contain pigments with antioxidant activity, the addition of these materials may improve the quality of *bugak*. Therefore, this study was performed to investigate the effects of the addition of gardenia seed, green tea, and cactus pear to rice batter on the chemical quality of lotus root *bugak*, including lipid oxidation, contents of antioxidants and pigments, and *in vitro* antioxidant activity. The degree of oil oxidation and contents of pigments and antioxidants of frying oil were also evaluated.

Materials and Methods

Materials and reagents Fresh lotus root and glutinous rice were purchased from a local supermarket in Incheon, Korea. Powdered gardenia seed, green tea, and cactus pear were purchased from Nanumherb (Yeongcheon, Korea), Cholocwon (Gyeongsan, Korea), and Jeju Cactus (Jeju, Korea), respectively. Unroasted sesame oil was a product of Dubio Co., Ltd. (Eumsung, Korea). Caffeic acid, Folin-Ciocalteu's phenol reagent, *p*-anisidine, chlorophylls *a* and *b*, β -carotene, lutein, and α -, γ -, and δ -tocopherol were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). *n*-Hexane, isopropanol, ethyl acetate, toluene, ethanol, acetone, methanol, and water were of HPLC grade and obtained from J.T. Baker Ltd. (Phillipsburg, NJ, USA). All other reagents were of analytical grade.

Preparation of lotus root *bugak* Lotus root *bugak* was prepared according to the traditional Korean method using fermented glutinous rice batter and pan frying with unroasted sesame oil (1). A mixture of glutinous rice and water (1:2, w/w) was fermented at room temperature for 7 days and then ground. Six volume of water was added and boiled for 15 min with continuous stirring to make the rice batter. Powdered gardenia seed, green tea, or cactus pear was added to the cooled rice batter at 10% by weight. The rice batter with/without food coloring materials (gardenia seed, green tea, or cactus pear) was spread onto both sides of thin lotus root slices (0.9 mm thick), which were blanched for 5 min. The battered lotus root slices were air dried at room temperature and then fried for 3 s for each side in a shallow pan (28 cm diameter and 2.41 cm height) with unroasted sesame oil (smoke point of 195°C) at 180°C.

Determination of antioxidants and pigments in frying oil and *bugak*

The contents of antioxidants and pigments of frying oil were determined, and those of *bugak* were determined after lipid extraction by the Folch method using a chloroform and methanol mixture (2:1, v/v) as an extraction solvent (1). The polyphenol content, represented as caffeic acid equivalent, was determined by the Folin-Ciocalteu method, and tocopherols were quantified using HPLC (YL 9100 HPLC; Younglin Co., Anyang, Korea) with a μ -porasil™ column (3.9 × 300 mm, 10 μ m i.d., Waters Co., Milford, MA, USA), a fluorescence detector (excitation 290 nm, emission 330 nm), and *n*-hexane and isopropanol mixture (99.8:0.2, v/v) as an eluent at 2 mL/min (9).

Chlorophylls and carotenoids were identified by comparing their retention times with those of standard compounds and then quantified based on the calibration curves using HPLC. The Younglin SP 930D HPLC (Younglin Co., Ltd.) was used, and the wavelengths of the UV detector for chlorophylls and carotenoids were 438 and 436 nm, respectively (1). The HPLC column for chlorophylls and carotenoids was a Symmetry C18 column (5.0 μ m, 4.6 × 250 mm; Waters Co.) and a μ -porasil column (10 μ m, 3.9 × 300 mm; Waters Co.), respectively. The eluting solvents for chlorophylls and carotenoids were a mixture of ethyl acetate, methanol, and water (50:37.5:12.5, v/v/v) at 1.5 mL/min and a mixture of *n*-hexane and isopropanol (97:3, v/v) at 1 mL/min, respectively.

Evaluation of lipid oxidation of frying oil and *bugak* The degree of frying oil oxidation was evaluated based on the conjugated dienoic acid and *p*-anisidine values by the AOCS method (10) Ti Ia-64 and Cd 18-90, respectively. Lipid oxidation of *bugak* was determined with the same methods after lipid extraction by the Folch method.

Evaluation of *in vitro* antioxidant activity of lotus root *bugak* The reducing power and DPPH radical scavenging activity of lotus root *bugak* were evaluated after obtaining the *bugak* extract with 80% ethanol (9). α -Tocopherol and ascorbic acid at the same concentration

were used as positive controls. DPPH radical scavenging activity was calculated from the absorbance (A) at 517 nm using a UV-Vis spectrophotometer (HP 8453; Hewlett Packard, Wilmington, DE, USA) as follows:

DPPH radical scavenging activity (%) = $100 \times (A \text{ of samples without } bugak \text{ extract} / A \text{ of samples with } bugak \text{ extract})$

The reducing power was evaluated with an absorbance at 700 nm (9). After mixing the *bugak* extract with a mixture of 0.2 M phosphate buffer (pH 6.6) and 1% potassium ferricyanide solution for 30 min, 10% trichloroacetic acid was added and then centrifuged. Water and 0.1% ferrous chloride were added to the upper layer, and the absorbance was read.

Statistical analysis All samples were prepared in duplicates, and the measurement for each sample was replicated. Data were statistically analyzed with SAS/PC (SAS version 9.2; SAS Institute Inc., Cary, NC, USA) including *t*-test, Duncan's multiple range test, and regression analyses.

Results and Discussion

Pigment and antioxidant contents of food materials No pigment was detected in lotus root, but powdered gardenia seed, green tea, and cactus pear contained chlorophylls and/or carotenoids. Chlorophylls *a* and *b* were detected only in green tea powder at 59.46±0.77 and 151.91±7.26 mg/kg, respectively, which were lower than the reported values (11). A wide range of the chlorophyll contents in green tea depending on the cultivating area and processing method has been previously reported (12,13). Carotenoids detected in the food materials included β -carotene and lutein. The contents of β -carotene and lutein were 847.13±2.51 and 4.86±0.18 mg/kg in gardenia seed, 22,402.77±1,206.95 and 223.48±6.16 mg/kg in green tea, and 663.81±24.14 and 14.41±0.93 mg/kg in cactus pear, respectively. These values were higher than the previously reported values in gardenia seed (12.7 mg/kg; 14) and green tea (700 mg/kg; 15); such differences were thought to be due to different genotypes, cultivating conditions, and/or processing conditions (12,13).

Powdered gardenia seed, green tea, and cactus pear contained α -tocopherol and polyphenols at 100.33±4.99 and 5,973.56±98.92 mg/kg, 423.53±0.90 and 34,617.32±397.58 mg/kg, and 74.55±1.09 and 3,623.59±39.32 mg/kg, respectively. The lotus root contained 13.69±0.27 mg/kg α -tocopherol and 255.51±32.58 mg/kg polyphenols. The hot water extract of green tea contained 85,620 mg/kg polyphenols (16), whereas gardenia seed and cactus pear contained 17,000 (14) and 1,210 mg/kg polyphenols (17), respectively. Lipids of gardenia seed and green tea were reported to contain 522 (18) and 1,100 mg/kg tocopherols (19), respectively, whereas skins and flesh lipids of cactus pear contained 21,820 (20) and 6,120 mg/kg tocopherols (21), respectively.

Table 1. Pigment and antioxidant contents (mg/kg) of lotus root *bugak* with different food coloring materials

	No (control)	Gardenia seed	Green tea	Cactus pear (<i>Opuntia ficus-indica</i>)
Chlorophyll <i>b</i>	ND ¹⁾	ND	13.83±0.19	ND
β-Carotene	21.45±1.44 ^{b2)}	23.08±0.45 ^b	175.09±7.60 ^a	22.38±1.05 ^b
Carotenoids	ND	ND	0.73±0.02	ND
Lutein	ND	ND	0.73±0.02	ND
Total	21.45±1.44 ^b	23.08±0.45 ^b	175.82±7.60 ^a	22.38±1.05 ^b
Polyphenols	25.59±2.25 ^a	26.47±3.99 ^a	25.12±2.62 ^a	29.52±1.82 ^a
γ-Tocopherol	469.46±16.17 ^c	571.32±3.90 ^a	547.50±12.89 ^b	456.89±10.38 ^c

¹⁾ND, not detected.²⁾Different superscripts mean significantly different values among the samples with different food coloring materials in each attribute by Duncan's multiple range test at 5%.**Table 2.** Pigment and antioxidant contents (mg/kg) of sesame oil after frying for lotus root *bugak* with different food coloring materials

Heated at 180°C but before frying		After frying			
		No (control)	Gardenia seed	Green tea	Cactus pear (<i>Opuntia ficus-indica</i>)
Chlorophyll <i>b</i>	ND ¹⁾	ND	ND	ND	ND
β-Carotene	21.19±1.45 ^{a2)}	14.30±0.43 ^b	19.91±2.16 ^a	15.73±0.12 ^b	14.73±0.22 ^b
Polyphenols	19.22±2.33 ^a	23.53±2.87 ^a	22.98±0.65 ^a	24.04±3.16	24.08±0.99 ^a
γ-Tocopherol	638.25±0.80 ^a	382.16±4.58 ^d	445.61±3.08 ^b	399.49±0.76 ^c	271.67±0.13 ^e

¹⁾ND, not detected.²⁾Different superscripts mean significantly different values among the oils after frying for lotus root *bugak* with different food coloring materials in each attribute by Duncan's multiple range test at 5%.**Pigment and antioxidant contents of lotus root *bugak* and frying oil**

The chlorophyll, carotenoid, polyphenol, and tocopherol contents of lotus root *bugak* are shown in Table 1. Chlorophyll *b* was detected only in green tea-added *bugak* at 13.83 mg/kg, which was derived from green tea. The absence of chlorophyll *a* in *bugak* in spite of its presence in green tea was thought to be due to its lower stability compared with chlorophyll *b* during frying. The low heat stability of chlorophyll *b* compared with that of chlorophyll *a* was previously observed (22). The β-carotene content was significantly ($p<0.05$) high in green tea-added lotus root *bugak* (175.09 mg/kg), and no significant difference was found among the control and gardenia seed- or cactus pear-added *bugak* (21.45, 23.08, and 22.38 mg/kg, respectively). This finding could be partly due to the high degradation of carotenoids during frying (23), especially during the preparation of green tea-added *bugak*. Some of the carotenoids in frying oil could have been transferred to *bugak*, which resulted in a similar β-carotene content in the control *bugak* and *bugak* with gardenia seed or cactus pear. The carotenoid content of *bugak* with gardenia seed or cactus pear was low compared with that of green tea-added *bugak*. The sesame oil used in this study contained 23.05 mg/kg β-carotene, and the presence of carotenoids in unrefined sesame oil was also reported elsewhere (24). Lutein was detected only in green tea-added *bugak* at 0.73 mg/kg.

Polyphenols were present in the control and gardenia seed-, green tea-, or cactus pear-added *bugak* at 25.59, 26.47, 25.12, and 29.52 mg/kg, respectively; the differences were not significant ($p>0.05$). However, a significant difference ($p<0.05$) was noted in the polyphenol

contents among gardenia seed, green tea, and cactus pear powder. This difference possibly resulted from the high degradation of polyphenols during frying. In addition, the polyphenol composition of gardenia seed, green tea, or cactus pear varied, which might cause different degradation behaviors (25,26). Among tocopherol isomers, only γ-tocopherol was detected in *bugak*, which suggested that tocopherols in *bugak* were mainly transferred from frying oil. All food materials used to prepare *bugak*, except sesame oil, contained α-tocopherol not γ-tocopherol; sesame oil contained 1446.47±97.85 mg/kg γ-tocopherol before heating. The tocopherol content was the highest in gardenia seed-added *bugak* (571.32 mg/kg), followed by that in green tea-added *bugak* (547.50 mg/kg). No significant difference ($p>0.05$) was found in the tocopherol content between the control (469.46 mg/kg) and cactus pear-added *bugak* (456.89 mg/kg). These results suggested that the addition of gardenia seed and green tea to rice batter protected γ-tocopherol present in oil from degradation during frying.

The pigment and antioxidant contents of frying oil are shown in Table 2. No chlorophyll was detected in frying oil before and after frying, even after frying green tea-added *bugak*. This finding could be related to the high susceptibility of chlorophylls to heat, as oil-soluble chlorophylls were possibly transferred to oil from the green tea-added batter for *bugak* during frying. The frying oil contained 21.19 mg/kg β-carotene after heating at 180°C but before frying. Frying the control and green tea- or cactus pear-added *bugak* significantly ($p<0.05$) decreased the β-carotene content in frying oil to 14.30, 15.73, and 14.73 mg/kg, respectively. However, no significant

Fig. 1. Conjugated dienoic acid value (%) and *p*-anisidine value of lotus root *bugak* with different food coloring materials (Different superscripts mean significantly different values among the samples with different food coloring materials in each attribute by Duncan's multiple range test at 5%.)

decrease ($p > 0.05$) was found in the β -carotene content of frying oil after frying the gardenia seed-added *bugak* (19.91 mg/kg). Moreover, lutein was not detected in frying oil. Carotenoids have been known to be degraded during heating (27). Thus, gardenia seed protected β -carotene in frying oil from degradation during frying, even though it contained a lower amount of β -carotene than green tea. More studies are required to elucidate this mechanism.

Frying oil heated at 180°C contained 19.22 mg/kg polyphenols before frying and slightly increased, but not significantly ($p > 0.05$), after frying. The polyphenol content slightly increased to 23.53, 22.98, 24.04, and 24.08 mg/kg in the control and gardenia seed-, green tea-, or cactus pear-added lotus root *bugak*, respectively. This finding indicated that the addition of gardenia seed, green tea, or cactus pear to rice batter did not cause a significant change in the polyphenol content of frying oil. Considering the significant degradation of polyphenols during frying at high temperatures (26,28), this insignificant change in the polyphenol content of frying oil upon frying was thought to be possibly due to the supply of additional polyphenols from the lotus root, gardenia seed, green tea, or cactus pear. The tocopherol (only γ -isomer) content of frying oil upon heating at 180°C but before frying was 638.25 mg/kg, and it significantly ($p < 0.05$) decreased to 382.16, 445.61, 399.49, and 271.67 mg/kg after frying the control and gardenia seed-, green tea-, or cactus pear-added lotus root *bugak*, respectively. These findings confirmed the significant degradation of γ -tocopherol in oil during frying and the lowest degradation in oil for gardenia seed-added *bugak*. Meanwhile, the addition of cactus pear to batter increased the degradation of γ -tocopherol in frying oil. Green tea did not show any significant effects.

Lipid oxidation of lotus root *bugak* and frying oil The degree of lipid oxidation of lotus root *bugak* is shown in Fig. 1. The conjugated dienoic acid content and *p*-anisidine value of the control lotus root *bugak* without any food coloring material were 23.04% and 19.07, respectively. However, *bugak* with gardenia seed powder showed

significantly ($p < 0.05$) low conjugated dienoic acid and *p*-anisidine values (21.80% and 12.33, respectively). The value of *p*-anisidine was significantly lower (16.17) in *bugak* with green tea powder than in the control *bugak* ($p < 0.05$). The conjugated dienoic acid content and *p*-anisidine value of *bugak* with cactus pear powder was 25.26% and 22.54, respectively, which was significantly higher than that of the control *bugak* ($p < 0.05$). These results suggested that gardenia seed and green tea decreased lipid oxidation, whereas cactus pear increased it during the preparation of *bugak*. Pigments, particularly crocin, present in gardenia seed possess antioxidant activity by scavenging reactive oxygen species, such as hydroxyl radicals (29). Phenolic compounds such as catechin in green tea also exhibit strong activity to scavenge radicals (30). Although studies have reported that phenolic compounds present in cactus pear show antioxidant activity in terms of radical scavenging activity and reducing power (31), its water-soluble betalain is highly sensitive to heat (17,32). Thus, the antioxidant activity of cactus pear on the lipid oxidation of lotus root *bugak* might not have been demonstrated in this study. In addition, a previous study showed that indicaxanthin from cactus pear acts as a prooxidant at the membrane level (33). However, the effect of cactus pear on lipid oxidation in foods during cooking has not been reported.

The conjugated dienoic acid contents and *p*-anisidine values of frying oil showed similar patterns (Fig. 2) to the results of lotus root *bugak*. The conjugated dienoic acid content and *p*-anisidine value of frying oil heated at 180°C but before frying were 17.00% and 5.17, respectively. After frying the control and gardenia seed-, green tea-, or cactus pear-added *bugak*, the conjugated dienoic acid contents and *p*-anisidine values of oil significantly ($p < 0.05$) increased to 27.54% and 32.12, 23.33% and 23.89, 27.57% and 30.50, and 36.37, respectively. As expected, increased conjugated dienoic acid contents and *p*-anisidine values of frying oil indicated oil oxidation during frying. Significantly lower conjugated dienoic acid contents and *p*-anisidine values in frying oil for the gardenia seed-added *bugak* and higher values in oil for the cactus pear-added

Fig. 2. Conjugated dienoic acid value (%) and *p*-anisidine value of sesame oil after frying for lotus root *bugak* with different food coloring materials (Different superscripts mean significantly different values among the oils after frying for lotus root *bugak* with different food coloring materials in each attribute by Duncan's multiple range test at 5%.)

Fig. 3. DPPH radical scavenging activity and reducing power of lotus root *bugak* with different food coloring materials (Different superscripts mean significantly different values among the samples with different food coloring materials in each attribute by Duncan's multiple range test at 5%.)

bugak compared with those in oil for the control *bugak* suggested antioxidant and prooxidant activities of gardenia seed and cactus pear, respectively, in frying oil during *bugak* preparation. The conjugated dienoic acid content and *p*-anisidine value of frying oil were not significantly different between the green tea-added *bugak*

and control *bugak*. This finding could be partly due to the high amount of carotenoid degradation products in frying oil derived from *bugak* with green tea. The carotenoid contents of *bugak* with green tea were 26 and 33 times higher than those of *bugak* with gardenia seed or cactus pear, respectively. Degradation products of carotenoids accelerate lipid oxidation by increasing radical production (34).

In vitro antioxidant activity of lotus root *bugak* The *in vitro* antioxidant activity of lotus root *bugak* was higher in gardenia seed-, green tea-, or cactus pear-added lotus root *bugak* than in the control *bugak*, as shown in Fig. 3. The ethanol extract of the control and gardenia seed-, green tea-, or cactus pear-added lotus root *bugak* (1 g/30 mL) showed 2.26, 0.98, 81.75, and 8.46% of DPPH radical scavenging activity, respectively. The reducing power, which was represented as the absorbance at 700 nm, was 0.09, 0.14, 0.57, and 0.18 by the control and gardenia seed-, green tea-, or cactus pear-added *bugak*, respectively. In particular, DPPH radical scavenging activity of the green tea-added lotus root *bugak* was higher than α -tocopherol (77.07%) and corresponded to 87.2% of ascorbic acid (93.73%). The reducing power of green tea-added lotus root *bugak* corresponded to approximately 68% of both α -tocopherol and ascorbic acid. These results clearly indicated that the addition of gardenia seed, green tea, or cactus pear to rice batter significantly increased the *in vitro* antioxidant activity of lotus root *bugak*. These finding also suggested that the addition of gardenia seed, green tea, or cactus pear to rice batter for *bugak* preparation was a good method to improve the health functionality of lotus root *bugak*. A previous study reported that the *in vitro* antioxidant activity of lotus root is significantly impaired during *bugak* preparation (9).

In conclusion, the addition of gardenia seed powder to rice batter significantly decreased lipid oxidation in both frying oil and lotus root *bugak*, whereas cactus pear increased it. Only green tea added to batter reduced the lipid oxidation of *bugak*. The *in vitro* antioxidant activity of lotus root *bugak* was improved by the addition of gardenia seed, green tea, or cactus pear to rice batter, with the best effect

demonstrated by green tea. Therefore, green tea and gardenia seed are the best additives to rice batter at 10% for lotus root *bugak* preparation as they provide health and food functionality as antioxidants, respectively.

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