

Influence of Extraction Method on Quality and Functionality of Broccoli Juice

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ABSTRACT: This study was performed to compare the quality and functionality of broccoli juice as affected by extraction method. Broccoli juice was extracted using method I (NUC Kuvings silent juicer), method II (NUC centrifugal juicer), and method III (NUC mixer), and the quality properties of the broccoli juices were analyzed using three different methods. Additionally, the antioxidative, anticancer, and anti-hyperglycemic activities of broccoli juice prepared by the three different methods were investigated *in vitro*. The broccoli juice made by method I contained the highest polyphenol and flavonoid contents at 1,226.24 mg/L and 1,018.32 mg/L, respectively. Particularly, broccoli juice prepared by method I showed higher DPPH and ABTS radical scavenging activities than those of the other samples. Additionally, broccoli juice made by method I showed the highest growth inhibitory effects against HeLa, A549, AGS, and HT-29 cancer cells. Broccoli juice prepared by method I had the highest α -glucosidase inhibitory effects. These results indicate that there are important differences in chemical and functional qualities between juice extraction techniques.

Keywords: broccoli, extraction, juice, antioxidative, anticancer

INTRODUCTION

The current market demands and promotes intensively processed products with characteristics resembling fresh products, and preventive health measures, such as diets with high fruit and vegetable contents (1). Raw fruits and vegetables are an important natural source of many essential vitamins, minerals, and polyphenols, but processing can easily destroy some of these nutrients (2). However, the vitamins, minerals, polyphenols, and enzymes that are present in uncooked fruits and vegetables are the same as those found in their juices. Fresh fruit and vegetable juices are generally perceived to be more wholesome and to have better flavor than processed juice. Fresh juice may have flavor, total phenolics, antioxidant capacity, and phytonutrients such as flavonoids that differ from processed juice (3).

Previous work showed that processing fruit and vegetable juices results in big losses of polyphenols and flavonoids. However, several possibilities exist within the juice production chain to enhance the flavonoid content of fruit and vegetable juices (4,5). As a consequence, each extraction method may have its own characteristics

in terms of the concentration of bioactive compounds as well as juice quality (6).

Broccoli (*Brassica oleracea* L. var. *italica*), a vegetable crop belonging to the Brassicaceae family, is widely consumed throughout the year as a fresh vegetable in Western Europe, USA, and Far East Asian countries (7). Broccoli is known as the “crown jewel of nutrition” because of its vitamin-rich, high-fiber, and low-calorie properties. Broccoli sprouts contain substantial amounts of antioxidants, vitamin C, carotenoids, and phenolic compounds (8). Broccoli is also rich in sulforaphane, a phytochemical with anticarcinogenic properties (9,10). Sulforaphane shows antioxidative properties that prevent multiple diseases, including several types of cancer, high blood pressure, macular degeneration, and stomach ulcers (11,12).

The objectives of this study were to investigate changes in quality and functionality on the same batch of broccoli due to differences in juice extraction methods. Broccoli juice was extracted using three different juicing machines and then analyzed for quality properties and functionality (antioxidative, anticancer, and anti-hyperglycemic effects).

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MATERIALS AND METHODS

Materials

All chemicals were obtained from Sigma Chemical (St. Louis, MO, USA) unless otherwise indicated. Cell culture reagents were purchased from Gibco BRL (Rockville, MD, USA) and fetal bovine serum (FBS) was obtained from Hyclone (Logan, UT, USA). All cell lines were obtained from the Korean Cell Line Bank (Seoul, Korea).

Broccoli juice preparation

Broccoli (*B. oleracea* L. var. *italica* Plenck) was obtained from a South Korean market. Broccoli (100 g) was cut up and produced into broccoli juice using a slow juicer (Kuvings KNJ-992250W, NUC Electronics Co., Ltd., Daegu, Korea), a centrifugal juicer (NJ-9300A, NUC Electronics Co., Ltd.), and a hand blender (NFM-3003; NUC Electronics Co., Ltd.) under the following operating conditions:

- (1) Slow juicer (method I): Broccoli was ground by a rotating juicing screw at 80 rpm. The broccoli juice was extracted by passing it through a 0.35 ± 0.05 mm strainer.
- (2) Centrifugal juicer (method II): A centrifugal juicer cuts up the broccoli with a flat cutting blade and then spins the produce at a high speed (15,000 rpm) to separate the juice from the pulp.
- (3) Hand blender (method III): Broccoli was squeezed using a hand blender. Whole samples were used without separating the juice from the pulp.

Quality analysis

Moisture content of the broccoli juice samples was measured using a moisture determination balance (FD-720, Kett Electric Lab., Tokyo, Japan). Total soluble solids ($^{\circ}$ Brix) were measured using a hand refractometer (PAL-3, Atago, Tokyo, Japan). Juice yield was determined and expressed as a percentage of broccoli weight. The color of the broccoli juice was determined using a colorimeter (CR-400, Minolta Holdings Ltd., Tokyo, Japan) with the following operating conditions:

- Lightness: D65
- Measurement on a surface of 8 mm
- The mean of three values is recorded
- Colorimeter system: L, a, and b.

Determination of total polyphenols and flavonoids

The concentrations of total polyphenols and flavonoids were measured using the Folin-Denis method (13) and the method described by Nieva Moreno et al. (14), respectively. Total polyphenol and flavonoid contents were expressed as tannic acid and quercetin molar equivalents, respectively.

Free radical scavenging

The free radical scavenging activity of the samples was measured using α - α -diphenyl- β -picrylhydrazyl (DPPH). This assay was carried out as described by Blois (15) with some modifications. Briefly, 0.15 mM solution of DPPH \cdot radical in ethanol was prepared, and 40 μ L of this solution was added to 160 μ L of sample solution in ethanol at different concentrations. After 30 min, the absorbance was measured at 517 nm. A lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The free radical scavenging activity of each solution was then calculated as percent inhibition according to the following equation:

$$[(A_o - A_e)/A_o] \times 100$$

(A_o =absorbance without broccoli juice,

A_e =absorbance with broccoli juice).

The spectrophotometric analysis of ABTS $^{+\cdot}$ radical scavenging activity was determined according to the method of Re et al. (16). The ABTS $^{+\cdot}$ cation radical was produced by a reaction between 7 mM ABTS in H₂O and 2.45 mM potassium persulfate during storage in the dark at room temperature for 24 h. Before use, the ABTS $^{+\cdot}$ solution was diluted with phosphate buffer (0.1 M, pH 7.4) to obtain an absorbance of 0.70 ± 0.02 at 732 nm. Then, 990 μ L of ABTS $^{+\cdot}$ solution was added to 10 μ L of sample. After 1 min, the percent inhibition at 732 nm was calculated for each concentration relative to blank absorbance.

Cell culture

Human cervical cancer (HeLa), human liver cancer (HepG2), human lung cancer (A549), human stomach cancer (AGS), human breast cancer (MDA-MB-231), human colon cancer (HT-29), and human normal liver (Chang) cells were cultured in 100 μ L of Roswell Park Memorial Institute medium (RPMI) 1640 media (Invitrogen, Carlsbad, CA, USA) supplemented with 10% FBS, 100 U/mL penicillin, and 100 μ g/mL streptomycin. All cells were incubated overnight at 37°C in 5% CO₂ for cell attachment.

MTT assay

This assay was conducted as described by Carmichael et al. (17). Briefly, cancer cells were seeded in 96-well plates at a density of 1×10^4 cells/well in 100 μ L RPMI medium. After 24 h, the medium was removed, and the cells were incubated for 24 h with RPMI in the absence or presence of a 5% (v/v) broccoli juice. After the incubation, 5 mg/mL of MTT reagent was added to each well, and the plates were incubated again for 4 h in a CO₂ incubator at 37°C. DMSO (100 μ L) was added to each well to dissolve the cells. The plates were kept at room temperature for 10 min, and the absorbance was measured at 550 nm using a multi-well spectrophoto-

tometer (Molecular Devices, Sunnyvale, CA, USA).

$$\text{Growth inhibition rate (\%)} = (1 - S/C) \times 100$$

S: O.D. of sample, C: O.D. of control

α -Glucosidase inhibition assay

α -Glucosidase inhibitory activity was assessed by the microplate-based method using on *p*-nitro-phenyl- α -D-glucopyranoside (PNPG) as the substrate (18). α -Glucosidase (50 μ L, 0.2 U/mL), PNPG (100 μ L, 12 mM), sample (50 μ L, 10% (v/v)), and potassium phosphate buffer (50 μ L, 0.1 M, pH 6.8) were mixed and preincubated at 37°C for 20 min. The reaction was terminated by adding 100 μ L of 0.1 M NaOH solution, and absorbance was measured at 405 nm. Acarbose was used as the positive control.

$$\alpha\text{-Glucosidase inhibition activity (\%)} = (1 - S/C) \times 100$$

S: O.D. of sample, C: O.D. of control

Statistical analysis

Data are expressed as mean \pm standard deviation of at least three separate experiments. Statistical analyses were performed with a one-way analysis of variance followed by Duncan's multiple range test using SPSS version 17.0 for Windows (SPSS Inc., Chicago, IL, USA). A $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Quality characteristics

Yield, moisture content, and soluble solid content of the broccoli juice are shown in Table 1. The yields of brocco-

Table 1. Quality properties of broccoli juice made by three different methods

| Method ¹⁾ | Yield (%) | Soluble solids ($^{\circ}$ Bx) | Moisture (%) |
|----------------------|-----------|---------------------------------|--------------|
| Method I | 45.50 | 4.8 | 95.19 |
| Method II | 42.75 | 4.2 | 96.51 |
| Method III | 100.00 | 4.7 | 91.91 |

¹⁾Method I, slow juice; Method II, centrifugal juicer; Method III, hand blender.

Table 2. Color of broccoli juice made by three different methods

| Method ¹⁾ | Huter's color value | | |
|----------------------|----------------------------------|--------------------------------|-------------------------------|
| | L ²⁾ | a ³⁾ | b ⁴⁾ |
| Method I | 26.61 \pm 0.18 ^{ns5)} | -3.85 \pm 0.09 ^{ns} | 5.93 \pm 0.17 ^{ns} |
| Method II | 25.89 \pm 1.46 | -4.91 \pm 0.25 | 7.15 \pm 0.31 |
| Method III | 25.36 \pm 0.94 | -4.52 \pm 1.51 | 8.07 \pm 1.96 |

¹⁾Method I, slow juice; Method II, centrifugal juicer; Method III, hand blender.

²⁾L value (0: black, 100: white).

³⁾a values (+: red, -: green).

⁴⁾b values (+: yellows, -: blue).

⁵⁾Each value is mean \pm SD ($n \geq 3$).
ns: not significant.

li juice extracted using method I (slow juicer), method II (centrifugal juicer), and method III (hand blender) were 45.50, 42.75, and 100.00% respectively. The order of moisture content according to extraction method was method II > method I > method III, whereas that of soluble solid content was method II < method III < method I. The color values of the broccoli juice prepared using the three different methods are shown in Table 2. The values of L, a, and b in the broccoli juice were 25.36~26.61, -4.91~-3.85, and 5.93~8.07. No differences in color values were observed among the three methods.

Determination of total polyphenol and flavonoid contents in broccoli juice

Phenolic compounds are secondary metabolites widely distributed in plants. Phenolics have diverse structures and molecular weights and are mostly comprised of flavonoids. Phenolic compounds contain functional phenolic hydroxyl (OH) groups that bind readily to proteins and macromolecules and have antioxidation, anticancer, and various other physiological activities (19). Flavonoids are one of the most diverse and widespread groups of natural compounds that prevent tooth decay, suppress hypertension, and have anti-AIDS, anticancer, anti-oxidation, and whitening effects (20).

Total polyphenol and flavonoid contents contained in the broccoli juice prepared using the three different methods were measured using tannic acid and quercetin, respectively, as standard compounds (Table 3). The total polyphenol contents of the broccoli juices ranged from 723.79 to 1,226.24 mg/L and flavonoid content ranged from 601.64 to 1,018.32 mg/L. Particularly, broccoli juice prepared using method I showed the highest total polyphenol and flavonoid contents.

Free radical scavenging activities

Free radicals have been implicated in the pathogenesis of many diseases, including myocardial and cerebral is-

Table 3. Contents of total polyphenols and flavonoids in broccoli juice made by three different methods

| Method ¹⁾ | Total polyphenols ²⁾ (mg/L) | Total flavonoids ³⁾ (mg/L) |
|----------------------|---|--|
| Method I | 1,226.24 \pm 36.74 ^{a4)} | 1,018.32 \pm 57.80 ^a |
| Method II | 724.37 \pm 48.05 ^b | 616.64 \pm 26.44 ^b |
| Method III | 723.79 \pm 47.44 ^b | 601.71 \pm 66.99 ^b |

¹⁾Method I, slow juice; Method II, centrifugal juicer; Method III, hand blender.

²⁾Milligrams of total polyphenol content/L of juice based on tannic acid as standard.

³⁾Milligrams of total flavonoid content/L of juice based on quercetin as standard.

⁴⁾Each value is mean \pm SD.

Values with different superscripts within the same column are significantly different by Duncan's multiple range test at $P < 0.05$.

chemia, arteriosclerosis, diabetes, rheumatoid arthritis, inflammation, cancer initiation, and the aging process (21).

DPPH is reduced by L-ascorbic acid (L-AsA), tocopherol, and polyhydroxy aromatic compounds, and is dark purple in color, which is applied frequently for screening of antioxidant substances from various natural materials. The scavenging effect of prepared broccoli juice on DPPH radicals and the synthetic/natural antioxidants such as butylated hydroxyanisole (BHA) and L-AsA was compared using the three different juicing methods (Fig. 1). The scavenging effect of 10% (v/v) broccoli juice made by methods I and II on DPPH radicals was 52% and 17%, respectively. The scavenging effect increased after examining the relationship between polyphenol content and free radical scavenging activity.

The ABTS system has been commonly used to meas-

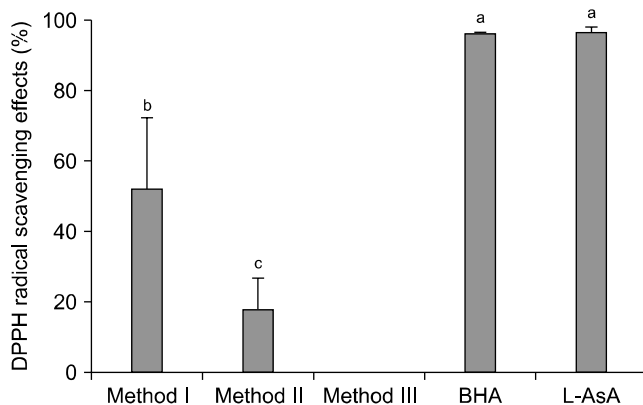


Fig. 1. DPPH radical scavenging effects of broccoli juice made by three different method. Broccoli juice: 10% (v/v), BHA and ascorbic acid: 10 μ g/mL. t-Butylatedhydroxyanisole (BHA) and L-ascorbic acid (L-AsA) were used a positive references. Each value is mean \pm SD. Values with different letters on the bars are significantly different by Duncan's multiple range test at $P<0.05$.

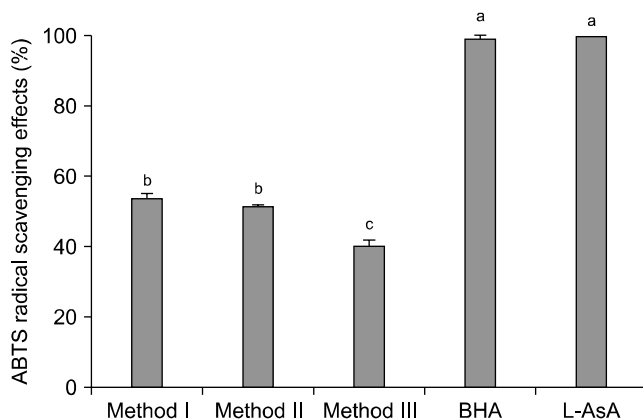


Fig. 2. ABTS radical scavenging effects of broccoli juice made by three different method. Broccoli juice: 1% (v/v), BHA and ascorbic acid: 10 μ g/mL. t-Butylatedhydroxyanisole (BHA) and L-ascorbic acid (L-AsA) were used a positive references. Each value is mean \pm SD. Values with different letters on the bars are significantly different by Duncan's multiple range test at $P<0.05$.

ure total antioxidative status of various biological specimens by measuring radical scavenging through electron donation (22). ABTS radical scavenging activity of 1% (v/v) broccoli juice made by the three methods is shown in Fig. 2. Although the results of the ABTS assay were similar to those of the DPPH assay, but the broccoli juices showed higher scavenging activity of ABTS than DPPH radicals.

Anticancer activity

The anticancer activity of broccoli juice prepared using the three different methods was investigated using the MTT assay and several human cancer cell lines (HeLa, HepG2, A549, AGS, MDA-MB-231, and HT-29 cells) and normal cell lines (Chang). The MTT assay measures purple formazan converted from the MTT reagent by succinate-dehydrogenase, a mitochondrial enzyme in living cells. The formazan produced is directly proportional to the number of viable cells present (23).

As shown in Fig. 3, broccoli juice expressed inhibitory activity against the six cancer cell lines when tested at a 5% (v/v) solution. Particularly, broccoli juice prepared by method I exhibited strong anticancer activity towards HeLa, A549, AGS, and HT-29 cells. In addition, the results showed that broccoli juice prepared by method I, method II, and method III does not have significant cell growth inhibition against normal human liver (Chang) cells.

α -Glucosidase inhibitory activity

Inhibiting α -glucosidases is an alternative therapeutic approach for treating non-insulin-dependent diabetes mellitus because these key enzymes are involved in starch

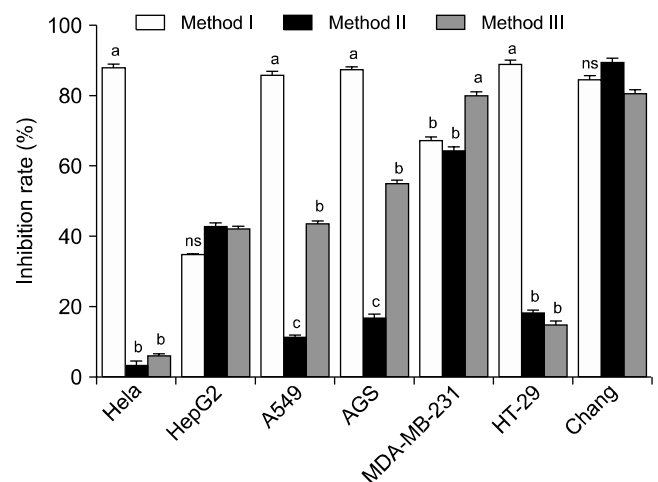


Fig. 3. Growth inhibition effects of broccoli juice made by three different method against human cancer and normal cells. Broccoli juice: 5% (v/v). Each value is mean \pm SD. Values with different letters among different methods are significantly different by Duncan's multiple range test at $P<0.05$. ns: Not significant.

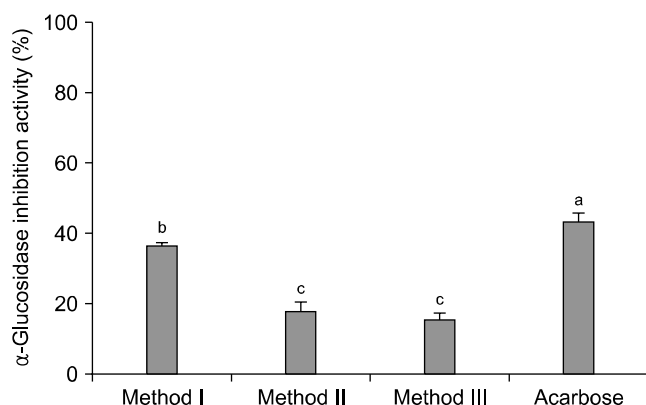


Fig. 4. α -Glucosidase inhibition activity of broccoli juice made by three different method. Broccoli juice: 10% (v/v), Acarbose: 5 mg/mL. Each value is mean \pm SD. Values with different letters on the bars are significantly different by Duncan's multiple range test at $P < 0.05$.

breakdown and intestinal absorption. The digestion and uptake of carbohydrates significantly decreases by inhibiting α -glucosidases, which decrease postprandial blood glucose levels in patients with non-insulin-dependent diabetes mellitus (24). Acarbose is presently used as an α -glucosidase inhibitor. Acarbose at 5 mg/mL can inhibit α -glucosidase activities by about 45%. Shai et al. (25) reported an IC_{50} value of 0.6 U in the reaction mixture of 17 mg/mL acarbose against yeast α -glucosidase; this disparity is a result of different concentrations of the enzymes (0.2 U in this study). α -Glucosidase inhibition activities of 10% (v/v) broccoli juice made by method I, method II, and method III were 36, 18, and 15%, respectively (Fig. 4). An important activity of polyphenols is the inhibition of digestive enzymes, especially carbohydrate-hydrolyzing enzymes such as α -glucosidase; inhibitors of these digestive enzymes are able to retard carbohydrate digestion, thus causing a reduction in glucose absorption rate. Effective α -glucosidase polyphenol-type inhibitors from natural resources have been reported to be useful in reducing postprandial hyperglycemia (26). The results indicate that among the extraction methods of broccoli juice, method I was the best for optimal α -glucosidase inhibition activity.

In conclusion, our results indicate that the extraction method may influence the quality and functional properties of broccoli juice. Particularly, broccoli juice prepared using method I showed higher antioxidative, anticancer, and anti-diabetic activities than those prepared by methods II and III.

AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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