Development of Low Calorie Roasted Radish Tea Beverage with Anti-oxidant Activity

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Abstract A functional radish tea beverage was developed using pressure roasted radish pieces, barley, and cassia seeds. Stevia, short-chain fructooligosaccharides (scFOS), and inverted sugar syrup were used as sugar substitutes. The formula developed for mass production was, pressure roasted radish pieces, barley, and cassia seed teas prepared separately, then mixed (3:1:1, v/v/v). Inverted sugar syrup (54.4 g/L), stevia (0.73 g/L), scFOS (17 g/L), citric acid (0.01%), and vitamin C (0.05%) were added. Sweetness and caloric contents of the roasted radish tea beverage were 6.5°Bx and 19.35 Kcal/100 mL, respectively. Based on consumer acceptance testing ($n=60$ persons), overall acceptance, taste, and sweetness scores of the roasted radish tea beverage were higher than for a commercially available H tea beverage. Caloric contents were reduced by approximately 23.5% using sugar substitutes. The IC_{50} value for the 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity of hot water extracts of dried radish increased 2.5-fold after pressure roasting.

Keywords: radish (*Raphanus sativus* L.), tea, beverage, anti-oxidant, sensory evaluation

Introduction

There are increasing concerns over sweetened beverages due to positive correlations between sugar contents of soft drinks and plasma triglycerides and/or blood glucose levels (1). However, consumers are still deeply attached to sweetened and/or carbonated drinks, such as cola. Stevia, which has a sweetness level 200–300% greater than sugar with no calories, has been used as a sugar substitute, but a bitter taste is a problem for use in beverage products (2). Short-chain fructooligosaccharides (scFOS), known to have prebiotic properties, have recently been introduced as a functional sugar (3). The sweetness and caloric content of scFOS are approximately 25 and 50%, respectively, of values for sugar (4,5). In the food industry, scFOS are targeted at customers who prefer products with greater health benefits despite higher costs (6).

Tea has been consumed in Asian countries for more than 2,000 years and has become a popular beverage worldwide due, at least in part, to health benefits (7). However, the process of traditional tea preparation is time-consuming thus beverage type tea packaged in a bottle has become popular. Consequently, the market for bottled tea has grown rapidly. In Korea, customers prefer bottled tea made from grain rather than leafy teas, such as green tea or oolong tea. Koreans traditionally drink water called *shoungnyung* made from leftover cooked rice after meals. *Shoungnyung* has a nutty flavor, which

provides a deep aroma. This could be a reason why Koreans prefer teas made from roasted grain. As barley, corn, brown rice, and sealwort provide a nutty flavor when roasted, these grains are frequently used to make tea at home and for commercial applications.

Radishes are the second largest vegetable crop in Korea. As volatile sulfur compounds in radishes are known to have pharmaceutical effects against coughing, phlegm, and dysentery, radishes have been used as a remedy in folk medicine for many years in Korea (8). Home-made roasted radish tea is widely consumed at home for general health purposes, in particular to prevent coughing (9). The anti-oxidant and radical scavenging activities of radishes are increased during roasting due to production of Maillard compounds (10).

In this study, beverage type radish tea with low calorie content and a sweet taste was developed. Sub-ingredients that could mask the radish flavor and increase the anti-oxidant properties of a roasted radish tea beverage were selected from grains commonly used for tea preparation. A formula for the roasted radish tea beverage was developed using pressure roasted radish pieces as a main material, and two other grains as sub-ingredients. Combined proportions of the roasted radish tea and other grain teas were then determined. Amounts of the sugar substitutes stevia, scFOS, and inverted sugar syrup were also determined. Finally, citric acid and ascorbic acid contents were determined to increase palatability and anti-oxidant properties of the roasted radish tea beverage. Sensory evaluation

was carried out at each step of the formula determination, and consumer preference testing was performed using the roasted radish tea beverage based on comparison with tea beverages available on the market. This study was approved by Institutional Review Board (IRB) of Pusan National University (PNU IRB/2014_89_HR) and conducted in agreement with the Helsinki Declaration of 1975, as revised in 2013. Informed consent was obtained from all subjects for inclusion in the study.

Materials and Methods

Materials Radishes (*Raphanus sativus* L.) were purchased at an agricultural wholesale market in Busan, Korea in September of 2014. Roasted grain products for tea making were purchased at a local market in Busan, Korea in September of 2014. Barley (Sempio Inc., Seoul, Korea), corn (Gangwonyacchonongsan, Changwon, Korea), sealwort (Gangwonyacchonongsan), *Cassia tora* L. seeds (Gangwonyacchonongsan), and brown rice (Jeongudang, Seoul, Korea) were used. Stevia sweetener (Joenwon Food, Gimpo, Korea), which is a mixture of stevia and glucose (1:3, w/w), scFOS (#71920; Allergy Research Group, Alameda, CA, USA), and inverted sugar syrup (Golden Syrup, Sejeon Co., Ltd, Jincheon, Korea), were used as sugar substitutes.

Preparation of pressure roasted radish pieces Radishes were washed to remove impurities and dirt, then sliced into 3x3x0.5 cm pieces. Radish slices were left in a room temperature (20°C) for a day, then hot air dried (USD-6533F; Kyung Dong Navien, Seoul, Korea) at 50°C for 24 h to produce dried radish pieces, which were then roasted at a pressure of 4.5 kg/cm² for 2 min to produce pressure roasted radish pieces, which were cooled to room temperature (20°C), then packed in airtight bags and stored in the dark.

Preparation of hot water extracts of tea samples Dried radish pieces, pressure roasted radish pieces, barley, corn, brown rice, sealwort, and cassia seeds were separately boiled in water at 1:20 (w/v) at 100°C for 1 h. Resulting extracts were filtered through Whatman No. 2 filter paper (Whatman, Springfield Mill, UK), concentrated in a vacuum evaporator (rotavator R-200; Bchi, Flawil, Switzerland) and freeze-dried (SFDSM06; Samwon, Busan, Korea), then stored at -80°C for subsequent *in vitro* testing.

Determination of DPPH radical scavenging activities and total phenolic contents The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was determined using a DPPH solution. The total phenol content was determined using Folin-Ciocalteu reagent (11). Total phenol contents of hot water extracts were expressed as tannic acid equivalents (mg of tannic acid/g of extract).

Tea brewing In order to develop a formula for the roasted radish

Table 1. Sugar substitutes used for adjustment of sweetness of the formulated roasted radish tea beverage

Ingredient	Degree Brix (°Bx)			
	5	6.5	8.5	12
Mixed tea (mL) ¹⁾	95.21	92.79	92.71	89.7
Inverted sugar syrup (g) ²⁾	3.2	5.44	5.44	7.68
ScFOS (g) ³⁾	1	1.7	1.7	2.4
Stevia (g) ⁴⁾	0.09	0.073	0.153	0.216

¹⁾Pressure roasted radish tea: Cassia seed tea: Barley tea (3:1:1, v/v/v).

²⁾78±2 Brix (Golden syrup; Sejeon Co.).

³⁾Brix of scFOS was calculated based on the relative sweetness of scFOS to sugar, which is 25%.

⁴⁾Brix of stevia was calculated based on the relative sweetness of stevia to sugar, which is 250%.

tea beverage, pressure roasted radish pieces and roasted grains were brewed. Pressure roasted radish pieces were brewed in boiled water (1:100, w/v) for 2 min (12). Hot water extracts of barley and cassia seeds exhibited significantly ($p<0.05$) greater DPPH radical scavenging activities than that of other roasted grain were selected and subsequently used as sub-ingredients for development of the roasted radish tea beverage. Barley was boiled in water (1:40, w/v) for 10 min, and cassia seeds were boiled in water (1:100, w/v) for 30 min, following supplier guidelines. After brewing, tea solids were removed via filtering through a stainless steel strainer (100 mesh). Bottled water (Jeju Samdasoo; Jeju Province Development Co, Jeju, Korea) was used for tea brewing.

Development of a formula for the roasted radish tea beverage

Pressure roasted radish tea, cassia seed tea, and barley tea were mixed for development of the roasted radish tea beverage. The proportion of pressure roasted tea in beverage formulations varied from 1 to 4 parts, while proportions of both cassia seed tea and barley tea were fixed at a single part each. Ratios used for this study were determined based on sensory evaluations (data not shown). Minimum amounts of cassia seed tea and barley tea were used to mask the radish flavor of the developed beverage.

Sweetness values of formulated tea beverages were in a range of 5 to 12°Bx using stevia, scFOS, and inverted sugar syrup sugar substitutes (Table 1) for a sweet, low calorie drink. The 5 to 12°Bx sweetness values used in this study was based on several beverages favored by Korean consumers, including cola, carbonated barley tea beverage, and lemon drinks. The amount of stevia used was restricted to avoid a bitter taste. Finally, to increase palatability and anti-oxidant properties of the tea beverage, ascorbic acid and citric acid were added. The ascorbic acid content of the final product was fixed at 0.05% (w/v) or 100 mg/day based on Dietary Reference Intakes for Koreans (KDRIs) (13) at a 200 mL volume for a single cup. The citric acid content varied from 0.001 to 0.05%. Citric acid can be used at a concentration of up to 1% (w/v) as a food additive (14).

Carbonation of the roasted radish tea beverage Seven hundred

mL of the tea beverage was put into a specially designed 1 L bottle for carbonation (Twist'n Sparkle; iSi GmbH, Vienna, Austria), followed by carbonic acid (gas type) injection. The amount of carbonic acid dissolved in the roasted radish tea beverage was 8 g.

Sensory evaluation Sensory evaluation was carried out at each step of the formula determination. The tea beverage was chilled in a refrigerator at 4°C and 80 mL of tea was served to panelists in a 73x75x50 mm white paper cup (DaeHeung Industry, Jeonju, Korea) labeled with a random 3 digit code. Ten panelists were intensively trained for 1 week with different tea beverages prior to evaluation. A nine point hedonic scale where 9=like extremely and 1=dislike extremely (15) was used for evaluation of the 4 attributes of overall acceptance, color, flavor, and taste.

Consumer acceptance testing Consumer testing was conducted after approval by the IRB of Pusan National University (PNU IRB/2014_89_HR). The roasted radish tea beverage developed in this study was compared with commercially available grain tea beverages (Haneulbori; Woongjin Foods Co., Ltd., Seoul, Korea), hereafter labeled as H. A carbonated version of the roasted radish tea beverage was also compared with carbonated grain tea beverages on the market (McCol; Ilhwa Co., Ltd., Seoul, Korea), hereafter labeled as M. A total of 60 consumers consisting of 24 males and 36 females aged from 23 to 38 years were recruited through a posting recruitment notice on an online bulletin board. The number of participants ($n=60$) for consumer acceptance testing was determined to obtain meaningful statistical results (16,17). Two sets of consumer testing were separately performed. All beverages were chilled in a refrigerator at 4°C and 80 mL of tea was provided individually. Tea was served in a 73x75x50 mm white paper cup (DaeHeung Industry) labeled with a random 3 digit code. Bottled water (Jeju Samdasoo; Jeju Province Development Co.) at room temperature was provided to participants for palate rinsing prior to each evaluation. Consumers rated tea beverages on a 9 point hedonic scale (15) for the 5 attributes of overall acceptance, color, flavor, taste, and sweetness. Evaluation of the carbonated tea beverage was also carried out.

Statistical analysis Data were analyzed using a one-way analysis of variance (ANOVA), followed by Duncan's multiple range test for determination of significance differences among beverage samples using SPSS version 20 (SPSS Inc., Chicago, IL, USA) at $p<0.05$. Consumer acceptance testing data were analyzed using Student's t -test at $p<0.05$.

Results and Discussion

Selection of grains as sub-constituents in the roasted radish tea beverage DPPH radical scavenging activities and total phenolic contents of dried radish pieces, pressure roasted radish pieces, and

Table 2. DPPH scavenging activities and total phenolic contents of hot water extracts of dried radish pieces, pressure roasted radish pieces, and other grains

Sample ²⁾	IC ₅₀ value of DPPH (mg/mL)	Total phenolic compound (mg/g)
DR	1.54±0.10 ^{1)b3)}	14.76±2.11 ^f
PRR	0.68±0.02 ^d	49.39±0.47 ^c
Barley	0.65±0.01 ^d	27.48±2.48 ^d
Corn	0.94±0.04 ^c	61.64±0.55 ^b
Brown rice	0.91±0.08 ^c	22.67±1.88 ^e
Cassia seed	0.68±0.03 ^d	68.72±1.55 ^a
Sealwort	2.61±0.10 ^a	22.24±1.84 ^e

¹⁾Data are mean±standard deviation (SD) ($n=3$).

²⁾DR: dried radish pieces, PRR: pressure roasted radish pieces, Barley (*Hordeum vulgare* L.), Corn (*Zea mays* L.), Brown rice (*Oryza sativa* L.), Cassia seed (*Cassia tora* L.), Sealwort (*Polygonatum odoratum* var.).

^{3)a-f}Values with different letters within a column are significantly different based on a one-way ANOVA followed by Duncan's multiple range test at $p<0.05$.

several different roasted grains used for tea preparation were determined and expressed as IC₅₀ values (Table 2). DPPH is generally used for determination of free radical-scavenging anti-oxidant activities of plant extracts (18). Total phenolic contents are closely related to anti-oxidant activities of herbs (19) and are frequently used for determination of anti-oxidant properties of plant extracts. DPPH radical scavenging activities and total phenolic contents of pressure roasted radish pieces increased by 250 and 330%, respectively, compared with dried radish pieces (Table 2), in agreement with an *in vitro* study using LLC-PK₁ cells (10). The Maillard reaction product 5-hydroxyl methyl furfural (5-HMF) was detected in pressure roasted radish pieces but not in dried radish pieces (10). Among 5 different grains used, barley and cassia seeds exhibited significantly ($p<0.05$) increased DPPH scavenging activities, compared with other grains. On the other hand, corn and cassia seeds showed significantly ($p<0.05$) higher total phenolic contents than other grains.

Sensory evaluation was performed using mixed teas for determination of which grain tea most effectively masked the radish flavor of the formulated radish tea. Barley tea had the strongest effect (data not shown). Based on anti-oxidant properties of grains and sensory evaluations, barley and cassia seeds were selected as sub-constituents for development of the roasted radish tea beverage. Barley is a rich source of soluble fiber-glucans, which are known to have health beneficial effects against diabetes (20), hypercholesterolemia (21), and cancers (22). *Cassia tora* L. is a medicinal plant used as a diuretic and for treatment of diarrhea and an anti-oxidant effect of cassia seeds was reported to reduce symptoms of hypercholesterolemia and hypertension (23).

Determination of different tea proportions for development of the roasted radish tea beverage Sensory evaluations showed that acceptance levels of a mixed tea prepared with pressure roasted radish tea, cassia seed tea, and barley tea were significantly ($p<0.05$)

Table 3. Sensory evaluation of the roasted radish tea beverage prepared using different tea proportions

	Roasted radish tea ⁴⁾ : Cassia seed tea : Barley tea (v/v/v)			
	1:0:0	4:1:1	3:1:1	2:1:1
Overall liking	3.57±1.27 ^{1)b2)5)}	6.43±1.19 ^a	6.60±1.07 ^a	6.00±1.00 ^a
Color liking	6.75±1.58 ^{NS3)}	6.89±1.36	7.11±1.05	6.78±1.20
Flavor liking	3.88±1.36 ^b	6.00±0.87 ^a	6.44±1.33 ^a	6.56±0.73 ^a
Taste liking	3.86±0.90 ^b	6.38±1.77 ^a	6.44±1.51 ^a	6.33±0.87 ^a

¹⁾Values are mean±SD (n=10 persons).^{2)a,b}Values with different letters within a row are significantly different based on a one-way ANOVA followed by Duncan's multiple range test at $p<0.05$.^{3)NS}Values in a row are not significantly different at $p<0.05$.⁴⁾Tea was prepared using pressure roasted radish pieces.⁵⁾9=like extremely, and 1=dislike extremely.**Table 4.** Sensory evaluation scores for the roasted radish tea beverage with different sweetness values

	Degree Brix (°Bx)			
	5	6.5	8.5	12
Overall liking	4.13±1.13 ^{1)b2)}	6.38±1.06 ^a	5.63±1.41 ^{ab}	5.25±1.98 ^{ab}
Color liking	5.25±0.89 ^{NS3)}	6.25±1.39	5.25±1.28	5.13±2.23
Flavor liking	5.50±0.76 ^{NS}	5.63±0.92	4.63±1.77	5.13±1.13
Taste liking	3.88±1.13 ^b	6.13±1.13 ^a	5.50±2.00 ^{ab}	4.88±2.36 ^{ab}
Sweetness liking	4.13±1.36 ^{NS}	6.00±1.07	5.50±1.93	5.25±2.55

¹⁾Values are mean±SD (n=10 persons).^{2)a,b}Values with different letters in a row are significantly different based on a one-way ANOVA followed by Duncan's multiple range test at $p<0.05$.^{3)NS}Values in a row are not significantly different at $p<0.05$.

better than for pressure roasted radish tea alone, regardless of proportions of different teas used (Table 3). In particular, flavor and taste evaluations of tea beverages were significantly ($p<0.05$) improved by mixing of different teas with increased overall acceptance scores. Among different proportions used for tea beverage preparation, a mixed roasted radish tea beverage prepared using 3 parts of pressure roasted radish tea, a single part of cassia seed tea, and a single part of barley tea was most favorable based on taste, color, and overall acceptance. Recently, tea made from roasted radishes has been widely consumed in the home for promotion of general health, in particular to prevent coughing and other diseases associated with lung or bronchioles (9). Volatile sulfur compounds of radishes are known to have pharmaceutical effects and these compounds have long been used as a folk medicine. However, volatile sulfur has a strong smell (24). Sensory evaluation produced a lower score for the flavor of pressure roasted radish tea than for the mixed tea. Thus, the typical radish flavor must be addressed prior to development of a roasted radish tea beverage. In this study, sensory evaluation scores for the flavor of a mixed pressure roasted radish tea consisting of a single part of barley and a single part of cassia tea increased 2-fold compared to that of pressure roasted radish tea. The nutty flavor of roasted barley (25) appears to have been responsible for masking the radish flavor, while cassia tea was probably ineffective and did not contribute to the masking effect but cassia tea increased anti-oxidant effects of mixed tea. The functional radish tea beverage reported herein was useful as a consumer oriented product based on use of radishes, which constitute the second largest vegetable crop harvested in Korea.

Determination of sweetness values for the roasted radish tea beverage prepared using different sugar substitutes For preparation of a tea beverage with a sweet taste and a low calorie content, the sugar substitutes stevia, scFOS, and inverted sugar syrup were used. The roasted radish tea beverage was prepared via mixing of 3 portions of roasted radish tea, a single portion of cassia seed tea, and a single portion of barley tea. Amounts of sugar substitutes used for adjusting sweetness values (°Bx) of tea beverages are shown in Table 1. Overall acceptance and taste scores of tea beverages with a sweetness level over 6.5°Bx were significantly ($p<0.05$) higher than for beverages with lower sweetness values (Table 4). However, there were no significant differences in scores among tea beverages with a sweetness level ranging from 5 to 12°Bx. Sweetness scores showed a range of 2 points on the 9 point hedonic scale. Nevertheless, 6.5°Bx tea beverages were most preferred based on color, flavor, taste, sweetness, and overall acceptance. Total caloric contents of 6.5°Bx tea beverages decreased 23.5% after use of sugar substitutes.

Determination of citric acid contents Based on sensory evaluation testing (Table 5), over 0.05% (w/v) citric acid contents of tea beverages caused sourness acceptance scores and overall acceptance, flavor, and taste scores to decrease significantly ($p<0.05$), compared with tea beverages with less citric acid, indicating that sourness is a critical factor affecting preferences for tea beverages. The amount of ascorbic acid was fixed at 0.05% (w/v) to meet the daily recommended amount of ascorbic acid, and the synergistic effect of citric acid on the overall acceptance of tea was monitored. A citric acid content of 0.01% (w/v) in tea beverages increased palatability. Apparently a

Table 5. Sensory evaluation results for the roasted radish tea beverage with varying citric acid contents

	Citric acid (%)			
	0.005	0.01	0.05	0.1
Overall liking	5.80±2.78 ^{1)ab2)}	7.00±1.41 ^a	3.40±1.67 ^{bc}	2.40±1.67 ^c
Color liking	5.80±1.30 ^{NS3)}	5.60±1.34	6.40±0.89	6.20±0.84
Flavor liking	6.00±1.58 ^{NS}	7.20±0.84	3.80±1.48	3.20±1.64
Taste liking	6.00±1.58 ^a	7.20±0.84 ^a	3.80±1.48 ^b	3.20±1.64 ^b
Sourness liking	5.80±1.09 ^a	5.60±1.34 ^a	3.60±1.95 ^b	2.40±1.14 ^b

¹⁾Values are mean±SD (n=10 persons).

^{2)a-c}Values with different letters within a row are significantly different based on a one-way ANOVA followed by Duncan's multiple range test at $p<0.05$.

^{3)NS}Values in a row are not significantly different at $p<0.05$.

Table 6. Consumer preferences for the roasted radish tea beverage and commercial barley tea beverage

	Roasted radish tea beverage	Barley tea beverage ²⁾	Carbonated tea beverage	
			Developed in this study ³⁾	Available on the market ⁴⁾
Overall liking	7.30±0.95 ^{1)*5)}	6.13±1.60	5.06±1.52 ^{NS}	4.48±1.94
Color liking	5.72±1.88 ^{NS6)}	5.07±1.62	6.02±1.46 ^{***}	4.93±1.75
Flavor liking	6.33±1.21 ^{NS}	5.92±1.43	4.69±1.29 ^{NS}	4.52±1.89
Taste liking	7.17±0.75 ^{**}	5.92±1.63	5.07±1.55 ^{NS}	4.60±1.92
Sweetness liking	6.03±1.53 ^{***}	4.55±2.16	5.70±1.54 ^{NS}	5.22±1.99
Carbonated taste liking	-	-	5.56±1.28 ^{NS}	5.23±1.57

¹⁾Values are mean±SD (n=60 persons).

²⁾Haneulbori (Woongjin Foods Co., Ltd.).

³⁾Carbonated roasted radish tea beverage.

⁴⁾McCol (Ilhwa Co., Ltd.).

⁵⁾Significantly different based on student's *t*-test at $p<0.05$.

^{**}Significantly different based on student's *t*-test at $p<0.01$.

^{***}Significantly different based on student's *t*-test at $p<0.001$.

^{6)NS}Values in a row are not significantly different at $p<0.05$.

nutty flavor and taste for the formulated tea beverage were eliminated by a sour tea taste. The final formula for the roasted radish tea beverage was a mixture of pressure roasted radish, barley, and cassia seed teas prepared separately (3:1:1, v/v/v) with addition of 54.4 g/L inverted sugar syrup, 0.73 g/L stevia, 17 g/L scFOS, 0.01% citric acid, and 0.05% vitamin C.

Consumer acceptance testing Consumer acceptance testing was carried out using 60 participants between 23 and 38 years of age. Scores for overall acceptance, taste, and sweetness of the formulated tea beverage were significantly ($p<0.05$) higher than for the H tea beverage available on the market (Table 6). The caloric content of the tea beverage developed in this study was 19.35 Kcal/100 mL while the content of H tea beverage was 0 Kcal. The radish flavor of tea was weakened by mixing barley and cassia seed teas into the formulated roasted radish tea. The flavor score for the tea beverage was 6.3 on a 9 point hedonic scale, which was acceptable. Thus, the formula developed in this study for the roasted radish tea beverage can be used for mass production. Consumer acceptance testing is designed to support product development based on consumer reactions to new or revised products, and to provide estimates of product acceptance (26).

The carbonated version of the roasted radish tea beverage was also evaluated in comparison with the M carbonated tea beverage

available on the market. Overall acceptance and other attribute scores, including color, flavor, taste, sweetness, and carbonated taste, for both carbonated tea beverages were approximately 5 on a 9 point hedonic scale (Table 6), indicating that both carbonated tea beverages were not highly preferred by consumers. Sensory evaluation results demonstrated that consumers preferred the roasted radish tea beverage without carbonation.

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