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Evaluation of Quality Characteristics of Broth Packets with Different Treatment of Dolsan Mustard Seeds

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Dolsan mustard seeds (DMS) were added in whole, crushed, and roasted form at 0.5 g (S-1), 1.0 g (S-2), and 1.5 g (S-3), respectively to broth and heated for 10 or 15 min. After cooling, the quality characteristics were measured. Salinity and pH decreased with boiling time. The antioxidant activities of the experimental broth were measured in terms of total polyphenol content, total flavonoid content, electron donating ability (EDA), 2,2-azino-bis (3-ethyl-benzothizoline-6-sulfonic acid) (ABTS) radical scavenging activity, and ferric reducing antioxidant power (FRAP). The overall, antioxidant activity was higher in broths containing 1.0 g and 1.5 g DMS than in those containing 0.5 g DMS and the activity increased with increasing boiling time. Sinigrin was not detected in the control group, and no significant difference in sinigrin content was noted among broths containing different concentrations of DMS. A high glutamic acid content was detected in the control broth, whereas glutamic acid, aspartic acid, glycine, proline, alanine, and arginine were detected in the broths containing DMS. The free amino acid contents, particularly aspartic acid and glutamic acid contents, were high in umami. Volatile components, such as 2-propenyl-isothiocyanate (ITC), allylthiocyanate, n-butyl ITC, and 3-butenyl ITC, were detected in the DMS-containing broths. Sensory evaluation revealed that a higher amount of DMS added and a longer heating time increased the overall taste preference, and the difference was statistically significant. The purpose of this study was to present basic data on the quality characteristics of DMS-added broths to aid in the development of new products using DMS.

Key words : Antioxidant activities, Dolsan mustard seed, sinigrin, volatile components

Introduction

The rising market demand for readymade seasoned broth sauce has boosted, research on commercial broth production, development of standard cooking methods, and quality. Thus, ongoing research focuses, for example, on the quality characteristics of fish broth addes with white wine [9], on the standardization of cool noodle broth recipes [14], the quality characteristics of chicken broth added with tomato [23], and the struggle for purchase of commercial broth products [2, 9], among other subjects of interest to the food industry. However, concomitant with the increasing consumer demand for improved quality of soup sauces and simple and convenient products, consumer concerns regarding the use of food additives has also spiked. In addition, as public interest in diet and lifestyle-related diseases, such as obesity and hypertension, is increasing [9], the idea and practice of preparing soups from scratch, despite the effort and time required, slowly but gradually is gaining ground. However, recent studies have focused only the physicochemical and sensory characteristics of broth cooked by mixing various raw materials such as fish, meat like beef, chicken, fish, or vegetables, and spices [9, 13, 23]. Mushrooms, anchovies, kelp, chicken, clams, bonito, brisket, and shrimp are the ingredients most widely used for preparing broths. Further, as data on the standard manufacturing methods with respect to nutrient content information and cooking conditions are still insufficient, information on broth nutrient content is currently excluded and replaced with the quantity of water used, while the evaluation of nutritional properties relative to the recommended dietary allowances for Korean continues. Further, although several studies have focused on the antioxidant activity of the phytochemicals extracted from plant foods; studies on the

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antioxidant activities of broths are scarce.

Dolsan leaf mustard (DLM) is a vegetable that belongs to the cruciferous family, whose leaves and stems are widely used as ingredients for preparing kimchi, while the seeds are used as spice because of their strong acidity [19]. This study is expected to demonstrate the high antioxidant activity of Dolsan mustard seeds (DMS) due to its high β-carotene content, which shows the highest sinigrin and antioxidant activities, and the high chlorophyll content previously reported [6, 21]. In this study, we compared the antioxidant activity of raw cooking materials combined in different ratios and containing different concentrations of DMS, which showed excellent antioxidant properties. Boiled for 10 mins and 15 mins, and boiled for 10 to 15 mins, referring to the boiling time of most commercially available broths in three types: whole, crushed and roasted, it was used in the experiment. Additionally, a sensory evaluation of the experimental broths was conducted. Our objective is to provide, a variety of functional natural products suitable for developing new added broth packet sets to satisfy consumer needs according to age and income groups.

Materials and Methods

Materials and reagents

The DMS used in this experiment were purchased in 2019 from the Agricultural Technology Center in Yeosu, Jeonnam Province, and stored at -18℃. The anchovies, kelp, kuruma shrimp, dried radish, *Hovenia dulcis* Thunb, and lotus used for preparing the experimental broth packetsets were purchased from a local market. Whole, crushed, and roasted DMS were added 0.5 g, (S-1), 1.0 g (S-2), 1.5 g (S-3) to the broth according to the mixing ratio to make a broth packetsets. The packetsets were boiled for 10 mins and 15 mins in 500 ml water and then cooled before further use. Sinigrin, Folin-Ciocalteu's phenol reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2-azino-bis(3-ethyl-benzothizoline-6-sulfonic acid) (ABTS), sodium carbonate, gallic acid, 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ), iron (III) chloride hexahydrate, Iron (II) sulfate heptahydrate ($FeSO_4 \cdot H_2O$), potassium persulfate, ascorbic acid, sodium chloride (NaCl), aluminum nitrate, potassium acetate, quercetin, methanol and ethanol were all purchased from Sigma-Aldrich Co. (St. Louis, MO, USA).

pH measurement

The pH of each sample broth was measured using 25 ml of the tested broth and a pH meter (Orion 520A, Boston, USA). Each pH measurement was made in triplicate and mean values were used for statistical analysis.

Salinity measurement

Tested broth (35 ml) each were placed in polypropylene tubes and the salinity was measured with a digital salinity meter (Atago ES-421, Tokyo, Japan).

Measurement of total polyphenol content

Samples of each broth packets were boiled and cooled to determine total polyphenol content using colorimetry and Folin-Ciocalteu's phenol reagent [20]. Briefly, 200 µl of Folin-Ciocalteu's phenol reagent and 2.6 ml distilled water were added to 200 µl of the sample and incubated for 6 minutes at room temperature. Then, the sample was incubated with 2 ml of 7% (w/v) Na_2CO_3 solution for 90 minutes, and the absorbance was measured at 750 nm using a microplate reader (Infinite F50, Männedorf, Switzerland). A standard curve was constructed using gallic acid and polyphenol content was reported as mg of gallic acid equivalents (GAE)/g.

Measurement of total flavonoid content

Total flavonoid content of each broth was measured as previously described [17]. Briefly, 0.1 ml of 10% aluminum nitrate, 0.1 ml of 1 M potassium acetate and 4.3 ml of 80% (v/v) ethanol were sequentially added to 1 ml of the sample and the absorbance was measured at 415 nm after incubating in dark for 40 mins. A standard curve was constructed using quercetin and flavonoid content was reported as mg of quercetin equivalents (QE)/g.

Measurement of electron donating ability (EDA)

The electron donating ability (EDA), which estimates DPPH radical scavenging ability, was measured to calculate the reducing power of the broth. Briefly, 0.5 ml of 0.5 mM DPPH solution was added to a test tube containing 1 ml of the sample, 1 ml methanol, and 0.99 ml of 100 mM sodium acetate buffer (pH 5.5). The mixture was agitated and incubated in the dark for 5 mins. The absorbance of the solution was measured at 517 nm. EDA was claculated using to the following equation [22]:

$$\frac{\text{EDA}}{(\%)} = \left(1 - \frac{\text{absorbance of the solution with sample}}{\text{absorbance of the solution without sample}}\right) \times 100$$

2,2-azino-bis(3-ethyl-benzothizoline-6-sulfonic acid) (ABTS) radical scavenging activity

The ABTS radical scavenging activity was measured using

the method reported by Kriengsak et al. [16] after a slight modification. Briefly, equal volumes of 1.8 mM ABTS solution and 0.63 mM potassium persulfate were mixed and incubated in dark for 24 hr at 37℃ to prepare a solution with ABTS free radicals; the solution was modified such that absorbance at 735 nm was 1.4±0.1. Then, 5 ml of the solution was added to 0.1 ml of the sample and incubated for 7 minutes. Subsequently, the absorbance was measured at 735 nm and the ABTS radical scavenging ability was calculated according to the following equation:

$$\text{ABTS radical scavenging ability (\%)} = \left(1 - \frac{\text{absorbance of the solution with sample}}{\text{absorbance of the solution without sample}}\right) \times 100$$

Measurement of ferric reducing antioxidant power (FRAP)

Ferric reducing antioxidant power (FRAP) was measured using the method reported by Benzie et al. [1] after a slight modification. Briefly, 30 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyls-triazine (TPTZ) dissolved in 40 mM HCl, and 20 mM iron (III) chloride hexahydrate were mixed in a 10:1:1(v:v:v) ratio. Then, 150 µl of the broth sample was mixed with 2,850 µl of the reaction mixture and incubated for 30 minutes; subsequently, absorbance was measured at 593 nm; a standard curve was constructed using $FeSO_4 \cdot H_2O$, and FRAP content was quantified as mg $FeSO_4$ equivalents/g extract.

Sinigrin standard curve and high-performance liquid chromatography (HPLC) operating conditions

Sinigrin solution at 125, 250, 500, and 1,000 ppm were prepared; measurements using these solutions were repeated three times and the mean values were used to construct a standard curve, used to determine sinigrin concentration in the broth samples. HPLC was performed using a Shimadzu JP/LC-20Avp istrument, fitted with a UV-VIS detector set at 228 nm, and a Phenomenex Luna 5 um C18 column (4.6× 250 mm) with a flow rate of 1.0 ml/min and a 10 µl injection volume.

Sensory evaluation

Ten trained panelists, who were graduate students at the Chonnam National University, performed the sensory evaluation. The color, flavor, taste, texture and overall acceptability were evaluated using a 9-point scale in which (1= very weak, 3= weak, 5= moderate, 7= strong, and 9= very strong).

Total amino acid analysis

This solution was filtered through a 0.2 µm membrane filter (GE Healthcare Bio-Sciences, Pittsburgh, PA, USA). The total amino acid content of the filtered liquid was analyzed using an automatic amino acid analyzer S433-H (Sykam GmbH, Germany) and a cation separation column (LCA K06/Na, 4.6×150 mm) set at 57-74℃. Buffer pH ranged from 3.45 to 10.85, with the flow rates for the buffer and reagent set to 0.45 and 0.25 ml/min, respectively. An automatic injector was used to inject 130 µl of the sample and a fluorescence detector (Ex=440 nm, Em=570 nm) was used for fluorescence detection.

Free amino acid analysis

This solution was filtered through a 0.2 µm membrane filter (GE Healthcare Bio-Sciences, Pittsburgh, PA, USA) and the free amino acid content of the filtered liquid was studied using an automatic amino acid analyzer S430 (Sykam GmbH, Germany) and a cation separation column (LCA K07/Li, 4.6× 150 mm) set at 37-74℃. The buffer pH ranged 2.90-7.95, with the flow rates of the buffer and reagent being 0.45 and 0.25 ml/min, respectively. An automatic injector was used to inject 130 µl of the sample and a fluorescence detector (Ex=440 nm, Em=570 nm) was used for fluorescence detection.

Gas chromatography-mass spectrometry (GC-MS)

Broth samples were mixed with 5 ml of distilled water and analyzed by GC-MS using a QP-2010 Ultra gas chromatograph (Shimadzu, Kyoto, Japan) and a Restek Rtx-5MS capillary column (30 m × 0.25 mm, 0.25 µm film thickness; Bellefonte, PA, USA). Initial GC oven temperature was maintained at 40℃ for 2 min, which was programmed to reach 250℃ at a rate of 6℃/min, and held at that point for 13 min. High-purity helium was used as carrier gas at a flow rate 1.0 ml/min. Injector temperature was maintained at 250 ℃and injection volume was 1.0 ml in split mode (10:1). Ionization source and interface temperatures were 200 and 250℃, respectively. Mass spectroscopy was performed at 70 eV, with a 50-500 m/z mass range. Three replicates were performed for each sample. The identity of volatile compounds was confirmed by comparison of spectra with published spectra in Wiley's database.

Statistical analysis

All tests and analyses were repeated at least three times. Results are expressed as the means ± standard deviation (SD). One-way analysis of variance (ANOVA) and Duncan's test

were used for multiple comparisons using the SPSS version 21.0 (SPSS Institute, Chicago, IL, USA). Differences among values were considered statistically significant at $p<0.05$ in all experiments.

Results and Discussion

pH and salinity of the DMS added broths

Broth packets were prepared according to the ingredient mixture ratios shown in Table 1, which were dissolved in 500ml of water and boiled. After boiling for 10 and 15 minutes, the broth was allowed to cool and their pH and salinity were measured Fig. 1. The pH of the control broth without DMS were 6.780 and 6.735 at 10 and 15 minutes after boiling, respectively and significantly decreased with increasing DMS concentration and boiling time. This finding is consistent with previous observations of the broth becoming acidic upon heating of the meat juice at a high temperature for an extended time [15]. Further, the salinity of the broth tended to decrease significantly as the concentration of DMS increased. In addition, salinity increased with increasing in boiling time, which could be due to the increase in the solid content of the broth as moisture content decreases with boiling time [11].

Table 1. Ingredients used to prepare the tested broths

Ingredient (g)	Control	S-1	S-2	S-3
Anchovy	6.3	6.0	6.2	6.2
Kelp	1.95	1.9	1.8	1.8
Kuruma shrimp	1.95	1.9	1.7	1.8
Dried radish	1.95	1.9	1.7	1.8
Hovenia dulcis Thunb	0.9	0.9	0.9	0.9
Lotus	1.95	1.9	1.7	1.5
Dolsan mustard seed		0.5	1.0	1.5
Total	15.0	15.0	15.0	15.0

Antioxidant activity of DMS added broths

The antioxidant activity of the experimental broths was

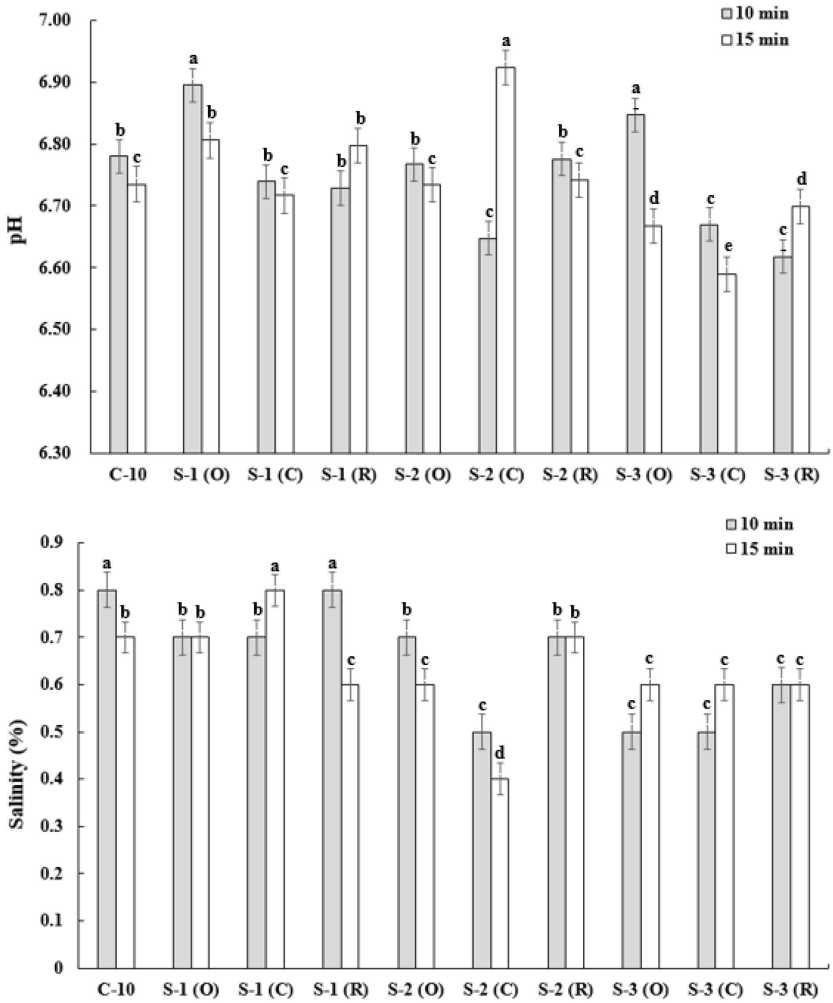

Fig. 1. pH and salinity values of the broths containing whole (W), crushed (C) and roasted (R) Dolsan mustard seeds. Data represent the mean ± SD of triplicate experiments (n=3). Columns with the same lower-case letters (a-c) are not significantly different by Duncan's multiple range test ($p<0.05$).

Table 2. Total polyphenol (TP) and total flavonoid (TF) contents and antioxidant activities of the broths containing whole (W), crushed (C) and roasted (R) Dolsan mustard seeds

10 min	Control	S-1			S-2			S-3		
		W	C	R	W	C	R	W	C	R
TP[1)]	83.98±0.69^{aB}	252.30±4.97^{bC}	339.77±4.73^{dC}	369.58±6.41^{dD}	298.72±5.42^{cC}	303.52±3.54^{cD}	281.98±1.44^{cB}	240.52±6.18^{bC}	381.57±1.80^{dD}	373.37±1.75^{dC}
TF[2)]	20.63±0.29^{aA}	26.75±4.16^{aA}	33.17±1.70^{bB}	41.96±8.06^{cC}	42.46±15.63^{cA}	54.25±6.30^{dB}	45.67±3.24^{cA}	45.17±2.40^{cA}	54.33±0.09^{dB}	63.85±7.21^{dB}
EDA (%)	25.45±2.85^{aA}	36.25±0.68^{bB}	33.02±1.16^{bB}	40.53±0.34^{cC}	38.43±3.92^{bA}	41.12±0.11^{cA}	46.42±3.20^{cA}	54.01±5.10^{dB}	58.80±2.04^{dB}	56.81±3.45^{dA}
ABTS (%)	24.48±0.27^{dA}	31.99±0.26^{bB}	32.82±0.30^{bB}	33.72±0.24^{bB}	40.11±0.30^{cA}	40.91±0.70^{cA}	42.01±0.59^{cA}	45.98±0.22^{dA}	47.60±0.35^{dA}	50.37±4.25^{dA}
FRAP[3)]	26.60±2.60^{aA}	28.70±0.70^{aA}	26.80±0.35^{aA}	28.67±2.46^{aA}	185.82±4.13^{bB}	208.10±7.75^{bC}	327.57±8.57^{dC}	192.98±7.21^{bC}	270.08±4.90^{cC}	328.40±1.28^{dC}
15 min	Control	S-1			S-2			S-3		
		W	C	R	W	C	R	W	C	R
TP	90.02±0.03^{aC}	351.60±2.24^{cC}	318.25±4.35^{cC}	341.67±2.51^{cD}	292.35±2.13^{bE}	300.95±2.59^{cD}	250.87±3.47^{bD}	446.82±7.00^{eD}	380.25±2.43^{dE}	429.45±8.82^{eE}
TF	43.05±6.76^{cB}	34.02±1.14^{bB}	32.38±5.25^{bA}	32.47±6.58^{bA}	35.05±1.46^{bB}	27.07±3.68^{aA}	48.79±0.57^{dA}	41.31±1.62^{cA}	47.37±3.13^{dA}	23.16±3.28^{aA}
EDA (%)	28.57±6.33^{aA}	40.84±0.26^{bB}	52.72±0.95^{cB}	64.70±0.23^{dC}	44.32±1.72^{bC}	45.57±0.69^{bB}	69.31±1.05^{dC}	68.51±0.47^{dB}	76.26±0.30^{eC}	77.56±0.20^{eC}
ABTS (%)	23.88±0.20^{aA}	31.77±0.56^{bA}	31.87±0.45^{bA}	41.53±0.30^{cB}	54.07±0.20^{dD}	55.61±0.47^{dC}	57.99±0.19^{dB}	61.94±0.34^{eB}	62.14±0.45^{eB}	64.64±0.27^{eB}
FRAP	29.50±1.15^{aA}	28.62±0.40^{aA}	29.63±1.28^{aA}	28.03±3.64^{aA}	28.83±2.62^{aA}	27.10±3.05^{aA}	234.0±2.42^{cD}	142.57±7.37^{bC}	284.52±3.33^{dD}	270.22±1.26^{dD}

[1)]Total polyphenols in mg GAE/100 g. [2)]Total flavonoids in mg QE/100 g.[3)]mg $FeSO_4$ eq./100 g

* Data represent the mean ± SD of the results of triplicate experiments. The different lower-case letters (superscript) in the same row (a-e) and column (A-E) indicate statistically significant difference by Duncan's multiple range test (p<0.05).

Table 3. Sinigrin content and sensory evaluation of the broths containing whole (W), crushed (C) and roasted (R) Dolsan mustard seeds

10 min	Control	S-1			S-2			S-3		
		W	C	R	W	C	R	W	C	R
Sinigrin (mg/100 g)	N.D.[1)]	0.11±0.02^{a}	0.12±0.01^{a}	0.12±0.02^{a}	0.13±0.02^{a}	0.13±0.03^{a}	0.14±0.03^{a}	0.14±0.04^{a}	0.14±0.03^{a}	0.14±0.03^{a}
Color	1.40±0.01^{aA*}	3.40±0.02^{bA}	5.00±0.02^{eC}	3.00±0.01^{bA}	3.40±0.01^{bA}	3.80±0.04^{cB}	4.00±0.03^{cA}	4.01±0.02^{cA}	4.60±0.02^{dB}	4.60±0.03^{dB}
Flavor	2.23±0.02^{aB}	3.40±0.02^{bA}	3.80±0.03^{cB}	3.40±0.02^{bA}	3.40±0.02^{bA}	3.40±0.03^{bA}	3.81±0.05^{cA}	4.00±0.04^{cA}	4.39±0.03^{dA}	4.40±0.03^{dA}
Taste	2.45±0.02^{aC}	3.80±0.02^{cB}	3.40±0.03^{bA}	3.30±0.01^{bA}	3.80±0.02^{cB}	3.80±0.03^{cB}	4.02±0.02^{dA}	4.11±0.03^{dA}	4.10±0.02^{dA}	4.30±0.03^{dA}
Overall acceptability	2.51±0.01^{aC}	3.40±0.01^{bA}	3.40±0.02^{bA}	3.39±0.03^{bA}	3.40±0.03^{bA}	3.80±0.06^{cB}	4.00±0.04^{dA}	4.20±0.02^{dA}	4.20±0.01^{dA}	4.30±0.04^{dA}
15 min	Control	S-1			S-2			S-3		
		W	C	R	W	C	R	W	C	R
Sinigrin (mg/100 g)	N.D.[1)]	0.12±0.01^{a}	0.11±0.02^{a}	0.13±0.03^{a}	0.13±0.04^{a}	0.14±0.04^{a}	0.14±0.05^{a}	0.14±0.03^{a}	0.14±0.04^{a}	0.14±0.05^{a}
Color	2.20±0.05^{aA}	3.50±0.02^{bA}	4.20±0.01^{cB}	4.19±0.01^{cC}	4.40±0.02^{cB}	4.40±0.02^{cB}	4.41±0.02^{cB}	4.80±0.02^{dB}	4.80±0.03^{dB}	4.80±0.08^{dB}
Flavor	2.20±0.06^{aA}	4.40±0.02^{cC}	4.00±0.01^{bB}	4.10±0.03^{bC}	4.20±0.03^{bB}	4.30±0.03^{bB}	4.40±0.05^{cB}	4.51±0.02^{cA}	4.50±0.02^{cA}	4.70±0.03^{dA}
Taste	3.00±0.06^{aB}	3.80±0.03^{bB}	3.70±0.02^{bA}	3.30±0.04^{aA}	3.80±0.04^{bA}	3.80±0.03^{bA}	4.00±0.08^{cA}	4.30±0.03^{cA}	4.30±0.03^{cA}	4.50±0.02^{dA}
Overall acceptability	3.01±0.04^{aB}	3.80±0.02^{bB}	3.80±0.04^{bA}	3.70±0.02^{bB}	4.41±0.02^{cB}	4.40±0.06^{cB}	4.50±0.04^{cB}	5.01±0.02^{dC}	5.10±0.03^{dC}	5.10±0.01^{dB}

N.D.[1)]: Not detected

* Data represent the mean ± SD of the results of triplicate experiments. The different lower-case letters (superscript) in the same row (a-d) and column (A-E) indicate statistically significant difference by Duncan's multiple range test (p<0.05).

estimated by measuring total polyphenol content, total flavonoid content, EDA, ABTS radical scavenging ability, and FRAP. The results, are shown in Table 2. The antioxidant activity of food is reportedly mainly due to the presence of polyphenol compounds. Total polyphenol contents of the control broth after boiling for 10 and 15 minutes was 83.98 and 90.02 mg GAE/100 g, respectively; however, it increased with the increasing concentration of crushed or roasted DMS added to the broth. In turn, total flavonoid content in the control broth after boiling for 10 and 15 minutes were 20.63 and 43.05 mg QE/100 g, respectively, which also increased with the increasing boiling time and concentration of crushed or roasted DMS added. EDA, ABTS radical scavenging ability, and FRAP increased in proportion to the concentration of DMS added; moreover, the increase was significant with the addition of crushed or roasted DMS. Similarly, Cheung

Table 4. Total amino acid content of the tested broths containing whole (W), crushed (C) and roasted (R) Dolsan mustard seeds and boiled for 10 mins and 15 mins (mg/100 g)

10 min	Control	S-1			S-2			S-3		
		W	C	R	W	C	R	W	C	R
Aspartic acid	23.47	22.84	24.66	57.45	27.12	28.68	42.33	38.26	67.52	81.14
Threonine	7.87	8.53	7.92	9.26	8.64	10.67	9.67	6.40	8.12	13.62
Serine	8.28	8.77	9.12	9.62	9.33	10.29	10.31	7.14	8.95	14.48
Glutamic acid	77.12	62.65	74.90	118.18	81.58	82.56	116.19	93.51	118.49	150.53
Proline	23.98	21.18	24.02	30.55	22.82	22.36	36.23	15.33	18.78	32.71
Glycine	33.72	41.15	33.93	38.13	42.02	38.61	49.19	30.16	33.98	52.28
Alanine	24.22	27.22	26.77	27.81	27.68	30.98	32.75	23.15	27.22	39.16
Cystine	N.D.[1)]	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Valine	9.48	10.10	9.63	10.84	10.1	12.30	12.26	8.43	8.58	14.77
Methionine	4.53	4.58	4.92	5.02	4.98	6.14	5.86	4.04	4.14	6.9
Isoleucine	6.64	7.13	6.35	7.53	6.86	9.03	8.30	5.67	6.19	11.2
Leucine	11.06	11.27	11.49	12.5	11.8	15.38	15.06	9.42	11.05	19.72
Tyrosine	4.51	4.57	4.27	5.34	4.35	5.58	5.35	3.53	4.46	8.05
Phenylalanine	5.72	5.83	5.93	6.42	5.99	7.76	6.89	4.79	5.86	9.94
Histidine	18.91	17.68	22.00	19.49	21.07	26.62	23.43	18.08	20.93	29.62
Lysine	13.34	12.79	12.94	14.18	13.3	17.25	18.64	12.04	13.23	24.12
Arginine	25.72	27.32	24.91	27.04	26.84	27.69	34.89	22.72	21.64	39.04
Total	298.57	266.29	278.85	372.32	297.64	324.21	392.46	279.95	357.5	547.28
15 min	Control	S-1			S-2			S-3		
		W	C	R	W	C	R	W	C	R
Aspartic acid	26.89	31.02	39.24	47.27	70.60	70.14	72.89	45.24	37.38	72.43
Threonine	9.57	7.61	8.00	10.50	11.60	10.62	10.60	9.98	11.46	13.04
Serine	10.41	8.21	8.86	11.13	12.31	10.72	11.22	9.93	12.09	13.49
Glutamic acid	87.73	98.10	87.07	105.26	128.11	138.35	149.20	109.55	98.05	149.35
Proline	25.19	21.97	26.87	22.29	25.90	26.46	23.12	23.84	30.81	32.13
Glycine	45.21	33.39	40.28	39.47	40.54	39.53	44.52	39.6	45.02	40.35
Alanine	29.88	24.50	29.53	32.32	34.76	34.01	35.40	30.69	36.77	36.86
Cystine	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Valine	11.78	9.27	10.33	13.36	13.64	12.11	11.68	11.35	13.84	13.20
Methionine	6.56	4.42	5.04	6.54	6.63	7.43	5.55	5.81	7.33	6.71
Isoleucine	8.58	6.25	6.91	9.28	9.70	9.28	8.73	8.69	10.1	10.03
Leucine	14.27	10.56	12.55	16.52	16.55	16.58	14.82	14.36	18.18	17.79
Tyrosine	5.38	4.05	4.61	5.93	6.46	6.14	5.57	5.57	6.34	6.98
Phenylalanine	7.21	5.20	6.38	8.12	8.18	7.76	7.74	7.29	8.92	9.15
Histidine	25.19	27.78	26.27	27.49	27.09	24.95	27.94	20.01	26.97	29.31
Lysine	15.82	12.26	14.84	20.04	20.97	19.21	15.56	15.58	20.06	20.60
Arginine	31.38	25.41	27.78	28.52	32.88	28.03	30.50	28.24	33.55	30.32
Total	361.04	329.98	354.55	404.04	465.90	461.30	475.03	385.73	416.87	501.74

N.D.[1)]: Not detected

Table 5. Free amino acid content in broths containing whole (W), crushed (C) and roasted (R) Dolsan mustard seeds and boiled for 10 mins and 15 mins (mg/100 g)

10 min	Control	S-1			S-2			S-3		
		W	C	R	W	C	R	W	C	R
Phosphoserine	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Taurine	24.93	27.94	32.54	28.07	29.05	35.80	33.02	24.93	31.73	30.69
Phosphoethanolamine	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Urea	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Aspartic acid	21.75	7.10	8.56	36.96	9.72	20.44	8.93	21.75	45.43	37.68
Hydroxyproline	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Threonine	2.84	3.88	3.56	4.69	5.36	4.35	5.36	2.84	3.71	3.19
Serine	2.85	3.95	3.61	4.23	3.92	3.88	3.92	2.85	3.42	3.39
Asparagine	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Glutamic acid	51.59	20.47	38.29	75.71	34.32	61.78	34.29	51.59	80.40	68.49
α-aminoadipic acid	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Proline	17.36	25.37	25.09	30.17	22.40	38.46	24.19	17.36	18.38	26.49
Glycine	19.72	28.83	16.69	24.93	25.69	31.88	23.68	19.72	19.01	26.08
Alanine	22.85	26.59	22.80	26.66	24.17	30.64	29.02	22.85	25.52	26.57
Citrulline	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
α-aminobutyric acid	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Valine	5.247	5.06	5.10	5.57	4.94	5.58	7.63	5.25	4.06	5.08
Cystine	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Methionine	2.15	17.1	1.37	1.74	1.50	1.98	2.08	2.15	1.35	2.50
Isoleucine	3.58	3.96	2.99	4.10	3.39	3.94	4.82	3.58	2.97	4.31
Leucine	5.20	5.53	4.83	6.48	5.44	6.57	8.09	5.20	4.60	7.29
Tyrosine	4.12	3.17	2.52	3.13	2.59	3.41	3.69	4.12	2.36	3.74
Phenylalanine	4.36	2.40	2.03	2.76	2.26	2.90	3.36	4.36	3.42	3.30
β-alanine	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
β-aminoisobutyric acid	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
γ-amino-n-butyric acid	3.42	2.61	2.61	3.57	2.91	3.38	3.07	3.42	2.49	2.35
Histidine	12.84	15.78	15.78	12.02	13.89	14.91	19.01	12.84	12.61	13.62
1-methylhistidine	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
3-methylhistidine	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Carnosine	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Tryptopan	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Ornitine	1.35	2.66	1.04	1.48	1.45	2.21	1.70	1.35	1.46	1.74
Lysine	5.15	5.32	4.72	6.25	5.26	6.96	7.76	5.15	5.02	7.54
Arginine	13.05	17.18	12.80	16.22	15.33	21.14	16.28	13.05	9.77	18.08
Total	224.36	206.04	206.94	294.72	213.58	300.22	239.87	224.36	277.69	292.09
15 min	Control	S-1			S-2			S-3		
		W	C	R	W	C	R	W	C	R
Phosphoserine	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Taurine	29.976	33.78	29.98	33.25	32.91	40.59	32.94	31.58	37.25	34.24
Phosphoethanolamine	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Urea	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Aspartic acid	14.85	25.50	14.85	21.65	44.29	46.04	47.47	24.49	43.02	12.45
Hydroxyproline	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Threonine	3.102	5.56	3.10	4.14	5.33	5.85	5.89	4.29	5.10	4.80
Serine	3.13	3.18	3.13	3.49	3.70	5.06	3.23	3.99	4.23	3.68
Asparagine	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Glutamic acid	15.473	15.72	15.47	14.40	14.51	21.34	15.66	10.77	14.37	10.51
α-aminoadipic acid	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Proline	25.41	35.22	25.41	28.08	30.76	29.62	26.06	19.91	26.67	30.69
Glycine	20.52	20.52	20.52	25.88	24.79	19.14	28.59	25.04	20.40	26.01

Table 5. Continued

15 min	Control	S-1			S-2			S-3		
		W	C	R	W	C	R	W	C	R
Alanine	22.63	6.10	22.63	28.55	32.84	28.29	35.82	28.50	32.41	26.66
Citrulline	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
α-aminobutyric acid	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Valine	4.16	8.02	4.16	5.78	8.25	6.95	7.15	3.63	7.03	8.26
Cystine	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Methionine	1.45	2.72	1.45	1.71	3.94	1.82	2.15	2.20	2.33	3.57
Isoleucine	3.01	4.61	3.01	3.79	6.18	4.41	4.15	4.48	4.68	6.24
Leucine	4.50	7.87	4.50	5.97	9.35	8.39	7.59	6.83	7.92	9.35
Tyrosine	2.39	3.02	2.39	3.87	5.65	4.11	5.14	3.30	3.30	5.38
Phenylalanine	1.68	4.18	1.68	3.24	9.01	2.81	6.08	3.08	4.20	3.27
β-alanine	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
β-aminoisobutyric acid	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
γ-amino-n-butyric acid	2.04	3.33	2.04	3.15	3.05	4.12	5.82	2.65	3.29	2.47
Histidine	20.96	20.80	20.96	18.99	17.43	18.12	19.88	13.67	21.31	18.87
1-methylhistidine	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
3-methylhistidine	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Carnosine	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Tryptopan	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Ornitine	1.61	1.48	1.61	2.05	1.92	3.33	2.11	1.65	1.42	1.61
Lysine	4.74	2.68	4.74	5.91	7.10	10.25	5.54	5.58	7.52	6.59
Arginine	14.31	16.14	14.31	18.97	14.78	21.25	17.46	15.81	15.48	24.51
Total	195.93	224.27	195.93	232.85	275.78	281.49	278.73	211.44	261.91	239.19

N.D.[1)]: Not detected

et al. [4] reported that, when total polyphenol content in food is high, its antioxidant activity increases proportionally. Therefore, when the antioxidant activity of the broth increases with DMS the increasing quantity of DMS, it is presumed that the concentration of polyphenol compounds present in the broth increases concomitantly.

Sinigrin content and sensory evaluation of the broths added DMS

Sinigrin content and sensory evaluation results are shown in Table 3. Sinigrin was not detected in the control broth but, its concentration increased with the increasing concentration of DMS added and boiling time. Moreover, it significantly increased upon addition of crushed or roasted DMS. Sinigrin content in DMS was higher than that reported for mustard roots, stems, and leaves which were similar to those reported by Oh et al. [18].

Broth color deepened with the concentration of DMS added and the boiling time. Spicy flavor increased with boiling time, and broths with 1.5 g of roasted DMS had the best flavor : however there were no significant flavor differences among the experimental broths. Moreover, taste and overall acceptability increased with increasing boiling time and DMS concentration. Thus, it was found that as the amount of DMS added and boiling time increased, the taste and overall acceptability of the broth increased [10, 24].

Total and free amino acid content of experimental DMS containing broths

The total and free amino acid contents are shown in Tables 4 and 5, respectively. Total amino acid contents were 298.57 and 361.04 mg/100 g, and glutamic acid contents were 77.12 and 87.73 mg/100 g, after the control broth was boiled for 10 and 15 minutes, respectively. A total of 16 amino acids were identified in the broth boiled for 10 minutes. Additionally, glutamic acid, aspartic acid, glycine, proline, alanine, and arginine contents were higher in the broth containing crushed or roasted DMS than in the broth containing whole DMS. However, in the broth boiled for 15 mins, the total amino acid content was higher when the broths crushed or roasted DMS than when the broth was contained whole DMS; furthermore, total amino acid content increased in proportion to the concentration of DMS added.

Free amino acids are divided into three main categories: essential amino acids, amino acids of delicious taste components, and all other amino acids, and umami (aspartic acid

and glutamic acid), sweet (threonine, serine, glutamine, proline, glycine, alanine and lysine) bitter taste (valine, methionine, isoleucine, leucine, phenylalanine, histidine and arginine), sulfur containing amino acids (cysteine and methionine) [3, 8]. Of the 19 different amino acids detected in broths supplemented with DMS, 10 were essential amino acids, including (threonine, valine, methionine, leucine, tryptophan, phenylalanine, lysine, isoleucine, histidine and arginine); additionally, five taste-related amino acids other than β-alanine were found, including, aspartic acid, serine, glutamic acid, glycine and alanine. Lastly, γ-amino-n-butyric acid (GABA), taurine, and ornithine were also detected. In particular, GABA, which is readily soluble in water, promotes brain cell metabolism by activating blood flow to the brain and increasing oxygen supply and helps in reducing blood pressure [5]. In turn, histidine and arginine, both of which are essential in growing children and recovering patients, were detected 1.2 times higher in DMS added broths than in the control broth.

Table 6. Volatile components in the broths containing Dolsan mustard seed as identified by gas Chromatography mass spectroscopy (%)

Compound	RT[1] (min)	Control	S-1			S-2			S-3		
		10 min	W	C	R	W	C	R	W	C	R
Toluene	3.710	0.80[2]	0.80	0.61	0.95	0.92	2.17	1.45	0.92	2.17	1.45
n-hexanal	4.425	0.28	0.28	0.40	0.73	0.29	1.14	0.97	0.29	1.14	0.97
Trimethylamine	5.450	8.91	8.91	7.86	7.19	-	6.29	5.39	-	6.29	5.39
Thiocyanic acid, 2-propenyl ester	6.016	-[3]	-	-	-	7.08	-	-	9.02	-	-
2-propenyl-isothiocyanate	6.319	-	40.18	44.13	49.06	50.11	53.10	58.43	62.18	66.52	69.46
Allyl-isothiocyanate	6.323	-	-	-	-	-	-	-	-	-	-
n-butyl isothiocyanate	7.580	0.64	0.64	0.38	0.70	1.07	0.94	1.08	1.11	1.27	1.21
1-isothiocyanato-butane	7.593	-	-	-	-	0.66	-	-	0.66	-	-
3-butenyl-isothiocyanate	8.905	0.64	0.64	4.46	4.50	2.72	2.86	2.98	2.81	2.97	3.08
Cymene	9.986	2.05	-	-	-	-	-	-	-	-	-
Acetic acid	10.880	0.70	0.70	-	0.43	-	-	-	-	-	-
2-methylbutyl isothiocyanate	10.884	-	-	-	-	0.72	-	-	0.72	-	-
2-phenylethyl-isothiocyanate	20.460	-	-	-	-	-	1.02	2.70	-	1.02	2.70
2-isothiocyanato-ethyl benzene	20.461	-	-	57.84	-	0.99	-	-	0.99	-	-
Total		13.22	52.15		63.56	64.56	67.52	73	78.7	81.38	84.26

Compound	RT[1] (min)	Control	S-1			S-2			S-3		
		15 min	W	C	R	W	C	R	W	C	R
Toluene	3.710	1.08	0.80	0.75	0.91	1.07	1.19	1.53	1.08	2.53	1.34
n-hexanal	4.425	1.12	0.41	0.52	0.86	1.34	1.27	1.17	0.62	1.45	1.15
Trimethylamine	5.450	9.37	7.93	8.07	7.43	0.04	6.83	5.46	1.03	6.47	5.87
Thiocyanic acid, 2-propenyl ester	6.016	-	-	-	-	-	-	-	-	-	-
2-propenyl-isothiocyanate	6.319	-	47.58	52.47	55.83	48.75	58.46	60.11	56.49	61.08	63.42
Allyl-isothiocyanate	6.323	-	-	-	-	-	-	-	-	-	-
n-butyl isothiocyanate	7.580	-	0.76	0.81	0.89	1.04	1.13	1.27	1.34	1.43	1.52
1-isothiocyanato-butane	7.593	0.71	0.78	0.22	0.62	-	-	-	-	-	-
3-butenyl-isothiocyanate	8.905	-	0.86	0.92	0.99	1.12	1.19	1.24	1.18	1.21	1.28
Cymene	9.986	0.88	0.64	1.18	1.24	1.83	2.86	2.98	3.11	3.24	3.29
Acetic acid	10.880	2.21	2.05	-	-	-	-	-	-	-	-
2-methylbutyl isothiocyanate	10.884	0.97	0.63	-	0.43	-	-	-	-	-	-
2-phenylethyl-isothiocyanate	20.460	-	-	-	-	1.14	-	-	0.72	-	-
2-isothiocyanato-ethyl benzene	20.461	-	-	-	-	-	1.02	1.18	-	1.02	2.43
Total		16.34	62.44	64.94	69.2	56.33	73.95	74.94	65.57	78.43	80.30

[1]Retention time (min).
[2]Peak area (%) = (peak area of each compound)/(total peak area of all compounds) ×100.
[3]Not detected.

Gas chromatography-mass spectrometry of the DMS added broths

As shown in Table 6, GC-MS analysis of the DMS added broths allowed the detection of volatile components related to flavor and taste, such as 2-propenyl-isothiocyanate (ITC), allyl thiocyanate (C_4H_5NS), n-butyl ITC, and 3-butenyl ITC. 2-Propenyl ITC (sinigrin) and phenylethyl ITC, a derivative of gluconasturtiin, were also detected; furthermore, sinigrin and its derivatives were detected even after boiling the broths. Hexanal is reportedly involved in imparting a grassy flavor to fresh fish [7] and exhibits the flavor of green, resin, and fat [12]. Trimethylamine is a fragrance component of the fishy flavor, which is thought to be caused by anchovies and kuruma shrimp.

The Conflict of Interest Statement

The authors declare that they have no conflicts of interest with the contents of this article.

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초록 : 돌산갓 종자를 첨가한 국물용 육수팩의 품질특성

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돌산갓 종자를 원형, 찧은 형태, 볶은 형태로 각각 0.5 g (S-1), 1.0 g (S-2), 1.5 g (S-3) 첨가한 국물용 육수팩를 10분, 15분간 가열한 육수를 냉각한 후 품질적 특성을 측정하였다. 가열시간이 짧을수록 pH와 염도는 감소하는 경향을 나타냈다. 항산화활성은 총폴리페놀, 총플라보노이드, 전자공여능(EDA), ABTS radical 소거활성 및 ferric reducing antioxidant power (FRAP) 항목으로 측정하였다. 전체적으로 항산화활성은 S-1보다 S-2와 S-3에서 높은 함량을 나타났고, 가열시간이 길수록 증가했다. Sinigrin 함량은 대조군에서 검출되지 않았고, 시료간 유의적 차이가 없었다. 총아미노산은 가열시간이 길수록 볶은 샘플에서 glutamic acid의 함량이 높게 나타났고, aspartic acid, glycine, proline, alanine, arginine등이 높은 함량으로 검출되었다. 유리아미노산 함량은 감칠맛 계의 aspartic acid, glutamic acid함량이 높게 나타났다. 휘발성 성분들은 갓종자를 첨가한 육수는 2-propenyl- isothiocyanate (ITC) (C_4H_5NS), allyl thiocyanate (C_4H_5NS), n-butyl ITC (C_5H_9NS), 3-butenyl ITC (C_5H_7NS) 등이 검출되었다. 관능검사는 돌산갓 종자 첨가량과 가열시간이 길수록 높은 점수를 받아 전체적인 기호도가 증가하였고 시료간의 유의적인 차이를 나타냈다. 돌산갓 종자를 첨가한 육수의 품질적 특성을 토대로 돌산갓 종자를 첨가한 제품 개발을 위한 기초자료로 제시하고자 한다.