

Full Length Research Paper

Addition of grape extracts can enhance quality of natural seasonings by changing their physicochemical properties

Il-Doo Kim¹, Sanjeev Kumar Dhungana⁶, Piao Mei Zi², Jung-Won Lee³, Hye-Kyung Moon⁴, Hwa-Seok Hwang⁵ and Dong-Hyun Shin^{6*}

¹Institute of Agricultural Science and Technology, Kyungpook National University, Daegu, South Korea.

²College of Food Science and Engineering, Qing Dao Agricultural University, Chanuang Road Chengyang District, Qingdao, China.

³Department of Global Tourism Management, Hanbuk University, Gyeonggi-do, South Korea.

⁴School of Food Science and Biotechnology, Kyungpook National University, Daegu 702-701, Korea.

⁵Institute of Regional Development, Kyungpook National University, Daegu, South Korea.

⁶School of Applied Biosciences, Kyungpook National University, Daegu, South Korea.

Received 14 September, 2013; Accepted 24 March, 2014

The present study was aimed to evaluate the application of different concentrations of grape extract on the physicochemical properties of natural seasonings. The results show that increase in concentration of grape extract (0 to 20%) significantly reduced the lightness (34.75 to 28.3) and yellowness (15.33 to 10.64), however increased the redness (0.98 to 1.5) values of natural seasonings. The mineral contents of zinc and iron were raised by the addition of grape extracts but potassium, magnesium, calcium and sodium were reduced. The antioxidant activity, as 1,1-diphenyl-2-picrylhydrazyl (DPPH), of seasonings increased by up to 6.21% with the addition of 20% grape concentration extract. Total phenolic content was also significantly increased with the addition of concentrated grape extract. Reactive oxygen species (ROS) as superoxide anion and hydroxyl radical were significantly reduced with the addition of grape extract. The amino acids analysis showed that concentration of few amino acids like aspartic acid, proline, alanine, cysteine, valine, methionine, isoleucine, leucine, and lysine were elevated, conversely while threonine, serine, glutamic acid, glycine, tyrosine, phenylalanine, histidine, and arginine were found declined with higher proportion of grape extract in the seasoning. Results suggest that addition of grape extract may enhance the physicochemical properties as well as health potential of natural seasonings.

Key words: Grape extracts, natural seasonings, physicochemical properties, quality characteristics.

INTRODUCTION

The terms spices and herbs, also known as seasonings, are usually used together to describe bark, buds, flowers, leaves, fruits, bulbs, roots or seeds derived from a group

of aromatic plants. In general, spices are highly aromatic due to their high contents of essential oils, whereas herbs are low in essential oils and usually used to produce

delicate or subtle flavors in food preparations (Chi and Wu, 2007). Spices have been recognized to possess medicinal properties and their uses in traditional system of medicines have been known since long. The use of spices might be whole spices, ground spices, or isolates from their extract (Srinivasan et al., 2004). Cooking with herbs, spices and seasonings can add flavor and variety to food. Seasonings are used as food additive for the purpose of flavor, medicine, color or as a preservative, that kills harmful bacteria or prevent their growth (Ernst and Pittler, 2000). Spices, like vegetables, fruit, and medicinal herbs, are known to possess a variety of antioxidant effects and properties (Zheng and Wang, 2001).

Most of the spices and herbs analyzed have very high antioxidant content (Paur et al., 2011). Phenolic compounds in the tested spices contribute significantly to their antioxidant capacity (Shan et al., 2005). Various spice-derived ingredients possess potential inhibitors of lipid peroxidation in cell and low density lipoprotein cholesterol in human (Naidu and Thippeswamy, 2002). It has been demonstrated that the antioxidant activity of bioactive compounds found in herbs and spices could play an important role in suppressing viral replication, inhibiting allergy and arthritis, preventing cancer and heart diseases (Aggarwal et al., 2002). Consumers' interest in food formulations containing "natural" ingredients has motivated the food industry to evaluate the effectiveness of naturally occurring components of food for functional purposes as compared to synthetic ones.

The nutritional and medicinal properties of spices and herbal plants may be interlinked through phytochemicals, both nutrient and non-nutrient (Ranhotra et al., 1998). Although spices are used primarily for their desirable flavor and odor, they may play other important roles in the food systems. From antiquity, in addition to spices and their derivatives being used for flavoring foods and beverages and for medication, they have also been highly valued for their use as antimicrobials (Koedam, 1986; Özcan, 2004), and antidiabetics (Tundis et al., 2010). Spices and herbs can be practical for antimicrobial effect as protecting seafood from the risk of contamination by *Vibrio parahaemolyticus*, a foodborne pathogen (Yano et al., 2006). Antioxidant food database (Carlsen et al., 2010) developed from the analysis of 3,100 foods, beverages, spices, and herbs, shows that the spices and herbs are the most antioxidant-rich products in the human diet, some of them exceptionally high. Various fruits, herbs, and spices have demonstrated the anti-inflammatory activity (Mueller et al., 2010). As grape is one of the major fruits in the world conferring

health benefits due to their antioxidant activity (Kedage et al., 2007), phytosterols (Ruggiero et al., 2013), also contains a characteristic color value for seasonings, the present study was aimed to evaluate the physicochemical changes in the natural seasonings by addition of various concentrations of grape extracts.

MATERIALS AND METHODS

Grape (*Vitis vinifera* L.) berries, *Saccharina japonica* powder, anchovy powder, shiitake (*Lentinus edodes*) powder, tomato (*Lycopersicon esculentum* Mill) puree, parched soybean (*Glycine max* L.) powder, hot pepper (*Capsicum annuum* L.) seed, Chinese radish (*Raphanus sativus* L.), root of Chinese bellflower (*Platycodon grandiflorum* A, DC.), lotus root (*Nelumbinis rhizoma*), sesame powder, roasted salt, grain syrup, black sugar, soy sauce and water were purchased from local markets in Korea.

Chemicals and reagents

Falin-Ciocalteu reagent, gallic acid, 1,1-Diphenyl-2-picrylhydrazyl (DPPH), pyrogallol, 2-deoxyribose, thiobarbituric acid (TBA), trichloroacetic acid (TCA), ethylenediaminetetraacetic acid (EDTA) and phosphate buffer were purchased from Sigma Chemical co. (St. Louis, Mo, USA). Iron (II) sulfate heptahydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) was purchased from Acro Organics (NJ, USA). Anhydrous sodium carbonate was purchased from J. T. Baker (NJ, USA). Ethanol and hydrogen peroxide were purchased from Merck (Darmstadt, Germany). All reagents used in the study were of analytical grade.

Preparation of grape natural seasoning samples

The ingredients of natural seasoning prepared for the experiment are given in Table 1. Grape berries cv. Muscat Bailey A, grown at Yeongcheon, Gyeongsangbuk-do, Korea, was harvested at the commercial maturity stage and transported to the laboratory. The fruits were washed several times with tap water. Fruit pulps and skins were separated from seed and heated in a pan over hot plate (Prestige Euro ER-822W, Sunny Tech Ltd, Korea) for 30 min and concentrated under vacuum to a final weight of 85%. The grape concentrates and the other ingredients mentioned in Table 1 were mixed thoroughly and simultaneously heated in a pan over hot plate (Prestige Euro ER-822W, Sunny Tech Ltd, Korea) for 45 min. The control seasoning sample was prepared by heating the ingredients (mentioned in Table 1 without grape extract) in a pan over hot plate (Prestige Euro ER-822W, Sunny Tech Ltd, Korea) for 45 min. The mixtures heated with different concentrations of ingredients were cooled to room temperature and freeze-dried (Virtis, 10-146-MRBA model). The freeze-dried mixture was milled with Speed Rotor Mill (Model: KT-02A) into powder and passed through a 100-mesh sieve. The strained samples were packed into airtight sample bottles and stored in refrigerator until analysis.

Extraction

Dried natural seasonings (1 g) were extracted in water (50 ml) for 4 h by using a Soxhlet extractor (Soxtec System HT6, Tecator,

*Corresponding author. E-mail: nsnishanim@gmail.com.

Table 1. Recipe of natural seasoning prepared by adding different grape concentrated extracts (%).

Ingredient	Sample ¹⁾				
	NSG-0	NSG-5	NSG-10	NSG-15	NSG-20
Grape concentrated juice	-	5	10	15	20
<i>Saccharina japonica</i>	3.1	3.0	2.9	2.7	2.6
Anchovy powder	2.3	2.3	2.2	2.0	1.9
Shiitake (<i>Lentinus edodes</i>) powder	3.2	3.0	2.8	2.7	2.5
Tomato puree	1.5	1.5	1.4	1.3	1.3
Parched soybean powder	1.5	1.5	1.4	1.3	1.3
Sesame powder	1.3	1.0	1.0	0.9	0.9
Hot pepper seed	0.8	0.8	0.7	0.7	0.6
Chinese radish	1.5	1.5	1.4	1.3	1.3
<i>Doraji</i> (root of Chinese bellflower)	0.2	0.1	0.1	0.1	0.1
Roasted salt	3.1	3.0	2.8	2.7	2.5
Grain syrup	7.7	7.3	6.9	6.5	6.2
Black sugar	1.5	1.5	1.4	1.3	1.3
Lotus root	0.2	0.2	0.2	0.1	0.1
Soy sauce	46.2	43.9	41.6	39.3	36.9
Water	25.7	24.4	23.1	21.8	20.5

¹⁾NSG-0, natural seasoning prepared by adding no grape concentrated extracts; NSG-5, natural seasoning prepared by adding 5% grape concentrated extract; NSG-10, natural seasoning prepared by adding 10% grape concentrated extract; NSG-15, natural seasoning prepared by adding 15% grape concentrated extract; NSG-20, natural seasoning prepared by adding 20% grape concentrated extract.

Hoganas, Sweden) under reflux conditions. The residues were then extracted in boiling water (50 ml). The extracts were filtered through Whatman No. 4 filter paper and then concentrated using a rotary evaporator (Büchi Rotavapor R-144, Switzerland) to get the extracts. The water extracts yielded 15.3% (w/w) were analyzed for their antioxidant activities.

Color measurement

L^* (lightness), a^* (redness, + or greenness, -), and b^* (yellowness, + or blueness, -) values of grape natural seasonings were measured using a Chroma Meter (CR-300, Minolta Corp., Japan). A Minolta calibration plate ($Y_{CIE} = 94.5$, $X_{CIE} = 0.3160$, $Z_{CIE} = 0.330$) and a Hunter laboratory standard plate ($L = 82.13$, $a = -5.24$, $b = -0.55$) were used to standardize the instrument with D65 illuminant. Color was measured directly on three zones of grape natural seasonings and the average was calculated (Son et al., 2013).

Determination of mineral content

Five hundred (500) mg of sample was taken into a cup and 15 ml of nitric acid (HNO_3) was added. The solution was diluted with distilled water. Mineral concentrations were determined by using Inductively Coupled Plasma Atomic Emission Spectrometer (ICP AES: Varian Vista) (Skujins, 1998). The instrument was calibrated using known standards for each mineral. Average values of two replicate samples were reported (Son et al., 2013).

Radical scavenging activity using DPPH

The scavenging activity of the extract from natural seasonings was measured with DPPH radicals according to the method of Blois (1958) with some modifications. DPPH solution was prepared at the

concentration of $4 \times 10^{-4} M$ in ethanol. A 0.1 ml aliquot of extract was mixed with 2.9 ml of DPPH solution and the mixture was incubated in the room temperature for 30 min. After standing for 30 min, absorbance was recorded at 516 nm by UV-VIS spectrophotometer (Opron 3000 Hanson Tech. Co. Ltd., Seoul, Korea). The inhibitory percentage of the DPPH radical by the samples was calculated according to Shyu and Hwang (2002) as:

$$\text{Scavenging effect (\%)} = [(A_0 - (A - A_b)) / A_0] \times 100$$

Where, A_0 is the absorbance of DPPH without sample (control), A is the absorbance of sample and DPPH, and A_b is the absorbance of sample without DPPH (blank).

Superoxide anion scavenging activity

Superoxide anion (O_2^-) scavenging activity was determined by measuring the inhibition of the auto-oxidation of pyrogallol following the method of Marklund and Marklund (1974) with a slight modification. A 0.3 g aliquot of sample and 2.61 ml of 50 mM phosphate buffer (pH 8.24) were added into freshly prepared 90 μL of 3 mM pyrogallol (dissolved in 10 mM HCl). The absorbance value was measured at 325 nm to determine the inhibition rate of pyrogallol auto-oxidation. The superoxide anion scavenging activity was calculated with inhibition rate of pyrogallol auto-oxidation, which was determined by the difference (the absorbance at 10 min - the absorbance at the starting time) in absorbance (325 nm) of each extract recorded at every 1 min interval for 10 min.

Hydroxyl radical scavenging activity

Hydroxyl radical (HO^\cdot) scavenging activity was determined according to the 2-deoxyribose oxidation method (Chung et al., 1997) with

Table 2. Hunters color values of natural seasoning prepared by adding different grape concentrated extracts.

Color value ²⁾	Sample ¹⁾				
	NSG-0	NSG-5	NSG-10	NSG-15	NSG-20
L (Lightness)	34.75 ± 1.01 ^{a3)}	33.00 ± 1.11 ^{ab}	31.36 ± 0.80 ^{bc}	30.82 ± 1.01 ^c	28.30 ± 1.32 ^d
a (Redness)	+0.98 ± 0.12 ^b	+0.79 ± 0.11 ^{bc}	+0.68 ± 0.11 ^c	+1.03 ± 0.21 ^b	+1.50 ± 0.11 ^a
b (Yellowness)	+15.33 ± 1.33 ^a	+14.27 ± 1.00 ^a	+13.81 ± 1.32 ^{ab}	+12.05 ± 0.98 ^b	+10.64 ± 0.63 ^b

¹⁾Abbreviations are defined in Table 1. ²⁾L: lightness (100, white ; 0, black), a: redness (-, green; +, red), b: yellowness (-, blue; +, yellow).

³⁾Values are mean±standard deviation of triplicate experiments. ^{a-d)}The values followed by the different superscripts in the same row are significantly different, according to Tukey test ($p < 0.05$).

some modifications. Fenton reaction in the presence of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ was used to generate hydroxyl radical. A reaction mixture containing each 0.2 ml of 10 mM $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 10 mM EDTA and 10 mM 2-deoxyribose was mixed with 0.2 ml of the extract solution and 0.1 M phosphate buffer (pH 7.4) was added into the reaction mixture to make the final volume of 1.8 ml. Then 0.2 ml of 10 mM H_2O_2 was added to the reaction mixture and incubated at 37°C for 4 h.

Then after that each 1 ml of 2.8% TCA and 1.0% TBA were added to the incubated mixture. Finally, the mixture was placed into a boiling water bath for 10 min and absorbance reading was taken at 532 nm.

Determination of total phenolic content

The amount of total phenolics (TPH) was determined using the Folin-Ciocalteu method (Zheng and Wang, 2001). A calibration curve of gallic acid was prepared, and the results were expressed as mg GAE (gallic acid equivalents)/g sample. Briefly, 5 ml of distilled water was put into a 10 ml volumetric flask. A suitable volume of the natural seasoning extract was transferred into the volumetric flask.

A 0.2 ml aliquot of Folin-Ciocalteu reagent was added into the flask and carefully mixed. After 3 min, 0.4 ml of saturated Na_2CO_3 solution was added, carefully mixed and made up to volume with distilled water. After 1 h of reaction in the dark, the absorbance was measured at 725 nm using a spectrophotometer (Hewlett-Packard 8452A diode-array).

Determination of amino acid content

Amino acid contents were analyzed following the procedure of Je et al. (2005) with some modification. Briefly, 1 g of freeze-dried sample powder was hydrolyzed with 6 N HCl (10 ml) in a sealed-vacuum ampoule at 110°C for 24 h. The HCl was removed from the hydrolyzed sample on a rotary evaporator, brought to a known volume (5 ml) with 0.2 M sodium citrate buffer (pH 2.2).

The sample was passed through a C-18 Sep Pak (Waters Co. Milford, USA) cartridge and filtered through a 0.22 μm membrane filter (Millipore, USA). Amino acids were determined on an automatic amino acid analyzer (Biochrom-20, Pharmacia, Biotech Co., Sweden).

Statistical analysis

Data were subjected to one-way or two-way analysis of variance (ANOVA) when required using Statistix version 4.0 package (Analytical Software, AZ, USA). Differences between means at $p < 0.05$ were analyzed using the Tukey test.

RESULTS AND DISCUSSION

Color of natural seasoning

Lightness (L), redness (a), and yellowness (b) color expressions of different natural seasonings prepared by adding concentrated grape extracts varied significantly depending on the concentration of the grape extract (Table 2). The seasonings showed a significant reduction in lightness and yellowness, whereas redness value was increased significantly with the increased amount of grape concentrated extract. Increase in concentration of the grape extract from 0 to 20% substantially reduced lightness (from 34.75 to 28.30) and yellowness (from 15.33 to 10.64). However, redness value was increased from 0.98 to 1.50 with the addition of 0 and 20% grape extract, respectively. This trend of color expression demonstrated that addition of higher concentration of grape extract would promote development of darker color of the natural seasonings. The darker color of seasonings at higher concentration of grape concentrated extract might be because of color of grapes itself and chemical reactions with other ingredients. Natural colorant areas like anthocyanins, betalains, chlorophylls, carotenoids, flavonoids, monascus, hemes, quinones, biliproteins, safflower, turmeric may be found as such and a variety of hues can be obtained ranging from green through yellow, orange, red, blue, and violet, depending on the source of colorant (Francis and Markakis, 1989). Color is a key factor in natural seasonings as different consumers prefer different hues of colors. Addition of grape extract improved the darkness of natural seasonings.

Mineral content of grape natural seasoning

The mineral contents of the natural seasonings were significantly varied with the concentration of the grape extract added in them (Table 3). Contents of minerals like K, Mg, Ca, and Na were significantly decreased while those of Fe, and Zn were increased after addition of grape extract to the natural seasonings. The seasonings were rich in K, the most abundant mineral element (ranging 26042.2 mg/100 g at 0% grape extract to 13759.6 mg/100 g at 20% grape extract) followed by Na (19454.4

Table 3. Mineral content of natural seasoning prepared by adding different grape concentrated extracts (mg/100 g sample).

Element	Sample ¹⁾				
	NSG-0	NSG-5	NSG-10	NSG-15	NSG-20
K	26042.2 ± 8.9 ²⁾	25730.3 ± 10.1 ^b	23688.3 ± 9.2 ^c	18951.5 ± 6.7 ^d	13759.6 ± 9.3 ^e
Mg	3192.2 ± 3.7 ^a	2882.3 ± 5.9 ^b	2859.2 ± 9.1 ^c	2800.3 ± 8.2 ^e	2821.8 ± 7.1 ^d
Ca	4295.5 ± 5.1 ^a	3737.3 ± 3.1 ^e	3756.6 ± 2.1 ^d	3866.6 ± 6.2 ^c	4147.7 ± 3.0 ^b
Na	19454.4 ± 10.2 ^a	17246.9 ± 9.9 ^b	16304.5 ± 9.1 ^c	12882.5 ± 13.1 ^e	12988.3 ± 8.1 ^d
Fe	43.3 ± 1.2 ^c	58.1 ± 0.9 ^b	68.5 ± 1.7 ^a	69.7 ± 0.8 ^a	69.8 ± 1.2 ^a
Zn	25.2 ± 0.5 ^c	24.2 ± 0.7 ^c	28.6 ± 0.6 ^{ab}	29.3 ± 0.9 ^a	27.6 ± 0.4 ^b
Mn	ND ³⁾	ND	ND	ND	ND

¹⁾Abbreviations are defined in Table 1. ²⁾Quoted values are mean±standard deviation of duplicate experiments. ³⁾ND: Not detectable. ^{a-e)}The values followed by the different superscripts in the same row are significantly different, according to Tukey test (p<0.05).

Table 4. Scavenging activity of reactive oxygen species and total phenolic contents of natural seasoning prepared by adding different grape concentrated extracts.

Sample ¹⁾	% Inhibition ²⁾			Total phenolics (mg GAE ³⁾ /g sample)
	DPPH	O ₂ ⁻	HO ⁻	
NSG-0	67.56 ± 0.31 ⁴⁾	19.17 ± 0.34 ^a	50.56 ± 1.83 ^a	153.3 ± 2.2 ^d
NSG-5	67.87 ± 0.39 ^c	17.21 ± 0.46 ^b	50.41 ± 2.21 ^a	148.8 ± 2.8 ^d
NSG-10	69.63 ± 0.23 ^b	8.82 ± 1.02 ^c	38.25 ± 1.82 ^b	164.0 ± 2.3 ^c
NSG-15	69.91 ± 0.16 ^b	5.91 ± 0.12 ^d	39.05 ± 1.66 ^b	201.2 ± 1.1 ^b
NSG-20	73.77 ± 1.01 ^a	5.41 ± 0.22 ^e	37.51 ± 0.61 ^b	229.2 ± 1.5 ^a

¹⁾Abbreviations are defined in Table 1. ²⁾DPPH: DPPH free radical scavenging activity; O₂⁻: superoxide anion scavenging activity; HO⁻: hydroxyl radical scavenging activity. ³⁾GAE: gallic acid equivalent. ⁴⁾Quoted values are mean±standard deviation of duplicate experiments. ^{a-e)}The values followed by the different superscripts in the same column are significantly different, according to Tukey test (p<0.05).

mg/100 g at 0% grape extract and 12988.3 mg/100 g at 20% grape extract). Zn and Fe were the least detected mineral elements in the seasonings; increased a little with the addition of grape extract from 0 to 20%. The increase in Zn and Fe content in grape extract added seasonings might be due to relatively higher content of these minerals in grape extract. It has been indicated that food seasonings contain plant sterols and/or stanols or their derivatives together with minerals, such as magnesium, calcium and potassium. Ingestion of food supplied with phytosterol leads to a significant decrease in both cholesterol level (Weingartner et al., 2008). The decrease is synergic, that is, larger than that expected from the sum of the effects of plant sterols and minerals (Karppanen et al., 2000) since grape berries contain phytosterols (Ruggiero et al., 2013). Thus addition of grape extract to the natural seasonings may enhance its nutritional and medicinal values even some of the minerals were significantly decreased.

Scavenging activities and total phenolic contents of natural seasoning

Scavenging activities of reactive oxygen species in the na-

tural seasonings were determined by analyzing DPPH, O₂⁻, HO⁻ and total phenolic content (Table 4). DPPH radical scavenging activities (73.77%) and total phenolic content (229.2 mg/g sample) were recorded the highest in the seasonings containing 20% grape extract whereas value of O₂⁻, and HO⁻ decreased with the increased concentration of grape extract. Addition of 5% concentrated grape extract did not show significant differences from the samples with no grape extracts in terms of DPPH, HO⁻, and total phenolic content. The increase in DPPH radical scavenging activity of the natural seasonings was accompanied by a significant reduction in O₂⁻ and OH⁻ activities in all the samples. The O₂⁻ content was the highest (19.17%) in the seasonings with no concentrated grape extract added and the least (5.41%) when the natural seasonings were prepared by addition of 20% concentrated grape extract. The HO⁻ levels were also reduced when the amount of concentrated grape extract added in preparation of the natural seasoning was increased from 5 to 20%. In effect, significant scavenging of reactive oxygen species (O₂⁻ and HO⁻) could be obtained in natural seasonings when 5 to 20% concentration of grape extract is added during preparation of the seasonings.

Table 5. Amino acids content (mg of amino acid per 100 g of sample) in natural seasonings prepared by adding different grape concentrated extracts.

Amino acid	Sample ¹⁾				
	NSG-0	NSG-5	NSG-10	NSG-15	NSG-20
Aspartic acid	4762.65 ²⁾	3138.49	4237.23	4241.89	4890.42
Threonine	1959.49	1149.31	1825.50	1584.32	1910.36
Serine	1953.80	1165.03	1801.41	1606.97	1909.92
Glutamic acid	6532.34	4675.94	6294.40	5587.96	6333.75
Proline	3185.39	451.76	3276.03	1855.69	3347.68
Glycine	2098.01	1319.91	1975.84	1787.60	2096.51
Alanine	2329.25	1495.15	2500.53	2221.01	2701.87
Cysteine	391.68	203.05	367.05	612.76	538.27
Valine	2260.78	1385.30	2382.38	2009.98	2316.45
Methionine	828.80	444.24	863.49	1017.91	1053.35
Isoleucine	2278.61	1150.29	2094.28	1954.22	2304.52
Leucine	3041.21	1851.56	3047.12	2757.16	3080.90
Tyrosine	1228.53	718.45	1113.92	1006.11	1166.47
Phenylalanine	2278.93	1396.35	2087.71	1823.80	2196.33
Histidine	1387.87	815.18	1237.72	1036.73	1233.45
Lysine	2535.10	1573.84	2350.16	2261.34	2619.90
Arginine	2442.28	1505.52	2255.45	2031.60	2347.34

¹⁾Abbreviations are defined in Table 1. ²⁾Values are the means of duplicate experiments.

Hydroxyl radical, superoxide anion radical, hydrogen peroxide, oxygen singlet, hypochlorite, nitric oxide radical, and peroxyxynitrite radical are highly reactive species, and capable of damaging biologically relevant molecules such as DNA, proteins, carbohydrates, and lipids (Young and Woodside, 2001). Alteration of lipids, proteins, and DNA by free radicals triggers a number of human diseases. Negative effects of free radicals can be corrected by the application of external source of antioxidants. Synthetic antioxidants like butylated hydroxytoluene and butylated hydroxyanisole have recently been reported to be dangerous for human health (Lobo et al., 2010). Grape berries are rich in antioxidants (Kedage et al., 2007) including melatonin (Vitalini et al., 2013), novel antioxidant. Thus addition of grape concentrated extract could increase the antioxidant potential of natural seasonings.

Total amino acid content of natural seasoning

Among the amino acids analyzed, glutamic acid (range 4675.94 - 6532.34 mg/100 g) was the most abundant followed by aspartic acid (range 3138.9 - 4890.42 mg/100 g) in the natural seasonings depending on the amount of concentrated grape extract added during preparation of the seasoning. Cysteine was the lowest constituent amino acid (range 203.05 - 612.76 mg/100 g) in the

seasonings. Some of the amino acids contents were increased while others decreased with the addition of different concentrations of grape extract in the natural seasoning (Table 5). Amino acids are the building blocks of protein (Ekeanyanwu, 2013) that plays an important role in biochemical, biophysical and physiological functions. The deficiency of proteins leads to weakness, anaemia, protein energy malnutrition (kwashiorkor and marasmus), delayed wound and fracture healing and also decreased resistance to infections.

Glutamic acid, glycine, alanine, proline and aspartic acid are recognized as being important in the taste and their presence in the natural seasoning with grape extract will enhance the properties of the seasoning (Choi et al., 1996). The results of this study showed that contents of amino acids aspartic acid, proline, alanine, cysteine, valine, methionine, isoleucine, leucine, and lysine could be increased in the natural seasonings with the addition of grape concentration extract.

Conclusion

Natural seasonings prepared by adding different concentrations of grape extracts could be used as good sources of antioxidants, proteins and minerals supplement in the human diet. This study established the physicochemical properties of natural seasonings when different concentra-

tions of grape extracts were added during preparation. Although some of the desirable physicochemical parameters were continuously improved with the gradual increase in the amount of grape extract, concentration beyond 20% resulted in poor quality seasonings by reducing their powdery structure making them difficult to use properly. Results of this study suggest that concentration of grape extract up to 20% could be added in natural seasonings to impart better physicochemical properties as well as to enhance their nutritional and medicinal value.

Conflict of Interests

The author(s) have not declared any conflict of interests.

ACKNOWLEDGEMENTS

This work was supported by a research grant from Rural Development Administration, Republic of Korea (Project No. PJ006949), and Kyungpook National University Research Fund, 2012.

REFERENCES

- Aggarwal BB, Ahmad N, Mukhtar H (2002). Spices as potent antioxidants with therapeutic potential. *In: Handbook of Antioxidants*. Cadenas E, Packer L (eds.). Los Angeles, USA: CRC Press.
- Blois MS (1958). Antioxidant determinations by the use of a stable free radical. *Nat*. 181: 1199-1200.
- Carlsen MH, Halvorsen BL, Holte K, Bohn SK, Dragland S, Sampson L, Willey C, Senoo H, Umezono Y, Sanada C, Barikmo I, Berhe N, Willett W, Phillips K, Jacobs D, Blomhoff R (2010). The total antioxidant content of more than 3100 foods, beverages, spices, herbs and supplements used worldwide. *Nutr. J.* 9:3.
- Chi SP, Wu YC (2007). Spices and seasonings. *In: Toldrá F (ed.) Handbook of Fermented Meat and Poultry*. 1st ed. Iowa, USA: Blackwell Publishing. P: 87.
- Choi YJ, Kim IS, Lee KW, Kim GB, Lee NG, Cho YJ (1996). Available components of cooking drips, dark muscle, head and raw viscera from skipjack. *J. Korean Fish. Soc.* 29: 701-708.
- Chung SK, Osawa T, Kawakishi S (1997). Hydroxyl radical scavenging effects of spices and scavengers from Brown Mustard (*Brassica nigra*). *Biosci. Biotechnol. Biochem.* 61: 118-123.
- Ekeanyanwu RC (2013). Evaluation of the crude protein and amino acid composition of Nigerian *Monodora myristica* (Ehuru). *Pakistan J. of Nutr.* 12 (3): 219-223.
- Francis FJ, Markakis PC (1989). Food colorants: Anthocyanins. *Crit. Rev. Food Sci. Nutr.* 28 (4): 273-314.
- Ernst E, Pittler MH (2000). Efficacy of ginger for nausea and vomiting: a systematic review of randomized clinical trials. *Br. J. Anaesth.* 84 (3): 367-371.
- Je JY, Park PJ, Jung WK, Kim SK (2005). Amino acid changes in fermented oyster (*Crassostrea gigas*) sauce with different fermentation periods. *Food Chem.* 91: 15-18.
- Karppanen H, Karppanen PH, Karppanen PKML, Nevalainen MLS, Vaskonen T (2000). Food seasoning, food ingredients and food item compositions and method for their preparation. United States Patent No. 6136349. P. 12.
- Kedage V, Tilak J, Dixit G, Devasagayam T, Mhatre M (2007). A study of antioxidant properties of some varieties of grapes (*Vitis vinifera* L.). *Crit. Rev. Food Sci. Nutr.* 47: 175-185.
- Koedam A (1986). Volatile oil composition of Greek mountain tea (*Sideritis* spp.). *J. Sci. Food Agric.* 37 (7): 681-684.
- Lobo V, Patil A, Phatak A, Chandra N (2010). Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacogenomics Rev.* 4 (8): 118-126.
- Marklund S, Marklund G (1974). Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur. J. Biochem.* 47(3): 469-474.
- Mueller M, Hobiger S, Jungbauer A (2010). Anti-inflammatory activity of extracts from fruits, herbs and spices. *Food Chem.* 122: 987-996.
- Naidu KA, Thippeswamy NB (2002). Inhibition of human low density lipoprotein oxidation by active principles from spices. *Mol. Cell. Biochem.* 229: 19-23.
- Özcan M (2004). Mineral contents of some plants used as condiments in Turkey. *Food Chem.* 84 (3): 437-440.
- Paur I, Carlsen MH, Halvorsen BL, Blomhoff R (2011). Antioxidants in herbs and spices: Roles in oxidative stress and redox signaling. *In Benzie IFF, Wachtel-Galor S (eds.) Herbal Medicine: Biomolecular and Clinical Aspects*. 2nd ed. Boca Raton, USA: CRS Press.
- Ranhotra GS, Leinen SD, Vinas MA, Lorenz KJ (1998). Nutritional profile of some edible plants from Mexico. *J. Food Compos. Anal.* 11:298-304.
- Ruggiero A, Vitalini S, Burlini N, Bernasconi S, Iriti M (2013). Phytosterols in grapes and wine, and effects of agrochemicals on their levels. *Food Chem.* 141: 3473-3479.
- Shan B, Cai YZ, Sun M, Corke H (2005). Antioxidant capacity of 26 spice extracts and characterization of their phenolic constituents. *J. Agric. Food Chem.* 53: 7749-7759.
- Shyu YS, Hwang LS (2002). Antioxidant activity of the crude extract of crude extract of lignan glycosides from unroasted Burma black sesame meal. *Food Res. Int.* 35:357-365.
- Skujins S (1998). Handbook for ICP AES (Varian Vista). A short guide to Vista series ICP-AES Operation. Varian Int. AG Zug, Version 1.0, Switzerland.
- Son JH, Kim ID, Kim MO, Gaveh EA, Shin DH (2013). Quality characteristics of rice inoculated with *Inonotus obliquus* mycelia and incubated under different cultivating conditions. *Afr. J. Biotechnol.* 12(33): 5131-5139.
- Srinivasan K, Sambaiiah K, Chandrasekhara N (2004). Spices as beneficial hypolipidaemic food adjuncts: A review. *Food Rev. Int.* 20: 187-220.
- Tundis R, Loizzo MR, Menichini F (2010). Natural products as alpha-amylase and alpha glucosidase inhibitors and their hypoglycaemic potential in the treatment of diabetes: An update. *Mini Rev. Med. Chem.* 10 (4): 315-331.
- Vitalini S, Gardana C, Simonetti P, Fico G, Iriti M (2013). Melatonin, melatonin isomers and stilbenes in Italian traditional grape products and their antiradical capacity. *J. Pineal Res.* 54: 322-333.
- Weingartner O, Bohm M, Laufs U (2008). Controversial role of plant sterol esters in the management of hypercholesterolaemia. *Eur. Heart J.* 30 (4): 404-409.
- Yano Y, Satomi M, Oikawa H (2006). Antimicrobial effect of spices and herbs on *Vibrio parahaemolyticus*. *Int. J. Food Microbiol.* 111 (1): 6-11.
- Young IS, Woodside JV (2001). Antioxidants in health and disease. *J. Clin. Pathol.* 54: 176-86.
- Zheng W, Wang SY (2001). Antioxidant activity and phenolic compounds in selected herbs. *J. Agric. Food Chem.* 49 (11): 5165-5170.