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Color Value, Free Amino Acid Content, and Antioxidant Potential of Seasoning Prepared with Hot Pepper Seeds

Kil Su Jang[1], Chan Yong Kim[1], Oh Hun Kwon[1], Jung-Bae Kwon[1], Sanjeev Kumar Dhungana[2], Yong-Sung Park[2], Hyeon-Min Do[2], Hye-Ryun Kim[3], Jin-Hwan Son[4], Il-doo Kim[5]

[1]Yeongyang Pepper Research Institute, Gyeongsangbuk-Do Agri. R&E Services, Yeongyangchangsu-ro 36531, Korea
[2]School of Applied Biosciences, Kyungpook National University, Daegu 41566, Korea
[3]Cheonnyeonmiin Co. Ltd., Gyeongju-si 38180, Korea
[4]Chachanson, 165-1, Yeonil-ro, Saengji-ri, Yeonil-eup, Nam-gu, Pohang-si, Gyeongsangbuk-do 37847, Korea
[5]International Institute of Agricultural Research and Development, Kyungpook National University, Daegu 41566, Korea

Abstract: Spices and herbs also known as seasonings are increasingly cherished not only for their culinary properties but also for their potential health benefits. Objective of the present study was to evaluate the physicochemical properties of seasonings containing different concentrations of pepper seed powder. Addition of hot pepper seed powder to the seasonings enhanced the color values and antioxidant potentials. The antioxidant activity, as 1,1-diphenyl-2-picrylhydrazyl (DPPH), of seasonings increased by up to 13.42%. Total phenolics content was also increased by 75.43% with the addition of 15% pepper seed powder. Although some of the amino acid contents were found to be reduced while others were increased, results of this study suggest that addition of hot pepper seed powder to the seasonings could impart better physicochemical properties as well as enhance the antioxidant potential.

Keywords: DPPH, Culinary, Physicochemical Property, Polyphenol, Spice

Introduction

Spices and herbs also known as seasonings have been used in a variety of cultures for a long time and contribute not only flavors but also serve as colorants and preservatives. The terms seasonings are usually used together to describe parts of aromatic plants like bark, buds, flowers, leaves, fruits, bulbs, roots or seeds.

Today, seasonings are increasingly cherished not only for their culinary properties but also for their potential health benefits. Generally, 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 are used in different forms; whole spices, ground spices, or isolates from their extract (Srinivasan et al., 2004). They can add flavor and variety to food. Seasonings are used for flavor, medicine, color as well as a preservative, that kills harmful bacteria or prevent their growth (Ernst and Pittler, 2000). They are known to possess a variety of antioxidant properties (Zheng and Wang, 2001). Many spices and herbs have been assayed and found to possess very high antioxidant potential (Paur et al., 2011). Phenolic compounds present in the spices contribute significantly to their antioxidant capacity (Shan et al., 2005). The antioxidant activity of 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). Various spice-derived ingredients possess potential inhibitors of lipid peroxidation in cell and low density lipoprotein cholesterol in human (Naidu and Thippeswamy, 2002). Because of increasing health

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Il-Doo Kim (Correspondence)
ildookim@hanmail.net
+82-53-950-5707, Fax: +82-53-958-6880

consciousness, consumers are found to be more interested in food formulations containing natural ingredients as compared to synthetic ones and it has motivated the food industry to evaluate the effectiveness of naturally occurring components of food for functional purposes.

The nutritional composition of hot pepper seeds showed that they could be considered as good sources of food ingredients and as new sources of edible oils (Zou et al., 2015). Hot pepper seed demonstrated significant health-promoting effects, having excellent anti-obesity activities (Jeon et al., 2010). In another study, hot pepper seeds showed strong antiproliferative activity against tumor cells (Jeon et al., 2012). As pepper seed is one of the major fruits in the world conferring health benefits due to their antioxidant activity (Kedage et al., 2007), phytosterols (Ruggieroa et al., 2013), and also contains a characteristic color value for seasonings, the present study aimed to evaluate the physicochemical changes in the seasonings by addition of various concentrations of pepper seed.

Materials and Methods

Materials

Hot pepper (*capsicum* annuum L.) cv. Longgreenmat was obtained from Yeongyang Pepper Experiment Station, Gyeongsangbuk-Do, Korea. All the other ingredients like *Saccharina japonica* powder, anchovy powder, shiitake (*Lentinus edodes*) powder, tomato (*Lycopersicum 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 granidiflorum* A, DC.), lotus root (*Nelumbinis rhzoma*), 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-pricrylhydrazyl (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 ($FeSO_4.7H_2O$) was purchased from Acro Orgamics (NJ, USA). Anhydrous sodium carbonate was purchased from J. T. Baker (NJ, USA). Ethanol and hydrogen peroxide were purchased from Merk (Darmstadt, Germany). All reagents used in the study were of analytical grade.

Preparation of seasoning samples

Hot peppers were harvested at the commercial maturity stage and transported to the lab within 2 h of harvest. The peppers were surface washed with tap water, kept for oven drying (60°C, 8 h), seeds were separated from the fruits manually, and ground into powder (60 mesh) using a mixer (HMF 3450S, Hanil Co., Seoul, Korea). The hot pepper seed powder was stored at 4°C and used within 3 d for making seasonings.

The formula of ingredients used for the preparation of hot pepper seed seasonings in the present study are given in Table 1. Hot pepper seed powder and other ingredients (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. After heating for 45 min, the samples were allowed to cool at room temperature and subjected to freeze dry. The freeze-dried mixture was milled (Speed Rotor Mill, KT-02A, Fukuoka, Japan) into powder and passed through a 100-mesh sieve. The strained samples were packed into airtight sample bottles and stored in refrigerator until analysis.

Table 1. Formula of recipe of seasoning prepared by adding different proportion of hot pepper seed powder (%)

Ingredient	Sample			
	NSP-0	NSP-5	NSP-10	NSP-15
Saccharina japonica powder	3.1	3.0	2.9	2.7
Anchovy powder	2.3	2.3	2.2	2.0
Oak mushroom powder	3.2	3.0	2.8	2.7
Tomato puree	1.5	1.5	1.4	1.3
Parched soybean powder	1.5	1.5	1.4	1.3
Sesame powder	1.3	1.0	1.0	0.9
Hot pepper seed powders	0	5.0	10	15
Chinese radish	1.5	1.5	1.4	1.3
Doraji (root of Chinnese bellflower)	0.2	0.1	0.1	0.1
Roasted salt	3.1	3.0	2.8	2.7
Grain syrup	8.7	7.3	6.9	6.5
Black sugar	1.7	1.5	1.4	1.3
Lotus root	0.2	0.2	0.2	0.2

Soy sauce	46.7	44.1	40.5	37.0
Water	25.0	25.0	25.0	25.0

NSP-0, seasoning prepared without adding hot pepper seed powder; NSP-5, seasoning prepared by adding 5% hot pepper seed powder; NSP-10, seasoning prepared by adding 10% hot pepper seed powder; NSP-15, seasoning prepared by adding 15% hot pepper seed powder.

Color measurement

L^* (lightness), a^* (redness, + or greenness, –), and b^* (yellowness, + or blueness, –) values of pepper seed seasonings were measured using a Chroma Meter (CR-300, Minolta Corp., Japan). A Minolta calibration plate (Y_{CIE} = 94.5, X_{CIE} = 0.3160, Y_{CIE} = 0.330) and a Hunter Lab 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 the seasonings and the average was calculated (Son et al., 2013).

DPPH radical scavenging activity

The scavenging activity of the extract from seasonings was measured with DPPH radicals according to the method of Blois (1958) with some modifications. One gram of pepper seed seasonings was extracted with 10 mL of ethanol for 12 h. The mixture was centrifuged (3000 rpm for 15 min) and supernatant was filtered using 0.22 µM membrane filter (Millipore, USA). DPPH solution was prepared at the concentration of 4×10^{-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 (Shyu and Hwang, 2002) as:

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

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

Determination of total phenolic content

The same ethanolic extract that was used for the DPPH radical scavenging assay was also used for measuring total phenolic content. The amount of total phenolics (TPH) was determined using the Folin-Ciocalteu method (Zheng and Wang, 2001). Five milliliter of distilled water was put into a 10-mL volumetric flask. A suitable volume of the 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 2% Na_2CO_3 solution was added, carefully mixed and made up to the mark 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). A calibration curve using gallic acid (GA) was prepared and the results were expressed as mg GAE.g^{-1} (milligram gallic acid equivalent per gram) sample.

Determination of free amino acid content

Amino acid contents were analyzed following the procedure of Je et al. (2005) with some modifications. One gram of 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, Pharacia, Biotech Co., Sweden).

Statistical analysis

Data were subjected to analysis of variance (ANOVA) using Statistix version 8.0 (Analytical Software, AZ, USA). Differences between means at $p<0.05$ were analyzed using the Tukey test.

Results

Color of seasoning

Significant variation in color value was observed among the seasoning samples prepared by adding hot pepper seeds (Table 2). The seasonings showed a significant increment in lightness, redness, and yellowness values with the increased amount of pepper seed powder. Increase in proportion of the pepper seed powder from 0 to 15% substantially increased lightness (35.12–60.17), redness (1.71–9.11), and yellowness (15.33–30.17).

Table 2. Hunters color values of seasoning prepared by adding different hot pepper seed powders

Color value	Sample			
	NSP-0	NSP-5	NSP-10	NSP-15
L (Lightness)	35.12±1.33^{d}	41.12±1.61^{c}	55.50±1.77^{b}	60.17±1.00^{a}
a (Redness)	1.71±0.31^{c}	5.31±1.18^{b}	9.00±0.51^{a}	9.11±0.81^{a}
b (Yellowness)	15.33±1.33^{d}	20.13±2.3^{c}	27.51±1.31^{b}	30.17±0.98^{a}

NSP-0, seasoning prepared without adding hot pepper seed powder; NSP-5, seasoning prepared by adding 5% hot pepper seed powder; NSP-10, seasoning prepared by adding 10% hot pepper seed powder; NSP-15, seasoning prepared by adding 15% hot pepper seed powder.
L: lightness (100, white ; 0, black), a: redness (–, green; +, red), b: yellowness (–, blue; +, yellow).
Values are mean±standard deviation of triplicate experiments.
The values followed by the different superscripts in the same row are significantly different, according to Tukey test ($p<0.05$).

Scavenging activities and total phenolic contents of seasoning

Free radical scavenging potential and antioxidant potential of the seasonings were determined by analyzing DPPH and total phenolic content (Table 3). Addition of hot pepper seed powder to the seasonings significantly enhanced the DPPH radical scavenging activities from 66.317% in control sample to 75.21% in the seasoning sample with 15% seed powder. Similarly, total phenolic content (200.1–244.2 mg GAE.g^{-1} sample) was also significantly high in the seasonings containing hot pepper seed powder as compared to control sample (139.2 mg GAE.g^{-1} sample).

Table 3. Scavenging activity and total phenolic contents of seasoning prepared by adding different pepper seed powders

Sample	NSP-0	NSP-5	NSP-10	NSP-15
DPPH (% Inhibition)	66.31±0.91^{d}	69.21±0.21^{c}	71.11±0.17^{b}	75.21±0.19^{a}
Total phenolics (mg GAE)/g sample)	139.2±1.9^{c}	200.1±2.1^{b}	241.6±2.7^{a}	244.2±3.9^{a}

Quoted values are mean±standard deviation of duplicate experiments.
GAE: gallic acid equivalent.
DPPH: DPPH free radical scavenging activity.
NSP-0, seasoning prepared without adding hot pepper seed powder; NSP-5, seasoning prepared by adding 5% hot pepper seed powder; NSP-10, seasoning prepared by adding 10% hot pepper seed powder; NSP-15, seasoning prepared by adding 15% hot pepper seed powder.
The values followed by the different superscripts in the same row are significantly different, according to Tukey test ($p<0.05$).

Total amino acid content of seasoning

Addition of hot pepper seed powder to the seasoning caused some of the amino acids to be increased while others (Table 4). Among the amino acids analyzed, glutamic acid (7012.77–7688.31 µg.mL^{-1} sample), which was increased with the addition of pepper seed powder, was the most abundantly found. Others like serine, aspartic acid, valine, leucine, phenylalanine, proline, and isoleucine, were also abundantly found in the seasonings. Amino acids like phospho-serine, taurine, phospho ethanol amine, α-aminoadipic acid, citrulline, α-amino-n-butyric acid, cystine, cystathionine, hydroxylysine, ornithine, histidine, anserine, carnosine, arginine and hydroxy proline were not detected.

Table 4. Amino acids content (μg.mL^{-1} of sample) in seasonings prepared by adding different pepper seed powders

Amino acid	Sample			
	NSP-0	NSP-5	NSP-10	NSP-15
L-Aspartic acid	3721.31	2711.31	2902.25	2812.31
L-Threonine	1521.00	1112.21	1070.01	998.31
L-Serine	1921.31	1032.00	1399.73	1300.66
L-Glutamic acid	6631.21	7012.77	7597.36	7688.31
L-Sarcosine	16.33	19.21	20.31	21.39
Glycine	1621.12	883.21	886.75	866.71
L-Alanine	2322.19	2521.00	2754.37	2933.71
L-Valine	2121.12	2009.71	1692.43	1566.71
L-Methionine	361.33	488.91	494.06	483.71
L-Isoleucine	2212.66	1721.66	1698.97	1598.77
L-Leucine	3002.54	2002.66	2378.51	2566.31
L-Tyrosine	921.31	200.00	212.54	231.44
L-Phenylalanine	2011.37	1066.31	1299.14	1361.21
β-Alanine	371.66	213.77	448.57	447.26
D,L-β-Aminoisobutyric acid	366.72	392.31	496.12	487.31
γ-Amino-n-butyric acid	355.12	512.77	633.84	662.31
Ethanolamine	132.37	66.77	99.56	89.31
Ammonia	721.33	381.33	423.12	4721.00
L-Lysine	2131.77	667.27	893.21	809.21
1-Methyl-L-histidine	1371.66	1521.33	1651.94	1777.37
3-Methly-L-histidine	71.77	65.31	67.06	63.21
Proline	3100.29	923.66	1318.23	1721.22
Total free amino acid	37007.49	27525.48	30438.08	35207.81

Values are the means of duplicate experiments.
NSP-0, seasoning prepared without adding hot pepper seed powder; NSP-5, seasoning prepared by adding 5% hot pepper seed powder; NSP-10, seasoning prepared by adding 10% hot pepper seed powder; NSP-15, seasoning prepared by adding 15% hot pepper seed powder.

Discussion

The change in color expression demonstrated that addition of higher concentration of pepper seed powder would promote development of darker color of the seasonings. The darker color of seasonings at higher concentration of pepper seed powder might be because of color of pepper seeds 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 one of the key factors in seasonings as they add different hues of colors to the foods.

Free radicals were 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 might cause a number of human diseases (Lobo et al., 2010). However, the detrimental effects of free radicals could be corrected by the application of external source of antioxidants. Synthetic antioxidants like butylated hydroxytoluene and butylated hydroxyanisole had been reported to be dangerous for human health (Lobo et al., 2010). Pepper seed berries were rich in antioxidants (Jeon et al., 2012; Kedage et al., 2007) including melatonin (Vitalini et al., 2013), a novel antioxidant. Thus addition of pepper seed powder could increase the antioxidant potential of seasonings.

Amino acids are the building blocks of proteins (Ekeanyanwu, 2013) that play an important roles in biochemical, biophysical, and physiological functions. Insufficient supply of proteins leads to different health disorders like 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 were recognized as being important in the taste and their presence in the seasoning with pepper seed powder will enhance the properties of the seasoning (Choi et al., 1996). The results of the present study showed that contents of amino acids aspartic acid, proline, alanine, cysteine, valine, methionine, isoleucine, leucine, and lysine could be increased in the seasonings with the addition of pepper seed concentration extract.

In conclusion, addition of hot pepper seed powder to the seasonings enhanced the color values and antioxidant potentials. Seasonings prepared by adding different concentrations of pepper seed powder could be used as good sources of antioxidants in the human diet. Although some of the amino acid contents were found to be reduced while others were increased, results of this study suggested that addition of hot pepper seed powder to the seasonings could impart better physicochemical properties as well as enhance the antioxidant potential.

Conflict of Interest

The authors declare no conflict of interest.

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