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Color and Antioxidant Potential of Seasoning with Butternut Squash (*Cucurbita moschata*) Powder

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Abstract: Seasonings, also known as spices and herbs, are appreciated for their culinary properties and potential health benefits. They are a rich source of beneficial bioactive phytochemicals. The objective of this study was to assess the effect of addition of different proportions of butternut squash seed (BS) powder on the quality characteristics and antioxidant potential of seasoning. The BS powder enhanced the color values and antioxidant potentials of the seasoning. The antioxidant activity, as 1,1-diphenyl-2-picrylhydrazyl (DPPH), of seasonings increased by up to 30.92% in the sample containing 5% BS powder. The total phenolics content was also increased by 91.40% with the addition of 5% BS powder. Seasonings prepared by adding BS powder could be used as good sources of natural antioxidants. This study suggests that the addition of BS powder could enhance the color value as well as antioxidant potential of seasonings.

Keywords: Antioxidant Potential, Butternut Squash Powder, Color Value, Seasoning

Introduction

Seasonings, sometimes interchangeably known as spices and herbs, have been used in a different ways in various cultures for a long time. In ancient times, spices were status symbols in many parts of the world for the wealthy who consumed them (Uhl 2000). Seasonings not only provide flavors but also play roles as colorants, preservatives, and source of phytochemicals. For example, turmeric is used for its yellow color but also contains anti-inflammatory, anti-mutagenic, anti-atherosclerotic, and anti-carcinogenic effects. Seasonings are generally a blended combination of more than one ingredients so as to provide a complete and balanced functional addition for further processing of meat and food products.

Seasonings are increasingly used not only for their culinary purposes but also for their potential health benefits because they are a good source of different phytochemicals. Formulation of seasonings is often dependent on the regional flavor preferences. Spices are, generally, highly aromatic because of the 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). Different forms of spices such as whole, ground, or extract are common in use (Srinivasan et al. 2004). In dietetic practice, herbs and spices are frequently used as an alternative to salt to prevent and manage of hypertension. In culinary art, they are primarily used to flavor foods and infuse vegetable oils and vinegars. Similarly, seasonings are used for flavor, medicine, color as well as a preservative for the prevention and destruction of harmful bacteria (Ernst and Pittler 2000). Many spices and herbs are gaining ever increasing interests because they possess a variety of phytochemicals such as polyphenols, menthol, retinol, carotenoids, which have antimicrobial, antioxidant, anticancer, and anti-inflammatory properties (Kaefer and Milner 2008; Viuda-Martos et al. 2010; Paur et al. 2011; Jungbauer and Medjakovic 2012). The antioxidant potential of herb and spices is mainly attributed to the phenolic compounds (Shan et al. 2005). They play an important role in controlling viral replication, inhibiting allergy and arthritis, preventing cancer and heart diseases (Aggarwal et al. 2002).

Butternut squash (*Cucurbita moshchata*) is a seasonal crop that is used as food and feed, and is rich in carotenoids, dietary fibers, and minerals (Jacobo-Valenzuela et al. 2011b). A scientific review report showed that it is a good source of α and β-carotene, lutein, vitamin C, dietary fiber, minerals, and phenolic compounds (Jacobo-Valenzuela et al. 2011a). Seeds and seed oil of butternut squash are also found to have antihypertensive and anti-inflammatory properties (Murkovic et al. 1996; Li 2020). Cultivation-wise, butternut squash is rather simple and able to adapt to organic farming. It can easily be grown in different parts of the world. It is one of the commonly grown vegetables in Korea. Considering the nutritional value and easy availability of butternut squash, the objective of this study was to investigate the color value and antioxidant potential of seasoning prepared with its

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addition.

Materials and Methods

Materials and chemical reagents

Butternut squash (*Cucurbita moschata*) was obtained from Namhea-Gun, Gyeongsangnam-Do, Korea. All the other ingredients like *Saccharina japonica* powder, anchovy powder, shiitake (*Lentinus edodes*) mushroom powder, tomato puree, parched soybean powder, sesame powder, Chinese radish (*Raphanus sativus* L.), root of Chinese bellflower (*Platycodon granidiflorum* A, DC.), roasted salt, grain syrup, black sugar, lotus root (*Nelumbinis rhizoma*), soy sauce and water were purchased from a local market in Daegu, Korea.

Falin-Ciocalteu reagent, gallic acid and 1,1-Diphenyl-2-pricrylhydrazyl (DPPH) were purchased from Sigma Chemical co. (St. Louis, Mo, USA). All reagents used in the study were of analytical grade.

Preparation of seasoning samples

Butternut squashes (BS) were harvested at the commercial maturity stage and transported to the lab within 2 h of harvest. The seeds were separated manually, were washed with tap water, and kept for oven drying (60°C, 8 h). The dried seeds were ground into powder using an electric grinder (HMF 3450S, Hanil Co., Seoul, Korea). The seed powder was stored at 4°C and used within 3 days for making seasonings.

The ingredients used for the preparation of BS seasoning are given in Table 1. The BS powder and other ingredients were mixed thoroughly and simultaneously heated in a pan over a 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) into powder. The samples were kept into airtight containers and stored in refrigerator until analysis.

Table 1. Formula of recipe of butternut squash powder seasonings (%)

Ingredient	Sample[1)]			
	BSP-0	BSP-1	BSP-3	BSP-5
Saccharina japonica powder	3.00	2.97	2.91	2.85
Anchovy powder	2.40	2.38	2.33	2.28
Shitake mushroom powder	3.20	3.17	3.10	3.04
Tomato puree	1.50	1.49	1.46	1.43
Parched soybean powder	1.50	1.49	1.46	1.43
Sesame powder	1.30	1.29	1.26	1.24
Butternut Squash powder	0.00	1.00	3.00	5.00
Chinese radish	1.50	1.49	1.46	1.43
Doraji (root of Chinese bellflower)	0.20	0.20	0.19	0.19
Roasted salt	3.00	2.97	2.91	2.85
Grain syrup	8.80	8.71	8.54	8.36
Black sugar	1.70	1.68	1.65	1.62
Lotus root	0.20	0.20	0.19	0.19
Soy sauce	46.70	46.23	45.30	44.37
Water	25.00	24.75	24.25	23.75
Total	100.00	100.00	100.00	100.00

[1)]BSP-0, seasoning prepared without adding butternut squash powder; BSP-1, seasoning prepared by adding 1% butternut squash powder; BSP-3, seasoning prepared by adding 3% butternut squash powder; BSP-5, seasoning prepared by adding 5% butternut squash powder.

Color measurement

L^* (lightness), a^* (redness, + or greenness, –), and b^* (yellowness, + or blueness, –) values of BS 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. The samples were placed into Petri plates and color was measured at three places (Kim et al. 2014).

DPPH radical scavenging activity

The radical scavenging potential of seasoning samples was measured through DPPH following the methods described ealier (Blois 1958; Dhungana et al. 2016). One gram of butternut squash seasonings was extracted with 10 mL of ethanol for 12 h. The mixture was centrifuged (3,000 rpm for 10 min) and supernatant was filtered using 0.22 µM membrane filter (Millipore, USA). DPPH solution was prepared at the concentration of 0.01% in ethanol. Equal volumes (100 µL) of sample extract and freshly prepared 0.01% (w/v) methanolic solution of DPPH were mixed in 96-microplate wells and left at room temperature for 30

min under dark condition. After 30 min, the absorbance values of the reaction mixtures were determined at 517 nm using a spectrophotometer (Multiskan GO, Thermo Fisher Scientific, Vantaa, Finland).

Determination of total polyphenols

The total polyphenol content of seasoning samples was determined using the Folin-Ciocalteu method (Singleton et al. 1999). Sample extracts (50 µL) (used for DPPH assay) and 1000 µL aqueous Na_2CO_3 (2%, w/v) were mixed and kept for 3 min at room temperature. Then, 50 µL 1 N Folin-Ciocalteau reagent was added into the reaction mixture and allowed to react for 30 min at room temperature under dark condition. The absorbance value of the reaction mixtures was measured at 750 nm using a spectrophotometer (Multiskan GO, Thermo Fisher Scientific, Vantaa, Finland). A calibration curve was plotted with gallic acid (GA) as a standard.

Statistical analysis

Analysis of variance was conducted using SAS 9.4 (SAS Institute, Cary, NC, USA) to compare the means of different samples, and the significant differences between the sample means were determined using Tukey test at $p<0.05$. Average values of three replicates were reported.

Results and Discussion

Color of BS seasoning

Significant variation in color value was observed among the seasoning samples prepared by adding different proportions of BS powder (Table 2). The seasonings showed a significant increment in lightness value of BSP-1 (36.17), however, the value decreased in BSP-3 (24.32) and BSP-5 (21.23) as compared to the control, BSP-0 (32.15). However, the redness and yellowness values were increased with the amount of BS powder. Increase in the proportion of the BS powder from 0% to 5% substantially increased redness (from 2.32 to 7.21), and yellowness (from 14.11 to 23.12). This trend of color expression demonstrated that addition of higher concentration of BS powder would promote development of darker color of the seasoning samples. The darker color of seasonings at higher concentration of BS powder might be because of color of the powder itself and/or 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 determining factors in seasonings as the seasonings add different hues of colors to the foods.

Table 2. Hunter color values of seasonings prepared by adding different proportions of butternut squash (BS) powder

Color value[2)]	BS powder	Sample[1)]			
		BSP-0	BSP-1	BSP-3	BSP-5
L (lightness)	64.21	32.15±1.32[b3)]	36.17±1.19[a]	24.32±1.66[c]	21.23±1.00[d]
a (redness)	11.18	2.32±0.37[d]	3.62±0.51[c]	4.37±0.81[b]	7.21±0.23[a]
b (yellowness)	32.71	14.11±1.31[d]	16.15±2.31[c]	20.23±0.69[b]	23.12±1.39[a]

[1)]BSP-0, seasoning prepared without adding butternut squash powder; BSP-1, seasoning prepared by adding 1% butternut squash powder; BSP-3, seasoning prepared by adding 3% butternut squash powder; BSP-5, seasoning prepared by adding 5% butternut squash powder. [2)]L: lightness (100, white ; 0, black), a: redness (–, green; +, red), b: yellowness (–, blue; +, yellow). [3)]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 ($p<0.05$).

Antioxidant potential of BS seasoning

The antioxidant potential of seasonings were determined through DPPH and total polyphenol content (Table 3). Addition of BS powder to the seasonings significantly increased the DPPH radical scavenging activities from 65.21% in the control sample (BSP-0) to 85.37% in the seasoning sample with 5% BS powder, BSP-5. Similarly, the total polyphenolic content was also significantly high in the seasonings containing BS powder (212.10−249.20 mg GAE/g sample) as compared to control sample (130.20 mg GAE/g sample).

Free radicals are highly reactive species and capable of damaging biologically important molecules such as DNA, proteins, carbohydrates, and lipids (Young and Woodside 2001). Significant alteration and damage to lipids, proteins, and DNA by free radicals may result in a number of health problems (Lobo et al. 2010). The detrimental effects of free radicals can be corrected by the supplementation of antioxidants. However, synthetic antioxidants like butylated hydroxytoluene and butylated hydroxyanisole have recently been reported dangerous for human health (Lobo et al. 2010). So, there is an increasing attention towards the natural dietetic antioxidants. The higher antioxidant potential of BS-added seasoning might be due to the phytochemicals and phenolic contents (Jacobo-Valenzuela et al. 2011a). Thus, addition of BS powder could increase the antioxidant potential of seasonings.

Table 3. Scavenging activity and total phenolic contents of seasonings prepared by adding different proportions of butternut squash powder

Sample[1)]	DPPH (% Inhibition[2)])	Total polyphenol content (mg GAE[3)]/g sample)
BSP-0	65.21±0.81[d4)]	130.20±1.10[d]
BSP-1	72.13±0.16[c]	212.10±3.20[c]
BSP-3	80.16±0.18[b]	219.10±2.20[b]
BSP-5	85.37±0.16[a]	249.20±2.90[a]

[1)]BSP-0, seasoning prepared without adding butternut squash powder; BSP-1, seasoning prepared by adding 1% butternut squash powder; BSP-3, seasoning prepared by adding 3% butternut squash powder; BSP-5, seasoning prepared by adding 5% butternut squash powder. [2)]DPPH: DPPH free radical scavenging activity. [3)]GAE: gallic acid equivalent. [4)]Values are mean±standard deviation of triplicate experiments. The values followed by the different superscripts in the same column are significantly different, according to Tukey test (p<0.05).

Conclusion

The effect of addition of butternut squash powder to seasoning was investigated with color and antioxidant potential. The addition of the powder enhanced the color values and antioxidant potentials. Thus, Natural seasonings prepared by adding different concentrations of butternut squash powder could be used as good sources of natural antioxidants in the human diet.

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