Progress in Nutrition 2017; Vol. 19, N. 2: 212-218 DOI: 10.23751/pn.v19i2.4826

Original Article

Antioxidant characteristics and quality assessment of soymilk supplemented with *Ocimum basilicum* L.

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Summary. Soymilk is used as a milk substitute for people with lactose intolerance. Recent research has aimed to fortify the health/functional aspect of soymilk. Hence, in order to investigate the potential of soymilk as a drink with improved functionality, *Ocimum basilicum* L. (Basil) was added to soymilk and the quality and antioxidant effects were comparatively analyzed. The addition of basil did not affect the water content or chromaticity of soymilk, and the pH of soymilk decreased as the concentration of basil increased. Although total phenol content significantly increased as the concentration of basil increased, total flavonoid content did not show a significant change. Moreover, the antioxidant effect significantly increased as the concentration of basil increased, suggesting that this effect is attributed to the phenol contained in basil. Results of the sensory evaluation suggest that overall evaluation was unfavorable since the aroma of basil grew stronger as the concentration of basil increased. Thus, while soymilk supplemented with basil has excellent antioxidant capacity, future studies on the growth conditions and processing of basil are needed to reduce its unique aroma and thus enable production of fortified soymilk with good flavor.

Key words: soymilk, *ocimum basilicum*, antioxidant, sensory evaluations

Introduction

Owing to the growing interest in health in today's society, there have been many changes in the choice of food consumed on a daily basis. Soybean (*Glycine max MERR*.) is consumed for increased nutrition, and research on its nutritional value and functionality is actively ongoing (1,2). Soybean is an important source of protein and is widely used to make fermented soybean lump, which is the basic ingredient of soybean paste (3). Soybean has a particularly high content of lecithin, saponin, isoflavones, and trypsin inhibitor, and these components exert an anticancer effect, decrease blood cholesterol, and prevent obesity by inhibiting lipid synthesis. Additionally, soybean activates bowel movement through intestinal regulation, and thereby, prevents constipation (4).

Soymilk, which is a processed food produced by extracting solid and protein from soybean, has a high content of protein, iron, unsaturated fatty acids, and niacin, and plays a critical role as a substitute for milk in cases of lactose intolerance. Furthermore, while soymilk contains hardly any cholesterol, it contains substances that are good for preventing adult diseases, and therefore it is becoming more widely accepted as a healthy vegetable drink (5). Various kinds of soymilk have been developed, such as soymilk supplemented with ginseng (6) and rice grains (7) to improve the quality, functionality, as well as sensory aspects of soymilk.

Ocimum basilicum L. (Basil) a type of herb, originates from hot and humid regions such as Africa and Asia, and is an annual plant which belongs to self-heal. It is cultivated throughout the world including Europe and grows to 0.4 ~ 0.9 m (8). The name basil is originated from *basileus*, which means 'prince' in Greek. Basil is widely used in western cuisines being referred to as 'kitchen herb,' and since it especially goes well with dishes made from tomatoes, it is commonly used in pizza, salad, and pasta. Its essential oil, made by distilling dried leaves and flowers, is used for fragrance in cosmetics, perfumes, soaps, and toothpaste, and it is also widely used for medicine and food (8). As it contains substances such as saponin, it is used as an antineuralgic in the medical field (9).

So far there have been hardly any studies on soymilk supplemented with a herb. Hence, in the present study, we produced soymilk supplemented with basil and comparatively analyzed its antioxidant effect and quality assessment in terms of basil content, and compared preferences through sensory evaluations. We aim to determine the potential of soymilk as a drink with improved functionality and antioxidant effects.

Materials and Methods

Materials

Basil and soybean used in the experiments were purchased from Chungwon Products (Incheon, South Korea). Four kinds of soymilk were produced using only basil at varying concentrations of (0%, 5%, 10%, 15%) and soybean, without any other ingredient.

Production of soymilk

Soybeans were macerated at room temperature for 8 hours and simmered in an amount of water that was twice the weight of the soybeans. From this, the concentrate of soybean was obtained. Basil was measured and added at 0%, 5%, 10%, and 15% of the total amount of soymilk.

Measurement of water content

Using the principle of drying method at high-pressure and heat, 2~5 g of soymilk was added to a weighing bottle, which was dried for 3~4 hours, and after taking it out from the dryer, it was cooled down for 30 minutes in the desiccator. After weighing, it was put back in the dryer and dried for 1 hour, cooled down, and weighted. The drying, cooling, and weighing processes were repeated until a constant weight was obtained.

The water content was calculated as follows:

- Water content (%) = (w2-w3)/(w2-w1) × 100 = weight of water (g) / weight of soymilk (g) × 100
- w1: constant weight of weighing bottle or weighing plate (g)
- w2: weight of soymilk + weight bottle(g)
- w3: weight of soymilk after drying of soymilk + weight bottle (g)

Measurement of chromaticity

To determine chromaticity, soymilk concentrate with 0% of basil was added to a transparent plastic cylinder container (35 × 10 mm), and the L value (lightness), a value (+redness, -greenness), and b value (+yellowness, -blueness) were measured using a spectrophotometer (CM-2500D, Minolta, Tokyo, Japan). Chroma values were calculated using the equation $(a2+b2)^{½}$, and Total Color Difference (TCD) values were calculated using the equation $\{(L–L^{control})\ 2+(a–a^{control})\ 2+(b–b^{control})\ 2\}^{½}$, which is the difference of color between the preprocessed group with basil added and the group without basil.

Measurement of viscosity

Viscosity was expressed in millipascal-second (mPa/sec) as shear rate was increased at 25°C using a Vibrio viscometer (SV-10, A&D Co. Ltd., Tokyo, Japan) (7).

Measurement of pH

pH was measured using a pH meter (model 420+, Thermo Fisher Scientific Inc., Waltham, MA).

Total phenol and flavonoid contents

The total polyphenol content was analyzed according to the method by Dewanto et al (10), which is based on Folin-Ciocalteu reagent (Sigma-Aldrich Co., St. Louis, MO) developing a molybdenum blue color after reduction by polyphenol compounds of 80% methanol extract. That is, 2 mL of 2% Na_2CO_3 solution (Junsei

Chemicals, Tokyo, Japan) was added to 100 μL of each extract, and after 3 minutes, 100 μL of 50% Folin-Ciocalteu reagent was added. After 30 minutes, absorbance of the reaction mixture was measured at 700 nm using a spectrophotometer. Gallic acid (Sigma-Aldrich Co., St. Louis, MO) was used as a standard material. A standard curve was generated (y=0.0018x; R^2=0.9991) and the total polyphenol content was expressed as mg gallic acid equivalents in g of specimen.

Total flavonoid content was determined according to the method by Kim et al (11). To 250 μL of methanol extract, 1 mL of distilled water and 75 μL of 5% $NaNO_2$ (Junsei Chemicals, Tokyo, Japan) were added, and after 5 minutes, 150 μL of 10% $AlCl·6H_2O$ (Sigma-Aldrich Co., St. Louis, MO) was added and the reaction was left to sit for 6 minutes. Then, 500 μL of 1N NaOH (Sigma-Aldrich Co., St. Louis, MO) was added, and after 11 minutes, absorbance of the reaction mixture was measured at 500 nm using a spectrophotometer. (+)-Catechin (Sigma-Aldrich Co., St. Louis, MO) was used as a standard material; a standard curve was generated (y=0.004x-0.0912; R^2=0.9993) and the total flavonoid content was expressed as mg catechin equivalents in g of specimen.

Antioxidant effect

Antioxidant activity was measured using the ABTS radical (2.2'-azino-bis-3-ethyl venzothiazoline-6-sulfonic acid, Sigma-Aldrich Co., St. Louis, MO) according to the method of ABTS radical cation decolorisation assay (12). Briefly, 7.4 mM ABTS and 2.45 mM potassium persulfate (Sigma-Aldrich Co., St. Louis, MO) was mixed at a final concentration, left in a dark room at room temperature for 24 hours to generate ABTS cations, and diluted with distilled water so that the absorbance at 700 nm would be 1.4~5. Then, 100 μL of methanol extract was added to 2 ml of the diluted ABTS solution and the absorbance was measured at 700 nm after exactly 5 minutes. ABTS radical scavenging activity was calculated using the following equation.

ABTS radical scavenging activity (%) = [1-(sample absorbance/control absorbance)× 100)]

DPPH radical scavenging activity was measured using the method by Blois (13). Briefly, 1.6 mL of 0.2 mM DPPH (1,1-diphenyl-2-picryl-hydrazyl, Sigma-Aldrich Co., St. Louis, MO) solution was added to 0.4 mL of the methanol extract, and after being left at room temperature for 10 minutes, the absorbance was measured at 520 nm. For the control group, 1.6 mL of DPPH solution was added to 0.4 mL of 80% methanol and left at room temperature for 10 minutes, and the absorbance was measured at 525 nm using a spectrophotometer. DPPH radical scavenging activity was calculated using the following equation:

DPPH radical scavenging activity (%)
= [1 - (sample absorbance/control absorbance) × 100]

Sensory evaluation

Sweetness, nuttiness, color, and overall evaluation were assessed by 10 students in the Department of Food Science and Nutrition at Gwangju University using random number. The assessment was based on a 5-point scale, where 1 indicated 'very bad' and 5 indicated 'very good.' Identical amounts of soymilk (10 ml) kept at refrigeration temperature were provided three times at 0%, 5%, 10%, and 15% in the sensory evaluation.

Statistical analysis

All tests were performed in triplicate. To statically analyze the total polyphenol and flavonoid contents, ABTS radical scavenging activity, and DPPH radical scavenging activity, descriptive statistics, such as mean and standard deviation, were calculated. To test statistical significance of our results, one way ANOVA (a=0.05) was performed using SPSS 20.0 (SPSS Inc, Chicago, IL, USA). In addition, Duncan's multiple range test was performed and correlation coefficients were calculated.

Results and Discussion

Physicochemical properties of soymilk supplemented with basil

The water content, pH, and chromaticity of soymilk supplemented with basil are shown in Table 1.The water content of the soymilk supplemented with basil was 91.21±0.34 when basil content was 0%, while it was 89.83±0.01 ($p<0.05$), 90.05±0.01 ($p<0.05$), and 89.74±0.07 ($p<0.01$) at 5%, 10%, and 15%, respectively, thus showing a decrease, increase, and decrease again.

This suggests that while the water content varied significantly for each concentration, the addition of basil did not have a huge effect on the water content. The pH was 6.75±0.01 when the basil content was 0%, while it was 6.47±0.00 (p<0.001), 6.61±0.00 (p<0.001), and 6.37±0.00 (p<0.001) at 5%, 10%, and 15%, respectively, showing significant results (p<0.001) with a decrease, increase, and decrease again. We found that the pH tended to decrease when basil was added. The L value was 47.84±1.06 when the basil content was 0%, while it was 43.80±0.90 (p<0.001), 45.56±1.16 (p<0.01), and 47.03±1.51 (p<0.05) at 5%, 10%, and 15%, respectively, showing a decrease and then increase. The a value, which indicates redness, was 5.72±0.27 when the basil content was 0% while it was 5.05±0.20 (p<0.01), 4.95±0.26 (p<0.05), and 5.05±0.09 (p<0.01) at 5%, 10%, and 15%, respectively. The b value, which indicates yellowness was 21.05±0.88, when the basil content was 0%, while it was 17.37±0.45 (p<0.001), 18.63±0.73 (p<0.001), and 18.69±0.75 (p<0.001) at 5%, 10%, and 15%, respectively, showing a decrease and then a gradual increase. There were significant differences in chromaticity when the basil content was high, but the trend as per the basil concentration changes could not be confirmed.

Although basil was added to soymilk, the changes in water content and chromaticity were not resulted with respect to its concentration. Boiling basil with soybeans is possibly similar to hot water extraction, and it can therefore be considered as unconcentrated hot water extract. The water content would not change even if the unconcentrated extract is added. Addition of basil decreased the yellowness; the leaves were dried and ground, and when concentration of basil increased, the bright yellow color unique to soymilk temporarily decreased when basil was first added and did not show further changes. Furthermore, since soymilk and basil are both weakly acidic, increasing the concentration of basil showed a tendency of lowering the pH.

Viscosity of soymilk supplemented with basil

Measurement of viscosity of soymilk with varying concentrations of basil (Table 2) showed that at 0% concentration the viscosity was 9.25±0.85 at 0 min and it decreased with time, reaching 4.8±0.90 at 7 min. The viscosity of soymilk supplemented with 5% basil was 15.55±0.75 (p<0.001) at 0 min and 7.5±0.80 (p<0.001) at 7 min, while that of soymilk supplemented with 10% basil was 7.95±2.25 at 0 min and 4.9±1.70 at 7 min, indicating no significant change. Viscosity of soymilk supplemented with 15% basil was 15.2±0.3 (p<0.01) at 0 min and 8.55 ±1.25 (p<0.05) at 7 min.
Viscosity of soymilk supplemented with basil did not show substantial changes with concentration of basil. As previously mentioned, since basil is similar to unconcentrated hot water extract, basil did not change the physical properties of soymilk.

Analysis of total phenol and flavonoid contents in soymilk supplemented with basil

The total phenol and flavonoid content of soymilk supplemented with basil are shown in Table 3. The total phenol content was 25.09±0.52 at 0%, while it was 43.27±0.46 (p<0.001), 51.42±0.49 (p<0.001), and 71.21±0.83 (p<0.001) at 5%, 10%, and 15%, respective-

Table 1. Changes in water content, pH, and chromaticity of soymilk supplemented with basil.

		Concentration (%)			
		0	5	10	15
Water content[1)]		91.21±0.34	89.83±0.01*	90.05±0.01*	89.74±0.07**
pH[1)]		6.75±0.01	6.47±0.00***	6.61±0.00***	6.37±0.00***
	L	47.84±1.06	43.80±0.90***	45.56±1.16**	47.03±1.51*
Chromaticity[1)]	A	5.72±0.27	5.05±0.20**	4.95±0.26*	5.05±0.09**
	B	21.05±0.88	17.37±0.45***	18.63±0.73***	18.69±0.75***

[1)] *Mean±SD*
** p<0.05 : Means in a column are significantly different at 5% significance level by Duncan's multiple range test.*
*** p<0.01 : Means in a column are significantly different at 1% significance level by Duncan's multiple range test.*
**** p<0.001 : Means in a column are significantly different at 0.1% significance level by Duncan's multiple range test.*

Table 2. Viscosity of soymilk supplemented with basil.

		Minute							
		0	1	2	3	4	5	6	7
Concentration	0	9.25±0.85	7.45±1.65	6.7±2.00	6.2±1.70	5.8±1.10	5.4±1.10	4.85±0.95	4.8±0.90
(%)[1)]	5	15.55±0.75***	12.05±1.55***	9.6±1.50**	9.3±1.69***	8.65±1.65**	8.65±1.55**	7.65±0.85***	7.5±0.80**
	10	10.25±1.25	7.95±2.25	7.25±2.05	6.6±1.80	6.4±1.70	5.9±1.60	5.3±1.71	4.9±1.70
	15	15.2±0.3**	11.95±0.95*	10.9±1.60	9.3±0.70	8.9±0.90	8.9±0.90*	8.65±1.35	8.55±1.25*

1) Mean±SD

** p<0.05 : Means in a column are significantly different at 5% significance level by Duncan's multiple range test.*

*** p<0.01 : Means in a column are significantly different at 1% significance level by Duncan's multiple range test.*

**** p<0.001 : Means in a column are significantly different at 0.1% significance level by Duncan's multiple range test.*

ly, showing a significant increase. Total flavonoid content was 12.55±0.14 at 0% and 9.8±1.77 at 5%, which showed no significant change, while it was 16.23±1.61 ($p<0.05$) and 16.44±1.88 ($p<0.05$) at 10% and 15% of basil content, showing a significant change at 10% or higher. The result that total phenol content significantly increased at higher concentrations of basil was not surprising. In a study on the phenol content in 37 species of basil produced in various regions showed that the phenol content was widely distributed in all species of basil from a minimum of 7.15 ± 2.11 (mg GAE/100 g dry wt) to a maximum of 107.43 ± 6.36 (mg GAE/100 g dry wt). However, the concentration of flavonoid did greatly change with a minimum of 12.89 ± 0.81 (mg QE/100 g dry wt) and maximum of 26.04 ± 4.16 (mg QE/100 g dry wt). The basil used in the present study had a higher total phenol content than flavonoid content, and therefore, basil is considered to have largely affected the total phenol content of soymilk supplemented with basil compared to its effect on the total flavonoid content (14).

Antioxidant effect of soymilk supplemented with basil

The experimental results of ABTS and DPPH methods are shown in Table 4. The antioxidant effect measured by ABTS method was 19.86±1.29 when the concentration of basil was 0%, while it was 46.61±0.93 ($p<0.001$), 54.99±1.08 ($p<0.001$), and 83.13±0.36 ($p<0.001$) at 5%, 10%, and 15%, showing a significant increase compared to that at 0%. With the DPPH method, the antioxidant effect was 11.16±0.26 at 0%, while it was 18.77±0.77 ($p<0.001$), 22.21±0.17 ($p<0.001$), and 25.76±2.02 ($p<0.001$) at 5%, 10%, and 15%, showing a significant increase compared to 0%. We found that soymilk with higher concentration of basil had a higher antioxidant effect. The phenol compound is one of the plant-derived metabolites widely distributed in plants, and because it has a phenolic hydroxyl group and can bind to proteins and other macromolecules, it has physiological activities such as antioxidant effects (15). The total phenol content largely increased ($p<0.001$) and the total flavonoid content increased only at 10%

Table 3. Total phenol and total flavonoid contents of soymilk supplemented with basil.

	Concentration (%)			
	0	5	10	15
Total phenol[1)]	25.09±0.52	43.27±0.46***	51.42±0.49***	71.21±0.83***
Total flavonoid[1)]	12.55±0.14	9.8±1.77	16.23±1.61*	16.44±1.88*

1) Mean±SD

** p<0.05 : Means in a column are significantly different at 5% significance level by Duncan's multiple range test.*

**** p<0.001 : Means in a column are significantly different at 0.1% significance level by Duncan's multiple range test.*

Table 4. Antioxidant effect of soymilk supplemented with basil.

	Concentration (%)			
	0	5	10	15
ABTS (%)[1)]	19.86±1.29	46.61±0.93***	54.99±1.08***	83.13±0.36***
DPPH (%)[1)]	11.16±0.26	18.77±0.77***	22.21±0.17***	25.76±2.02***

[1)] *Mean±SD*
*** *p<0.001 : Means in a column are significantly different at 0.1% significance level by Duncan's multiple range test.*

or higher. Therefore, we believe that the total phenol content is the main reason for the increase in the antioxidant effect at higher basil concentrations, and the total flavonoid content did not contribute much to the antioxidant effect. According to a recent study, phenolic acid has critical antioxidant effects among the phytochemicals contained in basil (16), thus supporting our finding that the total phenol content is closely related to antioxidant effects.

Sensory evaluations of soymilk supplemented with basil

Results of the sensory evaluation are shown in Table 5. Sweetness, nuttiness, and color of soymilk significantly decreased compared to that with 0% basil, and the overall evaluation became unfavorable as the concentration of basil increased.

Our study results suggest that, although there was no big difference in the appearance, the aroma that is unique to basil grew stronger as its concentration increased and this might have led to unfavorable overall evaluation. It has been reported that the content of volatile components in basil can change according to the process method of drying (17). In addition, a study by Moghaddam and Mehdizadeh (14) showed that the total phenol content in basil changed according to growing conditions, which suggests that volatile components of basil may also vary depending on the growing conditions. Therefore, future research on growing conditions for basil with low content of volatile components and process methods to reduce its aroma even at high concentration is necessary and it may allow the production of functional soymilk with a superior antioxidant effect.

Acknowledgments

This research was supported by Basic Science Research Program through the National Research Foundation of Korea(NRF) funded by the Ministry of Education, Science and Technology(NRF-2013R1A1A4A01013550). Moreover, this study was conducted in part by research funds from Gwangju University, South Korea in 2015.

Table 5. Sensory evaluation of soymilk supplemented with basil.

	Concentration (%)			
	0	5	10	15
Sweetness[1)]	2.37±0.87	1.90±0.67	1.37±0.88***	1.57±1.187***
Nuttiness[1)]	3.47±0.86	2.67±1.09**	1.80±0.71***	1.93±0.87***
Color[1)]	3.57±0.77	2.83±1.12**	2.53±1.04**	2.67±0.99***
Overall evaluation[1)]	3.30±1.12	2.67±1.18*	2.03±0.96***	1.93±0.69***

[1)] *Mean±SD*
* *p<0.05 : Means in a column are significantly different at 5% significance level by Duncan's multiple range test.*
** *p<0.01 : Means in a column are significantly different at 1% significance level by Duncan's multiple range test.*
*** *p<0.001 : Means in a column are significantly different at 0.1% significance level by Duncan's multiple range test.*

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