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Physiological Characteristics of Medicinal Herbs Soysauce with Ripening Period

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ABSTRACT

This study was carried out to investigate the physiological characteristics of medicinal herbs soysauce (MHSS), after adding 24 kinds of medicinal herbs, with ripening period. Total nitrogen compound content increased up to 5 years after which there were no significant differences; the peptide-N content increased up to 5 years and decreased thereafter, while formol- and amino-type N content gradually increased up to 10 years. The major isoflavones of MHSS were daidzin, genistin and genistein, accounting for 128.7~130.7, 113.7~128.5 and 96.8~104.6 µg/g, respectively. After 10 years ripening of MHSS, free type of daidzin doubled while conjugated type of genistin decreased by 30%. When the ripening period was increased, DPPH radical scavenging and SOD-like activity, as well as ACE inhibitory ability and nitrite scavenging effects increased: the IC_{50} value of MHSS ripened for 10 years for DPPH radical scavenging activity was < 0.1 mg, SOD-like activity was 82.5% at 5 mg/mL of MHSS, nitrite scavenging effect was 60.1% (pH 1.2) at 5 mg/mL of MHSS and the ACE inhibitory ability of 9-year-old ripened MHSS was highest, at 89.1 ± 1.5%.

Keywords: angiotensin converting enzyme, antioxidative activity, isoflavone, medicinal herb soysauce, nitrite scavenging effect
Abbreviations: ACE, angiotensin converting enzyme; **MHSS**, medicinal herb soysauce; **SOD**, superoxide dismutase

INTRODUCTION

Conventional fermented foods in South Korea generally use soybean paste and soysauce (SS). Through the action of the various enzymes which microbes such as molds, bacteria and yeast secretes during processing, protein and other constituents of soybean are changed to various organic ingredients, amino acids, peptides, nitrogen, melanoidins, among others. These organic materials control the taste of the various cooking foods; consequently, SS is the major seasoning foodstuffs in Korea (Kim *et al*. 1978a, 1978b; Kim *et al*. 1980; Oh *et al*. 2007). The SS prepared by Oh *et al*. (2007) involved submerging soybean in water, boiling and crushing it appropriately, molding it into a cube then fermenting in under traditional conditions (in a constant temperature room controlled naturally). The product is termed *meju*, which is soaked in salted water and fermented under natural conditions. The solution that is then obtained from this process is termed SS. Various physiological activities of soybean paste or *doenjang* have been studied such as the prevention of high blood-pressure, as an antimutagenic or anticancer agent, prevention of thrombosis, among others (Kim *et al*. 1991a, 1991b; Lee *et al*. 1991; Santiago *et al*. 1992; Seo *et al*. 1994; Kennedy 1995; Shin *et al*. 1995; Lee *et al*. 1997; Kim *et al*. 1999; Kwon *et al*. 2004; Lee *et al*. 2006). Although there are many reports about soybean paste, there are only few on the physiological activity of SS except for the antioxidative activity of SS and its products; the antioxidative compounds of SS are the melanoidins (Yamaguchi *et al*. 1979; Cheigh *et al*. 1993); the antioxidative activity of SS increased 3 times after a 6-month ripening period (Moon *et al*. 1987, 1990). Moon (1991) reported that antioxidative activity of SS products was highly related with total N-content and raw SS (unsterilized SS). Kang *et al*. (1999) reported that SS sauce prepared with mountain herbs increased the total content of N-containing compounds. Kataoka (2005) reported that *shoyu* (Japanese SS) has functional properties, including antihypertensive or anticarcinogenic effects. Recently, to enhance the quality and functional characteristics of soybean paste and SS, various additives – including medical plants – were added to soybean paste and SS during processing; after manufacturing, the functional characteristics of fermented products were investigated. Jang *et al*. (2003), Lee *et al*. (2003) and Park *et al*. (2006) reported that traditional soybean *doenjang* prepared with Korean herbal medicines had antioxidative activity and nitrite-scavenging activities. Lee *et al*. (2004, 2008) reported that physiological activity of *doenjang* increased when mushrooms were added. The antioxidative activity of *doenjang* containing *Astraglaus membranaceus* water extract (Min 2006), *Dioscorea* (Jang 2009), *Ulmi* cortex (Son 2008) or onion (Shin *et al*. 2008) was higher than control *doenjang*. Lee *et al*. (2009) reported that *doenjang* prepared using *Acanthopanax senticosus*, *Angelica gifgas* and *Corni fructus* reduced the negative flavor of Korean *doenjang.* Kwon *et al*. (2011) reported that the nitrite-scavenging ability of *doenjang* prepared using extract of rice fermented with *Poria cocos* mycelium was higher than the control. There are many quality characteristics of SS as mentioned, but there is no research on the physiological function of SS.

The possibility that the characteristics of fermented foodstuffs will change during fermenting and ripening, due to changes in ingredients, is large. In this study, we assessed the functional characteristics of SS made with 24 medicinal herbs – including *Angelica gigas* – during processing, including changes in nitrogen (N)-containing compounds and isoflavone content, antioxidant and superoxide dismutase (SOD)-like activity, angiotensin-converting enzyme (ACE) inhibitory activity and nitrite (NO) radical scavenging activity.

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MATERIALS AND METHODS

Materials

Soybean (*Glycine max* L., a Korean cultivar 'Daewon', genetically unmodifeied) used in this study was harvested in 2000 from Buyeo, Chungcheongnam-do, Korea. 24 dried medicinal herbs (*Angelica gigas*, *Cnidium officinale*, *Paeonia obovata*, *Rehmannia glutinosa***,** *Chaenomeles sinensis* Koehne, Omae *Prunus mume*, *Polygala japonica* Houtt, *Acorus graminei* Rhizoma, *Aralia contientalis* L., *Ostericum koreanum* Kitagawa, *Atractylodes japonica* Koidzumi, *Astragalus adsurgens*, *Ledouriella seseloides*, *Platycodon grandiflorum*, *Angelica decursiva*, *Angelicae dahuricae*, *Aurantii nobilis* Pericarpium, *Melia azedarach* L., *Pueraria thunbergiana*, *Paeonia suffruticosa* Andr. *Glycyrrhiza glabra*, *Poncirii fructus*, *Alpinia officinarum*, *Bupleurum falcatum* L.) were purchased from a medicinal herb market in Geumsan, Chungcheongnam-do, Korea. Refined salt (99.9%) was purchased from Hanju Salt (Hanju Co. Ltd, Ulsan, Geongsangnam-do, Korea). All chemicals (including daidzin, genistin and their aglycones) and reagents for verification of physiological characteristics were purchased from Aldrich Chemical Co. (Milwaukee, WI, USA) and Sigma Chemical Co. (St. Louis, MO, USA).

Preparation of medicinal herb SS (MHSS)

To investigate the quality characteristics of MHSS, it was prepared as follows, in a four-step process, and ripened for 10 years.

Step 1: Transforming soybean into meju

Soybean (10 kg) was soaked for 12 h in water (18°C) then boiled at 115°C for 40 min. Boiled soybean was crushed with a pestle and then shaped into cubes (20 × 20 × 15 cm) by hand using a mold. The surface of cubes was lightly dried for 1-2 days at 30°C. The resulting cube-shaped soybean is termed *meju*.

Step 2: Fermenting meju

Meju was fermented for 1 month on a shelf on top of clean rice straw in a fermentation room (25 ± 2°C, 70 ± 5% RH) and fermented *meju* was dried in the sun. Dried *meju* was ripened for 2 months in a fermentation room.

Step 3: Soaking meju in salted solution (preparation of MHSS)

10 kg of *meju* was soaked in a clay jar with 20 kg of 18-20% NaCl and 500 g of a mix of 24 medicinal herbs (i.e., 5% herbs: *meju*, w/w) were placed in a cotton bag and soaked in a clay jar. After this salted solution was fermented for 45 days, *meju* and the medicinal herb bag were taken out of the salted solution. This salted solution was named MHSS.

Step 4: Ripening of MHSS

MHSS was ripened under unsterilized conditions for 10 years at room temperature. An MHSS sample was taken in May of every year. MHSS samples were preserved at -70°C in a refrigerator until analysis.

Determination of nitrogen compounds

Total N content of MHSS was determined according to the micro Kjeldahl method (AOAC 1995). Peptide-, formol- and amino-type N content were determined according to the methods of the Korea Food and Drug Administration (2000).

Determination of isoflavones

The isoflavone content of MHSS was determined according to the method of Coward *et al*. (1993). Each MHSS sample was diluted with 80% aqueous methanol and filtered with a syringe filter (0.45 µm, Millipore Co., Bedford, MA, USA) for HPLC analysis. Reversed-phase HPLC analysis was carried out with an Agilent 1200 series system (Agilent Technologies, Santa Clara, CA, USA), using ZORBAX SB C-18 (4.6 × 250 mm, 5 µm, Agilent Technologies). The mobile phase was composed of 0.1% acetic acid in acetonitrile (solvent A) and 0.1% acetic acid in water (solvent B). Following the injection of 10 µL of sample, solvent A was increased from 15 to 35% over 50 min, and then held at 35% for 10 min. The solvent flow rate was 1 mL/min and the eluted isoflavones were detected at 254 nm. The quantitative data for daidzin, genistin, and their aglycones were obtained by comparison to known standards.

Determination of DPPH radical scavenging activity

The DPPH (2,2-diphenyl-1-picryl hydrazyl) radical scavenging activity of MHSS was measured according to the method of Blois (1958) with some modifications. A 0.9-mL aliquot of 0.2 mM DPPH ethanol solution was mixed with 0.1 mL of diluted MHSS. The mixture was then shaken vigorously and left to stand for 10 min under subdued light. The absorbance was measured at 525 nm using a spectrophotometer (Spectronic Genesys^{TM}2PC, Spectronic Instruments, Rochester, NY, USA).

$$\text{Radical scavenging activity (\%)} = (1 - A_{sample}/A_{control}) \times 100$$

where A_{sample} is the absorbance in the presence of sample and $A_{control}$ is the absorbance in the absence of sample.

Determination of SOD-like activity

SOD-like activity was measured according to the method of Marklund *et al*. (1974). 20 mL of diluted MHSS (1 mL MHSS + 99 mL distilled water) was added to 55 mM Tris-cacodylic acid buffer (TCB, pH 8.2) to pH 8.2 and was homogenized (2 min, Ultra-Turrax T25, IKA Labortechnik Co., Staufen, Germany), and filtered through Toyo No. 2 filter paper, and then filled up to 50 mL with 55 mM TCB (pH 8.2). 950 µL of this solution was mixed with 50 µL of 24 mM pyrogallol. The mixture was left to stand for 2 sec under subdued light. The absorbance was measured at 420 nm using a Spectronic Genesys^{TM}2PC spectrophotometer. SOD-like activity was expressed as % absorbance compared to the blank.

ACE inhibitory activity

ACE inhibitory activity was measured according to the method of Cushman *et al*. (1971). 50 µL of diluted MHSS was mixed with 50 µL of ACE solution and 10 µL of 10 mM sodium borate buffer (pH 8.3). The mixture was then shaken and pre-incubated for 5 min at 37°C in a shaking incubator (HB-201SF, HANBACK Science Technology, Bucheon, Korea) to which 50 µL of hippuryl-histidyl-leucine solution (HHL, 27 mg/25 mL in sodium borate buffer) was added, and then reacted for 30 min at 37°C in an incubator. To the reacted solution 250 µL of 1 N HCl was added to complete the reaction after which 1.5 mL of ethyl acetate was added and vortexed for 15 sec using a vortex-mixer (KMC-1300V, Vision Co. Ltd., Bucheon, Korea) and centrifuged (2100 × *g*, UNION 32R, Hanil Co., Incheon, Korea). 1 mL of the upper part of the centrifuged solution was dried using a Temp-Blok model heater and then dissolved with 3 mL of distilled water. The absorbance was measured on a Spectronic Genesys^{TM}2PC at 228 nm. ACE inhibitory activity was expressed as % absorbance compared to the blank.

Nitrite (NO) radical scavenging activity

NO radical scavenging activity was measured according to the method of Gray *et al*. (1975). 1 mL of diluted MHSS was mixed with 1 mL of 1 mM $NaNO_2$ and adjusted to pH 1.2, 3.0, 4.2 and 6.0 using 0.1 N HCl and 0.2 M citrate buffer solution, respectively, and then filled up to 10 mL. This solution was reacted for 60 min at 37°C. 1 mL of each pH solution was mixed with 5 mL of 25% acetic acid and 0.4 mL of Griess reagent (solution mixed with equal amounts of 1% sulfanilic acid and 1% naphthylamine) and left to stand for 15 min at room temperature. The absorbance was measured at 520 nm. NO radical scavenging activity was expressed as a percentage using the following formula:

NO scavenging activity (%) = {1-(absorbance of 1 mM $NaNO_2$ added sample) / absorbance of 1 mM $NaNO_2$} × 100.

Statistical analysis

All experimental data were analyzed by analysis of variance (one-way ANOVA) and significant differences among the means from triplicate analysis was determined by Duncan's multiple range test using SPSS 12.0 for Windows (SPSS Inc., Chicago, IL) at $P < 0.05$.

RESULTS AND DISCUSSION

Composition of nitrogen compounds

The composition of the N compounds of MHSS during the ripening period is presented in **Table 1**. The peptide-N content in MHSS increased up until 5 years then decreased. Formol- and amino-N content gradually increased up until 10 years. Thus, total N content increased up until 5 years, after which no significant differences were observed. However, among those different forms of N compounds, the increase in formol-N content was greatest, namely to 1.04% (after 10 years) from 0.84% (unripened). Joo *et al*. (1997) reported that the total N compound content increased as the ripening period of SS increased, as in our study, from 1.008% (unripened state) to 1.829% (after 2 years).

Isoflavones composition

The isoflavone composition of MHSS during the ripening period is presented in **Table 2**. The major isoflavones of MHSS were daidzin, genistin and genistein, accounting for 128.7~130.7, 113.7~128.5 and 96.8~104.6 µg/g, respectively. Only small amounts of daidzein (32.2~53.0 µg/g) were detected. Daidzin and genistin were primarily found in free form while genistein was found in conjugated form. As the ripening period of MHSS increased, there were no changes in the daidzin, a type of isoflavone. After ripening MHSS for 10 years, the free form of daidzein increased 2-fold while the conjugated form of genistin decreased by 30%. Total isoflavone content of MHSS was higher than in general SS. The results of these investigations suggest that more isoflavones in MHSS may have been due to the addition of medicinal herbs such as *Pueraria thunbergiana* which contain high levels of isoflavones (Oh *et al*. 1990), for example 0.73% daidzin and 0.58% purarin following the processing of MHSS. Isoflavone content in this study was higher than that detected by Lee *et al*. (2006), in which isoflavone content of soybean *meju* only was 20.53 µg/g. SS isoflavones differ in content depending on the processing method and are sensitive to the addition of salty water and the amount of SS processing; the more salt water that is added to *meju*, the less the isoflavone content, and the shorter the ripening period; the longer the ripening period, the higher the isoflavone content.

DPPH radical scavenging activity

The DPPH radical scavenging activity of MHSS and commercial antioxidant BHA during the different ripening periods are compared in **Table 3**. The radical scavenging activity of MHSS ripened for 1 year showed 8.6 and 96.2% DPPH radical scavenging activity at 0.1 and 5 mg/mL, respectively. By increasing the ripening period, the DPPH radical scavenging activity of MHSS increased, showing 100% scavenging activity at 5 mg/mL after 2-year ripening, while only 67.4% scavenging activity was observed with 5 mg/mL of general SS. The IC_{50} value of SS ripened for 10 years was 0.092 mg and similar to BHA activity (0.104 mg). A possible reason for the high radical scavenging activity of MHSS might be due to the addition of medicinal herbs, which contain polyphenolic compounds, and the formation of novel compounds such as Maillard reaction products with increasing ripening period (Moon *et al*. 1987; Moon 1991; Cheigh *et al*. 1993; Manzocco *et al*. 2001); the important antioxidative characteristics of SS are due to its higher concentration of N and melanoidin. Yamaguchi *et al*. (1981) reported that browning materials (i.e., melanoidins) produced by the xylose and glycine reaction have antioxidative activity, too. Thus, the main antioxidative activity of MHSS was not only due to the presence of N and melanoidin, but also because medicinal herbs were added.

Determination of SOD-like activity

The SOD-like activity of MHSS during different ripening periods and the commercial antioxidant BHA is compared in **Table 4**. SOD-like activity of 1-year-old ripened MHSS had no SOD-like activity: 0.3, 1.4, 2.1 and 6.2% at 0.1, 0.5,

Table 1 Changes of nitrogen compounds content during ripening period of MH soysauce (SS) during ripening periods. Values in g/100 g sample.

Nitrogen compound	MH SS (Ripening periods, years) 1	2	3	4	5	6	7	8	9	10	General SS 2
Peptide N	0.45 ± 0.12	0.48 ± 0.16	0.52 ± 0.15	0.56 ± 0.15	0.59 ± 0.17	0.52 ± 0.20	0.51 ± 0.20	0.49 ± 0.17	0.45 ± 0.22	0.43 ± 0.22	0.49 ± 0.16
Formol N	0.84 ± 0.20	0.95 ± 0.22	0.98 ± 0.21	0.97 ± 0.22	0.97 ± 0.21	1.00 ± 0.22	1.02 ± 0.21	1.03 ± 0.22	1.03 ± 0.21	1.04 ± 0.20	0.93 ± 0.20
Amino N	0.51 ± 0.17	0.54 ± 0.20	0.59 ± 0.22	0.60 ± 0.20	0.61 ± 0.18	0.64 ± 0.20	0.65 ± 0.20	0.66 ± 0.18	0.67 ± 0.16	0.68 ± 0.17	0.48 ± 0.14
Total N	1.80 ± 0.18	1.97 ± 0.21	2.09 ± 0.20	2.13 ± 0.20	2.17 ± 0.19	2.16 ± 0.21	2.18 ± 0.20	2.18 ± 0.20	2.15 ± 0.20	2.15 ± 0.20	1.90 ± 0.17

MH soysauce: soysauce made by adding medicinal herb. All values are mean ± SD of triplicate determinations. Means with the same letter are not significantly different by Duncan's multiple range test at $P < 0.05$.

Table 2 Changes of free and conjugated type isoflavones content (µg/g, dry basis) of MH soysauce during ripening period.

Sample	Ripening Periods (Years)	Daidzin Free type	Daidzin Conjugated type	Daidzein Free type	Daidzein Conjugated type	Genistin Free type	Genistin Conjugated type	Genistein Free type	Genistein Conjugated type	Total
MH soysauce	1	86.2 ± 1.3	44.2 ± 0.8	14.5 ± 1.5	17.7 ± 0.7	76.2 ± 2.6	52.3 ± 1.7	23.2 ± 0.9	73.6 ± 1.5	387.9 ± 9.2
	2	87.5 ± 1.4	42.5 ± 0.9	22.6 ± 1.6	20.4 ± 1.2	77.5 ± 2.2	48.7 ± 1.8	24.2 ± 1.1	74.3 ± 1.6	397.7 ± 10.3
	3	87.6 ± 0.9	43.4 ± 0.8	23.4 ± 1.4	22.6 ± 1.1	77.6 ± 2.4	46.0 ± 1.4	25.0 ± 1.2	76.5 ± 1.6	402.1 ± 8.7
	4	88.6 ± 1.0	43.6 ± 0.8	24.6 ± 1.2	23.4 ± 0.6	78.6 ± 1.9	42.1 ± 1.5	25.8 ± 0.8	75.4 ± 1.4	402.1 ± 9.6
	5	87.3 ± 0.8	42.5 ± 0.7	26.5 ± 1.3	24.2 ± 0.7	77.3 ± 2.0	38.5 ± 1.9	26.1 ± 0.9	74.6 ± 1.2	397.0 ± 8.4
	6	86.5 ± 0.6	42.2 ± 0.5	27.2 ± 1.4	23.5 ± 1.0	77.5 ± 1.8	38.0 ± 1.6	27.0 ± 0.7	75.3 ± 1.3	397.2 ± 7.7
	7	87.4 ± 0.5	43.0 ± 0.7	27.8 ± 1.2	24.6 ± 0.9	77.4 ± 1.7	39.0 ± 1.4	27.4 ± 0.8	77.2 ± 0.8	403.8 ± 8.0
	8	87.2 ± 0.5	42.5 ± 0.6	28.0 ± 1.0	24.2 ± 1.0	77.2 ± 1.7	37.1 ± 1.5	27.6 ± 0.6	75.6 ± 0.9	399.4 ± 6.8
	9	88.0 ± 0.4	42.7 ± 0.7	28.2 ± 1.0	25.0 ± 0.7	77.0 ± 1.8	37.5 ± 1.7	27.8 ± 0.7	75.2 ± 0.6	401.4 ± 7.1
	10	86.8 ± 0.3	42.6 ± 0.8	28.5 ± 0.9	24.5 ± 0.8	76.8 ± 1.7	36.9 ± 1.3	28.0 ± 0.7	72.6 ± 1.0	396.7 ± 6.9
General soysauce	2	75.8 ± 0.7	42.1 ± 0.3	13.4 ± 1.2	12.3 ± 0.8	75.8 ± 2.0	44.6 ± 1.4	22.5 ± 0.8	71.6 ± 1.1	358.1 ± 8.9

MH soysauce: soysauce made by adding medicinal herb. All values are mean ± SD of triplicate determinations. Means with the same letter are not significantly different by Duncan's multiple range test at $P < 0.05$.

Table 3 DPPH radical scavenging activity of MH soysauce during ripening period. (Units: %)

Conc. (mg/mL)	MH soysauce (Ripening period, years)										General soysauce	BHA
	1	2	3	4	5	6	7	8	9	10	2	
0.1	8.6 ± 2.3 g	11.9 ± 1.8 g	23.2 ± 0.8 fg	28.6 ± 0.8 f	30.5 ± 0.7 f	36.8 ± 0.9 e	43.5 ± 0.8 de	48.0 ± 1.0 de	52.1 ± 0.7 d	56.4 ± 0.9 d	10.5 ± 2.3 g	46.8 ± 2.1 de
0.5	32.4 ± 3.5 b	42.9 ± 2.4 de	53.4 ± 1.3 d	66.1 ± 0.9 cd	72.4 ± 1.0 c	77.9 ± 1.5 c	83.8 ± 1.2 c	87.7 ± 0.9 bc	92.5 ± 1.1 b	97.6 ± 1.0 a	22.3 ± 3.1 fg	98.3 ± 1.0 a
1	52.1 ± 0.9 d	68.7 ± 1.1 cd	78.6 ± 0.7 c	92.3 ± 0.6 b	95.8 ± 0.9 a	96.8 ± 0.8 a	97.0 ± 1.0 a	97.7 ± 0.8 a	98.1 ± 0.2 a	99.6 ± 0.2 a	36.4 ± 2.7 e	100 ± 0.0 a
5	96.2 ± 0.2 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	67.4 ± 0.5 cd	100 ± 0.0 a

MH soysauce: soysauce made by adding medicinal herb. All values are mean ± SD of triplicate determinations. Means with the same letter are not significantly different by Duncan's multiple range test at $P < 0.05$.

Table 4 SOD-like activity of MH soysauce during ripening period. (Units: %)

Conc. (mg/mL)	MH soysauce (Ripening period, years)										General soysauce	BHA
	1	2	3	4	5	6	7	8	9	10	2	
0.1	0.3 ± 0.2 j	1.2 ± 0.5 j	3.4 ± 0.7 j	4.6 ± 1.1 ij	5.4 ± 0.8 ij	7.2 ± 0.9 i	8.4 ± 0.8 i	9.5 ± 1.0 i	11.2 ± 1.7 i	15.7 ± 1.9 hi	0.0 ± 0.0 j	21.3 ± 2.1 h
0.5	1.4 ± 0.5 j	3.9 ± 0.7 jb	10.5 ± 1.1 c	9.8 ± 0.8 i	12.3 ± 1.2 i	17.6 ± 1.0 hie	20.4 ± 1.3 h	25.0 ± 1.9 fg	26.3 ± 1.4 fg	30.1 ± 1.7 f	1.1 ± 0.6 j	44.2 ± 1.0 de
1	2.1 ± 0.9 j	8.9 ± 0.9 i	19.6 ± 0.9 h	20.2 ± 0.6 h	24.1 ± 0.6 fg	26.7 ± 0.8 fg	28.1 ± 1.1 f	29.8 ± 0.7 f	32.2 ± 0.8 f	48.9 ± 0.8 d	1.6 ± 1.1 j	92.3 ± 0.7 a
5	6.2 ± 0.2 j	10.8 ± 1.2 b	38.2 ± 0.5 e	43.5 ± 1.0 ded	52.6 ± 0.7 d	65.3 ± 0.4 cd	70.8 ± 2.0 c	76.7 ± 1.6 b	78.1 ± 0.7 b	82.5 ± 0.5 b	7.2 ± 0.7 i	96.5 ± 0.3 a

MH soysauce: soysauce made by adding medicinal herb. All values are mean ± SD of triplicate determinations. Means with the same letter are not significantly different by Duncan's multiple range test at $P < 0.05$.

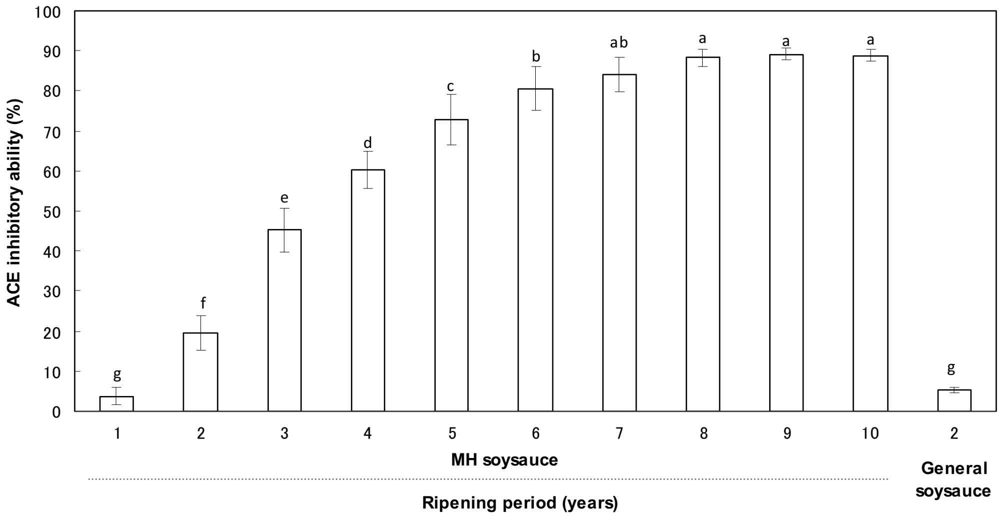

Fig. 1 ACE inhibitory ability of MH soysauce during the ripening period. Data are mean ± SD (n = 3). Values with the same superscript are not significantly different by Duncan's multiple range test at $P < 0.05$. MH soysauce was diluted 100-times.

1.0 and 5.0 mg/mL, respectively. However, SOD-like activity of MHSS increased as ripening period increased: 82.5% activity at 5 mg/mL after 10 years' ripening. The activity was less than that of BHA, but the activity after 10-years' ripened MHSS was higher than that after 1 year ripening. SOD is an enzyme that produces hydrogen peroxide when it reacts with a superoxide radical. The hydroxyl radical is an extremely reactive free radical formed in biological systems, and has been implicated as a highly damaging species in free radical pathology, capable of damaging the biomolecules of living cells (Sohal *et al.* 1989; Yang *et al.* 2011). Thus, suppression of the formation of reactive oxygen species can ultimately prevent or delay diseases caused by oxidative stress in the body. Thus, MHSS, which has SOD-like activity, could be used for preventing diseases related with oxidative stress and enhancing health.

ACE inhibitory activity

The antihypertensive ACE inhibitory activity of MHSS during different ripening periods is presented in **Fig. 1**. This activity increased significantly from 3.8 ± 1.1% in MHSS to 88.9 ± 1.5% after 7-years' ripening; in general SS ripened for 1 year, the value was 1.4 ± 0.6%. However, these values did not change significantly after ripening for 10 years. The ACE inhibitory ability of MHSS ripened for 9 years peaked at 89.1 ± 1.5%. Back *et al.* (2010) reported that soybean isolate protein hydrolysate had effective ACE inhibitory ability (IC_{50} = 79.94 μg/mL). Many peptides have an antihypertensive effect (Do *et al.* 2006) while peptides in salt-free SS have ACE inhibitory ability (Zhu *et al.* 2008). Seo *et al.* (1994) reported that the main materials of ACE inhibitor obtained from *deonjang* were a kind of amino acid; similarly, protein hydrolysate had an antihypertensive effect. In this study, MHSS made from soybean was hydrolyzed to peptides and amino acids during fermentation and ripening periods. We thus assume that this would explain why MHSS has increasing ACE inhibitory activity as ripening period increased.

Nitrite radical scavenging activity

Nitrite reacts with amines in protein-rich foods, medicines and residual pesticides and produces nitrosamine, which converts to diazoalkane, a protein, and intracellular components, which can increase the risk of cancer (Beckman *et al.* 1996). **Table 5** shows the nitrite-scavenging effect of MHSS during the ripening period under different pHs: 1.2, 3.0, and 6.0. Generally, nitrite radical scavenging activity was high at low pH, and this study has a similar to results, too. One-year-old MHSS had low nitrite scavenging activity at all pHs: 6.7 and 10.9% (pH 1.2), and 1.8 and 3.2% (pH 6.0) at 1.0 and 5.0 mg/mL, respectively. However, by in-

Table 5 Nitrite scavenging effects of MH soysauce during ripening period under different pH condition. (Units: %)

Conc. (mg/mL)	pH	MH soysauce (Ripening period, years)										General soysauce
		1	2	3	4	5	6	7	8	9	10	2
1.0	1.2	6.7 ± 1.0 a	12.4 ± 1.2 b	18.7 ± 1.5 c	20.1 ± 0.9 c	25.5 ± 1.1 d	32.7 ± 1.4 e	35.6 ± 1.0 f	34.9 ± 0.9 f	35.1 ± 1.1 f	36.2 ± 1.2 f	5.2 ± 0.7 a
	3.0	3.1 ± 0.8 a	5.2 ± 0.1 b	6.9 ± 1.4 c	7.4 ± 1.1 cd	7.9 ± 1.0 d	8.3 ± 0.8 de	9.2 ± 0.9 e	9.2 ± 1.1 e	9.6 ± 1.2 e	9.5 ± 0.9 e	2.3 ± 0.5 a
	6.0	1.8 ± 0.6	2.0 ± 0.7	2.8 ± 0.5	3.7 ± 0.3	4.5 ± 0.5	4.4 ± 0.6	4.4 ± 0.4	4.6 ± 0.5	4.5 ± 0.8	4.7 ± 0.6	0.2 ± 0.1
5.0	1.2	10.9 ± 0.8 a	19.2 ± 1.1 b	29.3 ± 0.9 c	37.8 ± 1.0 d	46.5 ± 0.8 e	52.8 ± 0.8 f	57.6 ± 0.9 g	59.3 ± 0.8 g	59.4 ± 0.6 g	60.1 ± 0.6 g	10.4 ± 0.6 a
	3.0	5.6 ± 0.6 a	7.5 ± 0.7 b	9.8 ± 0.7 c	12.2 ± 0.6 d	15.1 ± 0.5 e	17.6 ± 0.4 f	19.2 ± 0.5 g	20.3 ± 0.5 gf	21.9 ± 0.4 f	22.4 ± 0.4 f	4.8 ± 0.4 a
	6.0	3.2 ± 0.2	4.2 ± 0.5	5.0 ± 0.2	5.8 ± 0.2	6.5 ± 0.3	7.6 ± 0.1	8.4 ± 0.1	9.1 ± 0.2	9.5 ± 0.1	9.6 ± 0.1	2.2 ± 0.2

MH soysauce: soysauce made by adding medicinal herb. All values are mean ± SD of triplicate determinations. Means with the same letter are not significantly different by Duncan's multiple range test at $P < 0.05$.

creasing the ripening period, the nitrite scavenging activity of MHSS increased: 60.1% at pH 1.2, at 5 mg/mL after 10-year ripening. Thus, at pH 1.2, the pH in the human stomach, this should reduce the creation of nitrosamine, which is a carcinogen (Choi *et al*. 2008).

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