Changes in Antioxidant Components and Antioxidant Potentials of Soybean During *Monascus*-Fermentation

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Abstract

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This study was designed to investigate the changes in antioxidant components and the *in vitro* antioxidant activities of 80% ethanolic extracts from soybeans fermented with *Monascus pilosus* 60084. White and black soybeans were incubated during 45 days at 27 ℃ and collected at 5 days intervals of *Monascus*-fermentation. The concentrations of mevinolin, CoQ10, and tocopherol were analyzed by HPLC. The total phenols, total flavonoids, total carotenoids, and proanthocyanidins were determined by spectrophotometry. The highest contents of mevinolin (568.18 μg/ g dry weight, DW) and CoQ10 (65.59 μg/ g DW) were obtained from *Monascus*-fermented white soybean after 20 days of fermentation at

27℃. The highest yields of total flavonoids (0.57 mg catechin equivalents, CE/g DW), total phenols (10.49 mg gallic acid equivalents, GAE/g DW) and proanthocyanidins (15.31 mg CE/g DW) were obtained from white soybean at 35 days and 40 days of *Monascus*-fermentation, respectively. The maximum yield of total tocopherol (312.87 μg/g DW) was produced in unfermented white soybean. The highest production of total carotenoids (25.42 μg β-carotene equivalents, BCE/g DW) was obtained from black soybean at 45 days of fermentation. All fermented soybeans showed antioxidant potential (0.77-3.79 mg Trolox equivalents, TE/g DW), which was measured by radical scavenging activity and the reducing power. A strong correlation ($p<0.01$) was found between contents total phenolic ($r^2=0.847$), CoQ10 ($r^2=0.822$) and the antioxidant parameters. This suggest that total phenolic and CoQ10 contents are likely significant contributors to the antioxidant capacity of *Monascus*-fermented soybeans. The results indicate that *Monascus*-fermentation have great potential for the enrichment of natural antioxidants such as CoQ10 and phenolic compounds in soybeans.

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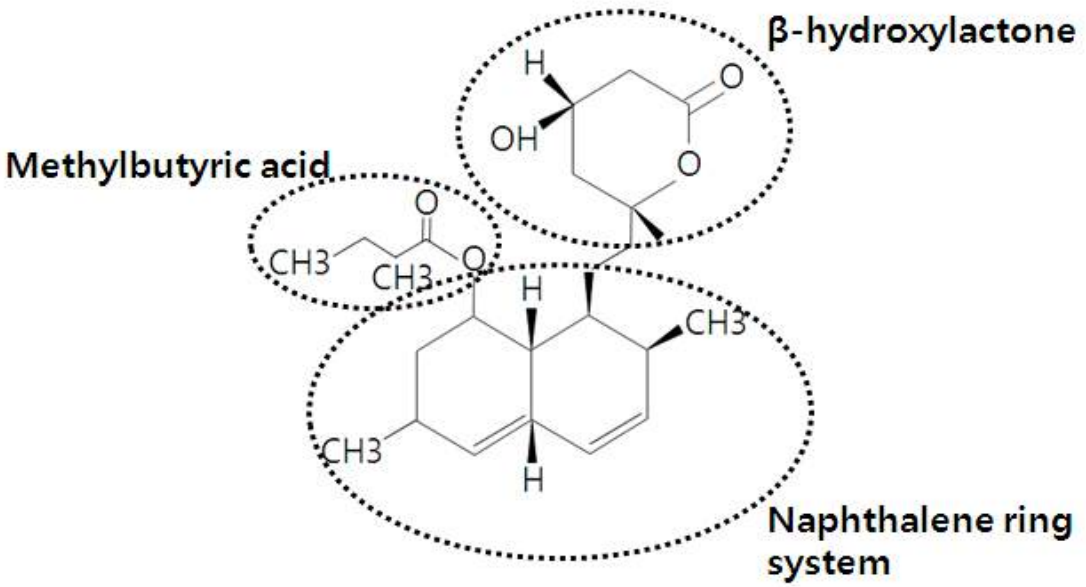
Ⅰ. Introduction

Coronary heart disease (CHD) is a disease occurring due to the atherosclerosis that narrowing of coronary arteries which supply blood to the hear(1). CHD can occur when the coronary arteries are blocked or narrowed partly insufficient supply of oxygen and nutrients to the heart. If CHD happens, angina pectoris can occur that cause chest pain(1). Coronary heart disease (CHD) is the largest cause of death in developed countries and one of the main cause of disease burden in developing countries(2). According to the mortality rate data of Korea in 2012 of Statistics Korea(3), circulatory system diseases including heart disease and cerebrovascular diseases is one of the leading causes of death accounting for 22 % of the total mortality that is almost 1/4 of the total mortality rate. WHO (The World Health Organization) predicted that 23 million people will die from CVD in 2030(4). Hypercholesterolemia is a major cause of coronary heart disease and the human death(5).

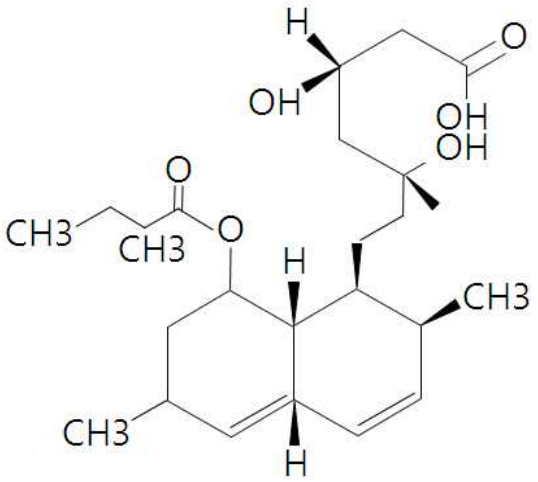
Genus *Monascus* fungi are classified as Monascaceae belongs to Hemiascomycetes and about 70 strains of *Monascus* are separated and identified(6). *Monascus* sp. fermented rice, known as red yeast rice and red koji, has traditionally been used as a natural coloring agent in wine, meat, and fish and flavoring agent, and food preservative in East Asia(7). In the Botanical list, red yeast rice helps digestion and blood flow and make internal organs stronger(6). Also *Monascus* sp. produce mevinoline (lovastatin, monacolin K) which is secondary metabolites(8, 9). Mevinolin is

3-hydroxy-3-methylglutaryl co-enzyme A (HMG-CoA) reductase inhibitor(10) that inhibits mediators of cholesterol biosynthesis by inhibiting and competitively binding the rate limiting enzyme HMG-CoA of the mevalonate pathway(11, 12). Mevinolin is a secondary metabolite biosynthesis by *Aspergillus, Monascus* and *Penicillium* via the polyketide pathway(8, 13). Naphthalene ring system, β-hydroxy lactone and Methylbutyric acid were contained in mevinolin(13) (Fig. 1). The physiologically active form is the β-hydroxy acid which is hydrolyzed of the lactone ring(8). Most of the mevinolins are produced by mixing in the lactone and β-hydroxyacid form(13). The mevinolin family comprises lovastatin, pravastatin, mevastatin and simvastatin, which are fungal derivatives, and atorvastatin, fluvastatin, cerivastatin, rosuvastatin and pitavastatin, which are synthetic compounds(14). Mevinolin therapy reduce the formation of LDL-cholesterol and up-regulate LDL receptor activity, lowering triglycerides and LDL- cholesterol and increasing HDL-cholesterol(15). So mevinolin is used to lower the blood cholesterol in patients who have cardiovascular diseases or to prevent cardiovascular events(16). Recent, some experimental reports manifested a potential anti-cancer activity of mevinolin(17, 18). Also, reduction in the size of lipoma was observed for mevinolin treat. However mevinolin affects the biosynthesis of ubiquinone (CoQ, Coenzyme Q) which share the same biosynthetic pathway with cholesterol until farnesyl pyrophosphate(19). The most frequent and serious side effects of mevinolin are a variety of myopathy ranging from mild myalgia to fatal rhabdomyolysis(11). In general, 5~10% of patients receiving treatment of mevinolin complain muscle symptom(10). Although the underlying mechanisms of

mevinolin-associated myopathy are not entirely elucidated, the most popular theory for mevinolin myopathy is CoQ10 deficiency(10).



Mevinolin lactone



Mevinolinic acid

Fig. 1. Chemical structure of mevinolin lactone and mevinolinic acid

Coenzyme Q10 (CoQ10) is a naturally occurring lipid-soluble compound (20) and present a small amount in all tissues and cells(21). CoQ10 found in humans is composed of a substituted benzoquinone moiety attached to 10 isoprenoid units(22). CoQ10 plays an important role in energy production and is an essential carriers of the electron transport system in mitochondria(19). Also CoQ10 has a role of lipid-soluble antioxidant(23). CoQ10 can be exists in a oxidized (ubiquinone-10) or a reduced (ubiquinol-10) form(24) (Fig. 2). And $CoQH_2$ (ubiquinol) reduces the initiating perferryl radical, with formation of ubisemiquinone and H_2O_2(25). Additionally, $CoQH_2$ eliminates LOO• directly(25). CoQ10 is endogenously synthesised via the mevalonate pathway, and some is obtained from the diet(23, 24). But, CoQ10 share the same biosynthetic pathway with cholesterol until farnesyl pyrophosphate(19). Thus, mevinolin decrease both cholesterol and CoQ10 biosynthesis(19). Nowaday, CoQ10 is widely approved as a potent antioxidant dietary supplement linked with immune enhancement, energy boosting, and ease of cardiovascular disease(26).

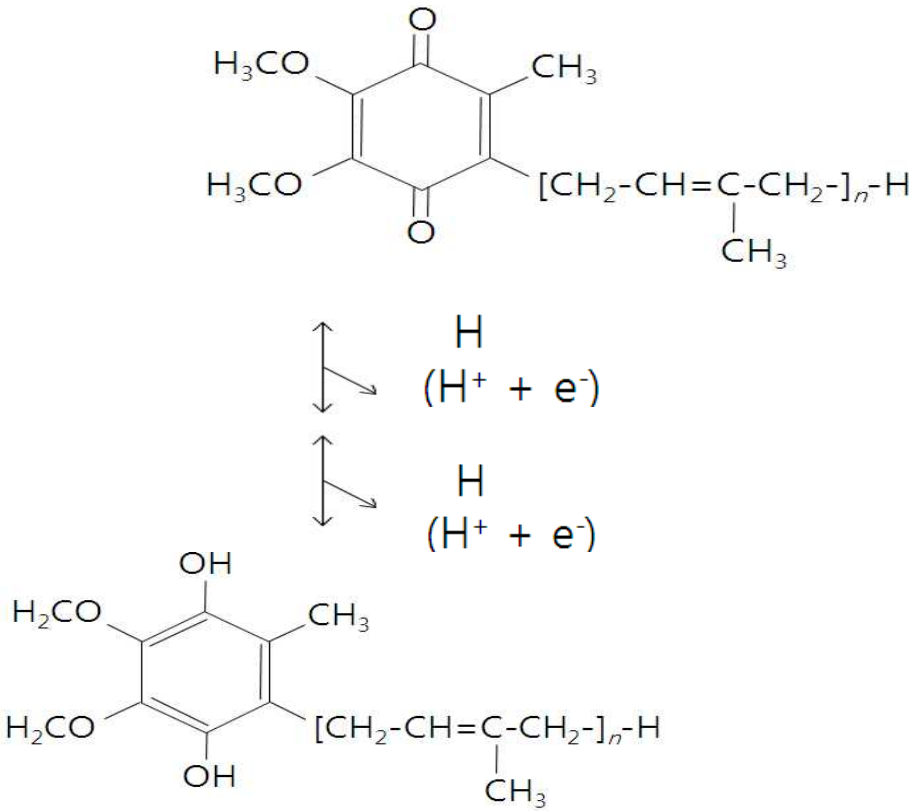
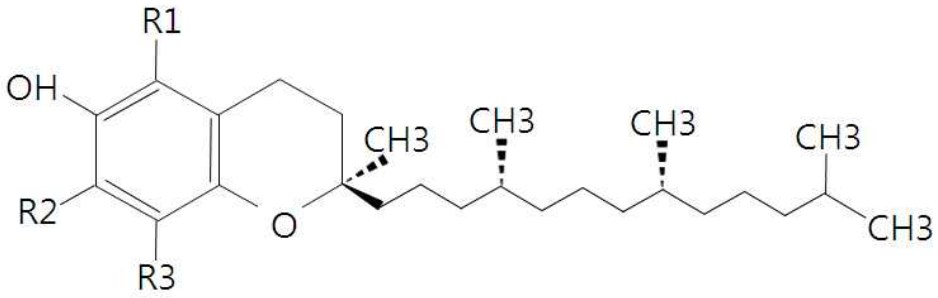


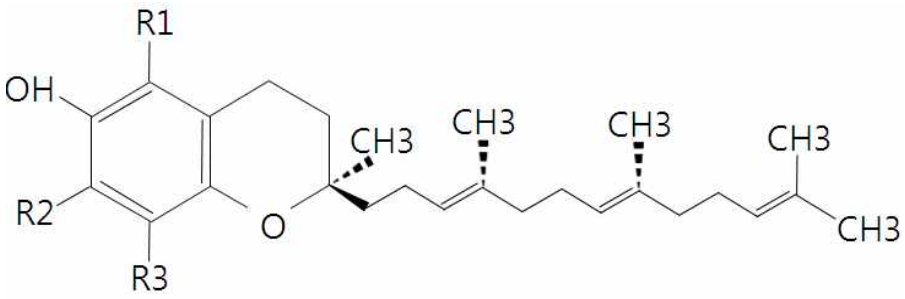
Fig. 2. Chemical structure of reduced (ubiquinol) and oxidazed (ubiquinone) coenzyme Q

Vitamin E consists of α-, β-, ɣ-, and δ-tocopherols and four unsaturated derivative α-, β-, ɣ-, δ-tocotrienols(27). Tocopherols and tocotrienols contain a hydrophobic phytyl side chain and a chromanol ring but there are difference in hydrophobic side chain(28, 29). Tocopherols have a saturated phytyl side chain (Fig. 3) while tocotrienols have unsaturated hydrocarbon tails (Fig. 4) in each of 3 isoprene units(29). Among the 4 isomers of tocopherol, it is α-tocopherol that has the most biologically active(27, 30). Tocopherols are synthesized in photosynthetic organisms and plants(27), but not synthesized in human body(31). So human must intake vitamin E by foods and supplements(31). Vitamin E is a strong chain-breaking lipid-soluble antioxidant that helps protect membrane from the free radicals that can induce potentially damaging of the body's metabolism(27, 32, 33). Typical functions of the vitamin E is well known to role of suppress the damage of lipid caused by active oxygen species(34) and also have anti-cancer effect(35, 36). The ranking of tocopherols content in soybean was ɣ> δ> α that reported by Murphy et al.(30) who showed the tocopherol ranking in 5 kinds of soybean. This result corresponded to the report by Ko et al.(37).



Homologues	R1	R2	R3
α-tocopherol	CH_3	CH_3	CH_3
β-tocopherol	CH_3	H	CH_3
γ-tocopherol	H	CH_3	CH_3
δ-tocopherol	H	H	CH_3

Fig. 3. Chemical structure of α, β, γ, δ-tocopherols



Homologues	R1	R2	R3
α-tocotrienol	CH_3	CH_3	CH_3
β-tocotrienol	CH_3	H	CH_3
γ-tocotrienol	H	CH_3	CH_3
δ-tocotrienol	H	H	CH_3

Fig. 4. Chemical structure of α, β, γ, δ-tocotrienols

The phenolic compounds are ubiquitous in all plants which are metabolites formed to protect themselves from photosynthetic stress, reactive oxygen species, cuts and herbivores(38, 39). The phenolic compounds contain more than one unit or building block(40). Phenolic compounds generally possess a common structural feature that contain an aromatic ring with more than one hydroxy substituents(41, 42). Most of the phenolic compounds in whole cereal are in the insoluble bound forms(43) and plant phenols are synthesized from carbohydrates through the shikimate pathway and phenylpropanoid metabolism(44, 41). Phenolic compounds contain over 8000 different known structures(45). But usually, phenolic compounds can be divided into ten types according to their basic structure: simple phenols, hydroxycinnamic acids, phenolic acids, coumarins and isocoumarins, xanthones, naphthoquinones, stilbenes, flavonoids, anthraquinones and lignins(46) (Fig. 5 & Table 1). The phenolic compounds have some physiological activities such as antioxidant activity(47), blood pressure decrease effect(48), preventing cardiovascular diseases, chronic inflammation, cancer and diabetes(43).

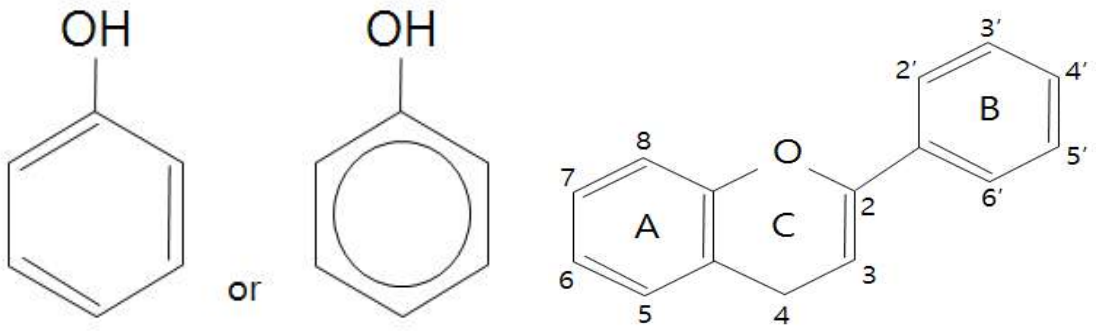


Fig. 5. Phenol, the simplest phenoilc compound and flavonoid skeleton

Table 1. The most important groups of plant phenolic compounds

Number of C-atoms	Basic skeleton	Class
6	C_6	simple phenols, benzoquinones
7	$C_6 - C_1$	phenolic acids
8	$C_6 - C_2$	acetophenone, phenylacetic acid
9	$C_6 - C_3$	hydroxycinnamic acid, polypropene, coumarin, isocoumarin
10	$C_6 - C_4$	naphtoquinone
13	$C_6 - C_1 - C_6$	xanthone
14	$C_6 - C_2 - C_6$	stibene, anthrachinone
15	$C_6 - C_3 - C_6$	flavonoids, isoflavonoids
18	$(C_6 - C_3)_2$	lignans, neolignans
30	$(C_6 - C_3 - C_6)_2$	biflavonoids
n	$(C_6 - C_3)_n$ $(C_6)_n$ $(C_6 - C_3 - C_6)_n$	lignins catecholmelanine (condensed tannins)

Flavonoids are plant secondary metabolites(49) and described over 5000 compounds(38). Flavonoids are benzo-ɣ-pyrone derivatives which consist of phenolic and pyrane rings(50). Flavonoids are diverse and differ according to hydroxylation pattern, conjugation between the glycosidic moieties, aromatic rings, and methoxy groups(50). In plants, flavonoids are most found as glycoside derivatives. But occasionally occur as aglycones(39). Flavonoids are known to have antioxidant activity(51). These antioxidant activity is generally linked with the degree and position of hydrosilylation of the B ring(46). And usually, aglycones are more active form than glycosides(46). Flavonoids are mainly classified into flavanols (catechins), flavones, isoflavones, flavonols, anthocyanins, and flavanones(38). Flavonoids are abundant in wine, grape fruit juice and green tea and they are reduce the risk of cardiovascular disease(52). Also flavonoids are reported to have anti-inflammatory activity(53), antiviral, anti-cancer, anti-allergic and anti-tumor properties(54-57).

Carotenoids are widespread natural pigments that recognizable from yellow, orange, red or purple colors and found in all kingdoms of the living world(58). Carotenoids comprise more than 700 structures(59). Carotenoids are C_{40} isoprenoid compounds which made of C_5 isopentenyl building block(60). The most widely distributed carotenoids contain the conjugated chain joined by two terminal ring systems(61). These include the simple hydrocarbon carotenes (e.g. β-carotene) and the xanthophylls which hydroxylated at the terminal rings (e.g. zeaxanthin, lutein)(61). Also, oxygen can be introduced, with or without additional hydroxyl groups (canthaxanthin, astaxanthin)(61) (Fig. 6). Carotenoids are syn- thesized by plants, photosynthetic bacteric, algae(62). But animals, include human, can not synthesize the carotenoids(61). There are high concentrations in carrot, paprika, spinach, pumpkin or tomato(58). The natural extract which contain carotenoids has been used in food coloring for a long time(63). Carotenoids have functions of the antioxidants, provitamin A(58, 61, 64) and anticancer effect(65-66).

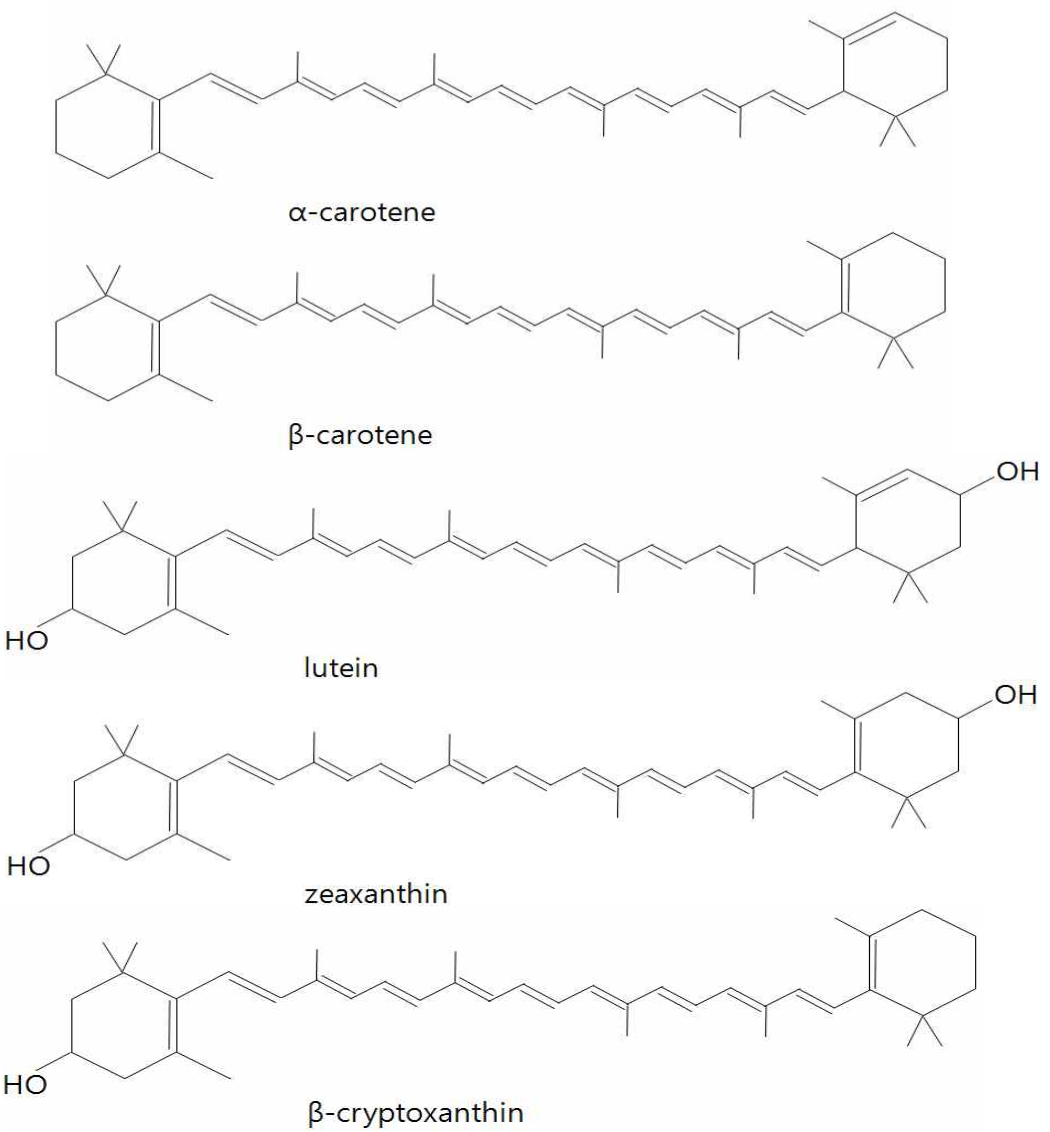


Fig. 6. Some of the carotenoids

Proanthocyanidins known as condensed tannins(67), are considered secondary metabolites in the plant(68). Proanthocyanidins are widely distributed in plant foods and our diet(69, 70). Proanthocyanidins, a major group of polyphenols, polymers made of elementary flavan-3-ol units which are linked by C-C and C-O-C bonds(69-70) (Fig. 7). The flavan-3-ol units typically have the C6-C3-C6 flavonoid skeleton(70). Proanthocyanidins have structurally differ according to the stereochemistry of the heterocycle and the hydroxylation pattern(70, 71). The most common proanthocyanidins in food are procyanidins (PCs) which is 3′,4′-dihydroxy-substituted procyanidins and prodelphinidins (PDs) which is 3′,4′,5′-trihydroxy-substituted prodelphinidins(70, 71). Recently, proanthocyanidins have received an attention in the fields of nutrition, health due to their physiological activities(68), such as antioxidant(72), anti-cancers and anti-cardiovascular diseases(70).

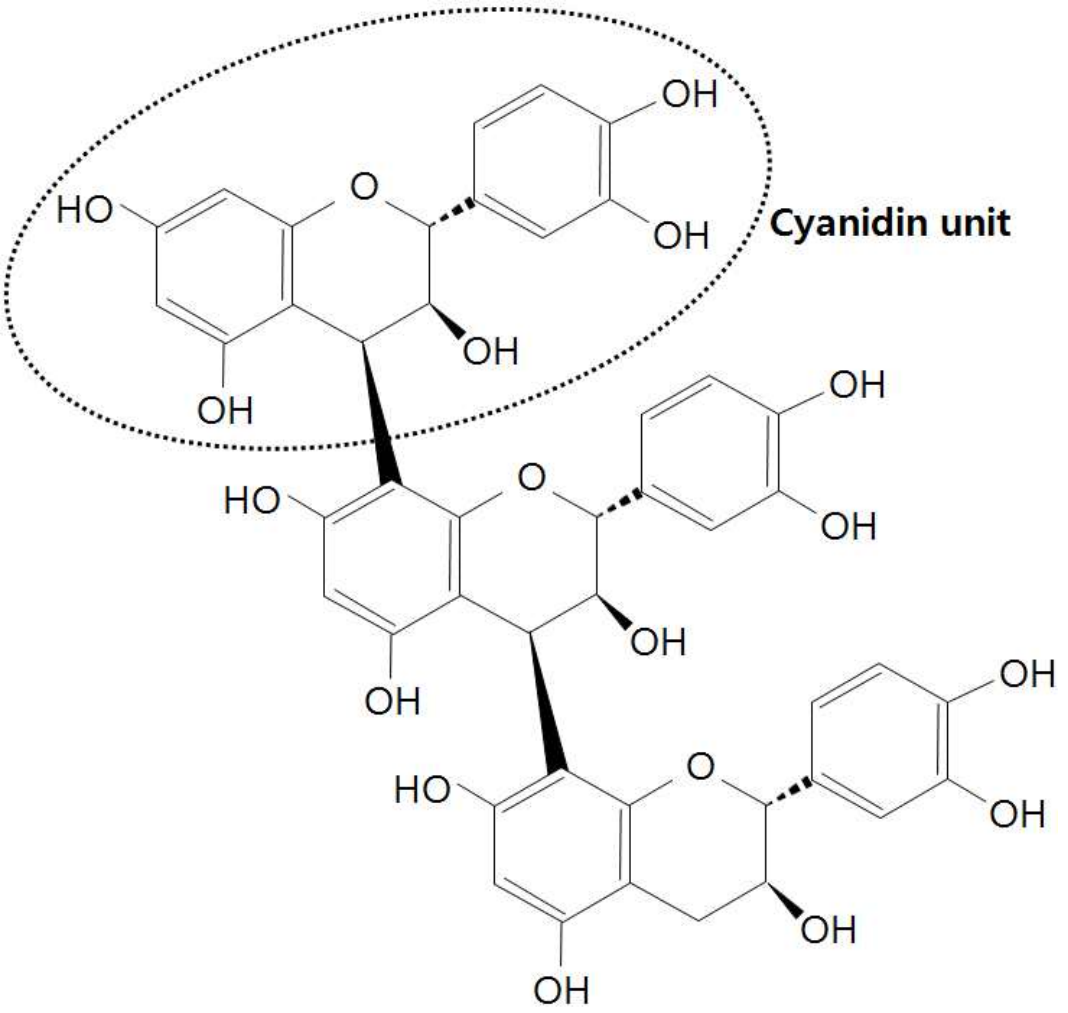


Fig. 7. Chemical structure of proanthocyanidin

Soybeans are one of the major crops grown in Asian countries, and very common foods in Asian countries, as a meat substitute(73, 74). Soybean products can be divided into two groups (non-fermented soy foods and fermented soy foods) and fermented soy foods include natto, soy sauce, doenjang, kochujang and temphe(73). Compared to unfermented soybean products, the fermented soybean products have higher antioxidant activity and total polyphenol content and the composition changes of isoflavones were observed(75). Moreover there were hypotensive effect(76) and anticancer effect(77) in fermented soybean products.

It has been known that bioactive compounds in *Monascus*-fermented soybean was increased during fermentation. For instance, The contents of essential amino acids (11.22 ± 0.15 mg/g), total free amino acids, and Gamma-aminobutyric acid (GABA) of *Monascus*-fermented soy products increased by 2.4, 5.6, and 2.5 times compared with non-fermented control, respectively(78, 79). This indicate that *Monascus* disassemble soybean protein very fast and increase the total free amino acids(79). Accdording to the Pyo(80), the maximum yield of mevinolin (0.42 - 2.94 mg/g) by *Monascus pilisus* IFO 480 was observed after 20 days of fermentation. In particular, mevinolinic acid (β-hydroxy acid) which contribute 86 - 97.3% of total mevinolin was the main component in *Monascus* fermented soybean(73, 80-82). It was also reported that total phenolics and total flavonoid contents of *Monascus*-fermented soybean was significantly increased($p<0.05$) during fermentation(83). Total phenolics contents of *Monascus*-fermented soybean was increased until 20 days (7.5 ± 0.4 mg GAE/g) and tended to decrease after 20 days of fermentation.(83)

There are also several studies that antioxidant activity of

Monascus-fermented products increase with fermentation time. For example, *Monascus*-fermented soybean had higher ABTS and DPPH radical scavenging activities than the non-fermented soybean(84). After 20 days of fermentation, *Monascus* fermented soybean showed 93.2% inhibition effects of linoleic acid peroxidation and 91.7% radical scavenging effect [(DPPH+ABTS)/2] which was increased by 2.7 - 3.1 fold compared to non-fermented products(83). There was a good correlation between the scavenging effects (DPPH and ABTS radicals) and the content of total flavonoids (r^2=0.81) and total phenols (r^2=0.84). This indicate that high antioxidant activity of *Monascus*-fermented soybean was responsible to contents of total phenolics compounds(83).

Unlike the well-known and commercialized red yeast rice, *Monascus* fermented soybean has a little report on the other bioactive compounds, like carotene, tocopherols and proanthosyanidins. Therefore, this study was evaluated on the changes of amount of bioactive compounds and the antioxidant activities of soybeans during *Monascus*-fermentation.

Ⅱ. Material and Method

1. Material

1) substrate

The white soybean and the black soybean were purchased wholesale mart in Korea, Seoul.

2) Fungal strain

The genus *Monascus* strains (*Monascua pilosus* 60160, *Monascua pilosus* 60084, *Monascua pilosus* 60396, *Monascua pilosus* 60399) were purchased from Korean Culture Center of Microorganisms.

2. Method

1) *Monascus*-fermentation

(1) Seed culture

The four kinds of *Monascus* strains from Korean Culture Center of Microorganisms used to find best strain for mevinolin and CoQ10 production. The selected strain (*Monascus pilosus* 60084) was inoculated onto dishes with potato dextrose agar(PDA; Becton, Dickinson and Co. USA). The dishes were incubated at 27℃ for 7 days. and then used to seed culture

(2) Nutrition broth

The colonies of spore that appeared on the petri dish were transferred (about 3cm^2) and inoculated into 100ml of nutrition broth [Rice powder, glucose(Junsei Chemical Co. Japan), maltose(Junsei Chemical Co. Japan), peptone(Becton, Dickinson and Co. Sparks, MD, USA • Le pont de claix, France), glycerol(Junsei Chemical Co. Japan), $NaNO_2$(DaeJung Chemical Co. Korea), MgSO4(Kanto Chemical Co. Japan), $(NH_4)_2SO_4$(Junsei Chemical Co. Japan), Yeast ext.(Alpha Biosciences. USA)]. And then incubated at 30℃ for 5 days with shaking incubator(Chang shin Scien- ce Co. Korea) at 150 rpm.

(3) Solid substrate fermentation

White soybean and black soybean were washed and soaked over 12 hr in running distilled water. After decanting the water, soybeans were weighted to 50 g in Erlenmeyer flask and autoclaved at 121℃ for 20 min. After cooling, the substrates were inoculated with 5 ml (10% V/W) of nutrition broth and incubated at 27℃ for 45 days. At 5 days intervals, samples were collected, freeze dried and homogenized. The sample not inoculated with *Monascus pilosus* 60084 was used as control.

2) Measurement of antioxidant components from *Monascus* fermented soybeans

(1) Determination of mevinolins

Each samples (0.1g) was extracted with 1 ml of 70% ethanol for 1hr with sonication(5510R-DHT, Bransonic Ultrasonics Clearance, USA). After centrifuging for 7 min at 13,500 rpm and filtering (0.20 μm membranes), filtrate was directly analyzed to HPLC. Reverse-phase high performance liquid chromatography (HPLC) analysis was carried with an HP 1100 series(Agilent Technologies, Palo Alto, CA, USA), using ZORBAX Edioase plus C_{18}(2.1×50mm; 3.5-micron particle size; Agilent). The solvent flow rate was 0.3 ml/min, injection volume was 5 μl, and eluted mevinolins were detected at 238nm. The mobile phase for mevinolins analysis is in Table 2. The acid form of mevinolin was obtained by alkaline hydrolysis from Friedrich(85) as described in Fig. 8. Quantitative data for mevinolin and mevinolinic acid were obtained by comparison to standards.

Table 2. Analysis of gradient elution condition for mevinolins with HPLC.

	1 % Acetic acid	D.W	Acetonitrile
0 min	10 %	75 %	15 %
30 min	10 %	15 %	75 %

1mM lactone form mevinolin 5ml + 0.1mM NaOH 500μl

↓

Sonication and heated at 50℃, 1hr

↓ Alkaline hydrolysis

Adjust to PH 7.7 using 1M HCl

↓

Filtering filtering (0.20μm membranes)

Fig. 8. Procedure for the preparation of acid form mevinolin

(2) Determination of CoQ10

To measure the ubiquinone (CoQ10) content in *Monascus* fermented soybeans, the saponification and solvent extraction process were used(86). For saponification, each sample (1 g) and 2.5 g of pyrogallol (Samchun Chemicals. Korea), 10 g of NaOH(Samchun Chemicals. Korea), 25 ml of water, and 75 ml of methanol(Samchun Chemicals. Korea) were added. The mixture was boiled into hot plate for 20 min. After saponification of the samples, CoQ10 was extracted using 60 ml of n-hexane and extracted tree times repeated. The organic layers were collected and evaporated with rotary evaporator(N-100, EY- ELA, Tokyo, Japan). The residue was dissolved in 2 ml of 2-porpanol and filtered(0.20μm membrane) and analyzed. The analysis for CoQ10 was performed by reversed-phase HP 1100 series followed by electrospray ionization in positive mode and MS detector(Agilent 6130 series). A ZORBAX Eclipase plus C_{18} column(2.1×50mm; 3.5-micron particle size; Agilent) was used for chromatographic separation. A mixture of 2-propanol: methanol (50: 50; v/v) containing ammonium formate (5 mmol/l) was used as the mobile phase with a flow rate of 0.3 ml/min. The temperature of the column was maintained at 30℃ and aliquots (1 μl) were injected automatically into the HPLC system. The concentration of CoQ10 (M+ 863.7, 880.7, 881.7 *m/z*) in sample extracts was determined by the peak area.

(3) Determination of Tocopherols

The tocopherol contents were measured by solvent extraction(37). 0.5 g of sample and 5 ml of hexane were added and extracted for 3 hr at 150rpm. After centrifuged, the organic layer was collected and evaporated with rotary evaporator. The residue was dissolved in 1 ml of 2-propanol and filtered(0.20μm membrane) and analyzed. The analysis for tocopherol was performed by HP 1100 series using SUPELC-OSIL^{TM} ABZ+plus column (25cm×4.6m, 5μm; SUPEL-CO). The injection volume was 20 μl and flow rate was 1 ml/min. The temperature of the column was 40℃ and a detection wavelength was 298nm. The mobile phase was described in Table 3. The standards were α-tocopherol, β-tocopherol, ɣ-tocopherol, δ -tocopherol (α, δ-tocophero: Sigma-Aldrich, U.S.A. β, ɣ-tocopherol: Supelco, U.S.A) and the concentration of tocopherol in sample extracts were determined by the peak area.

Table 3. Analysis of gradient elution condition of tocopherol with HPLC.

	Water	Acetonitrile
0 min	15 %	85 %
15 min	0 %	100 %
20 min	0 %	100 %

(4) Determination of total phenolics content

Total phenolics content was determined by Folin-Ciocalteu assay(87). Ground powder of each sample (2 g) was extracted by shaking at 150 rpm for 24 hr with 20 ml of 80% ethanol(Samchun Chemicals. Korea). The solvent centrifuged for 10 min at 3000 rpm and extract was used for determine antioxidant components. 100 μl of sample extract, 1 ml 2% Na_2CO_3(Samchun Chemicals. Korea) and 100 μl 50% Folin-Ciocalteu's reagent solution(Sigam-Aldrich, U.S.A) were mixed and allowed to react for 30 min in dark. The absorbance was measured at 750nm using spectrophotometer. The results were expressed as gallic acid equivalents (mg GAE/g DW).

(5) Determination of total flavonoids content

Total falvonoids content was measured based on colorimetric method (88). 1 ml of 80% ethanol sample extract, 30 μl of 5% $NaNO_2$(DaeJung Chemicals. Korea) were added. After 5 min, 30 μl of 10% $AlCl_3$ was added and allowed to stand for another 5 min before adding 200 μl of 1M NaOH. The mixture was measured the absorbance at 510nm. The total flavonoids content was expressed as catechin(Sigma-Aldrich, U.S.A) equivalent (mg CE/g DW) through the calibration curve of catechin.

(6) Determination of total carotene content

1 g of sample was mixed with 10 ml of solvent (n-hexane: acetone: ethanol, v/v; 50: 25: 25) and extracted for 15 min at 150 rpm. After centrifuged, upper layer was collected and measured the absorbance at 450nm(89). The total carotene content was expressed as β-carotene (Sigma-Aldrich, U.S.A) equivalent (μg CE/g DW) through the calibration curve of β-carotene.

(7) Determination of proanthocyanidins content

Proanthocyanidins content in sample was measured by vanillin- surfuric acid method(90). 1 ml of 80% ethanolic extract, 2 ml of 4% vanillin solution, and 2 ml of 25% H_2SO_4 solution were mixed and shaken for 15 min at 150 rpm. The solvent centrifuged for 10 min at 4000 rpm and upper layer was measured the absorbance at 500nm. The proanthocyanidins content was expressed as catechin equivalent (mg CE/g DW) through the calibration curve of catechin.

3) Measurement of antioxidant activities from *Monascus* fermented soybeans

(1) DPPH radical scavenging assay

The ability of each extract of 80% ethanol to scavenge the DPPH radical was estimated electron donating ability(91). A 100 μl aliquot of each extract in 80% ethanol was mixed in a test tube with 900 μl of 0.1mM DPPH (1,1-diphenyl-2-prcrylhydrazyl; Sigma-Aldrich) solution. The mixture was shaken vigorously and left to stand for 10 min in the dark. Then, the absorbance was measured at 517nm. Trolox(Acros organics, USA) calibration solutions were used to generate the standard curve. The radical scavenging activity of sample was expressed as mg trolox equivalent (mg TE/g DW).

(2) ABTS radical scavenging assay

ABTS radical scavenging was estimated by $ABTS^{+\cdot}$ cation decolorization assay(92). The blue ABTS was produced through the reaction between 7.4 mM ABTS (2,2'-azino-bis-3-ethylbenzo- thiazoline -6-sulfonic acid, Sigma-Aldrich) and 2.6 mM potassium persulfate in water. This solution stored in the dark for 24 hr before use. The concentrated ABTS solution was diluted with distilled water to a final absorbance of 1.45 ± 0.05 at 735nm. 50 μL of each sample extract was added to 950 μL of ABTS solution. The absorbance was measured 5 min after the mixing. The radical scavenging activity of samples was expressed as mg trolox equivalent (mg TE/g DW).

(3) Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was performed ability to reducing power which reduced ferric-tripyridyltriazine (Fe III-TPTZ) [Sodium acetate trihydrate (Junsei Chemical Co. Japan), Acetic acid glacial(Samchun Chemicals. Korea), HCl(Junsei Chemical Co. Japan), 2.4.6-Tris(2-pyridyl)-s-triazine (Sigma-Aldrich, U.S.A), $FeCl_3 \cdot 6H_2O$(Sigma-Aldrich, U.S.A)] to the ferrous (Fe II) form was estimated by Ferric reducing antioxidant power (FRAP)(93). Stock solution of 300 mM acetate buffer, 20 mM FeCl3·6H2O, and 10 mM tripyridyl trizine (TPTZ, in 40 mM HCL) were prepared. The stock solutions were mixed in a 10: 1: 1 ratio to prepared the FRAP reagent and maintained at 37℃. Then, 50 μl of each sample extract was mixed with 950 μL of FRAP solution. The mixture was shaken vigorously and left to stand for 20 min in the dark. The absorbance was measured at 593nm. The results were expressed mg trolox equivalent (mg TE/g DW).

4) Statistical analysis

All samples were analyzed in triplicate. The results were expressed mean ± standard deviation. The SPSS Inc. (version 21.0) was used and statistical comparisons were made by ANOVA procedure followed by Duncan's multiple range test, $p<0.05$ was considered significantly different.

Ⅲ. Result and Discussion

1. Antioxidant components in *Monascus* fermented soybeans

1) Mevinolin content

Mevinolin ($C_{24}H_{36}O_5$) is a member of 8 substituted hexahydro naphthalene lactones(94). The physiologically active form of mevinolin is the p-hydroxy acid (opened lactone) structure(8). Mevinolin is one of the most effective drugs for coronary heart disease as 3-hydroxy -3-methylglutaryl co-enzyme A (HMG-CoA) reductase inhibitor(10). Mevinolin competitively inhibit the HMG-CoA reductase, so inhibit the synthesis of mevalonate which is important mediators of the cholesterol synthetic pathway(11).

Mevinolin production from white soybeans (MFWS) and black soybean (MFBS) fermented with *Monascus pilosus* 60084 during 45 days at 27℃ show in Fig. 9. Mevinolinic acid was the main form of mevinolin in fermented soybeans. This result is similar to the data of Pyo(81), who showed that mevinolinic acid (β-hydroxy acid) was the main component in *Monascus* fermented soybean. The highest production of mevinolin in MFWS was 568.18 ± 6.13 μg/g in 20 days, and in MFBS was 502.36 ± 6.41 μg/g in 40 days. The highest production of mevinolin in MFWS was 1.1 times higher than MFBS. The amount of mevinolin content was

decreased after reaching the maximum value. This is thought to be because mevinolin was decomposed by *Monascus* consumed most nutrient source. This data was substantially lower than that of Pyo and Lee(82), who reported the mevinolin of 2.82 mg/g DW in *Monascus* fermented soybean. The reason for this difference is estimated to be due to the difference of *Monascus* strain used. This data indicate that white soybean was better substance than black soybean for *Monascus* fermentation($p<0.05$).

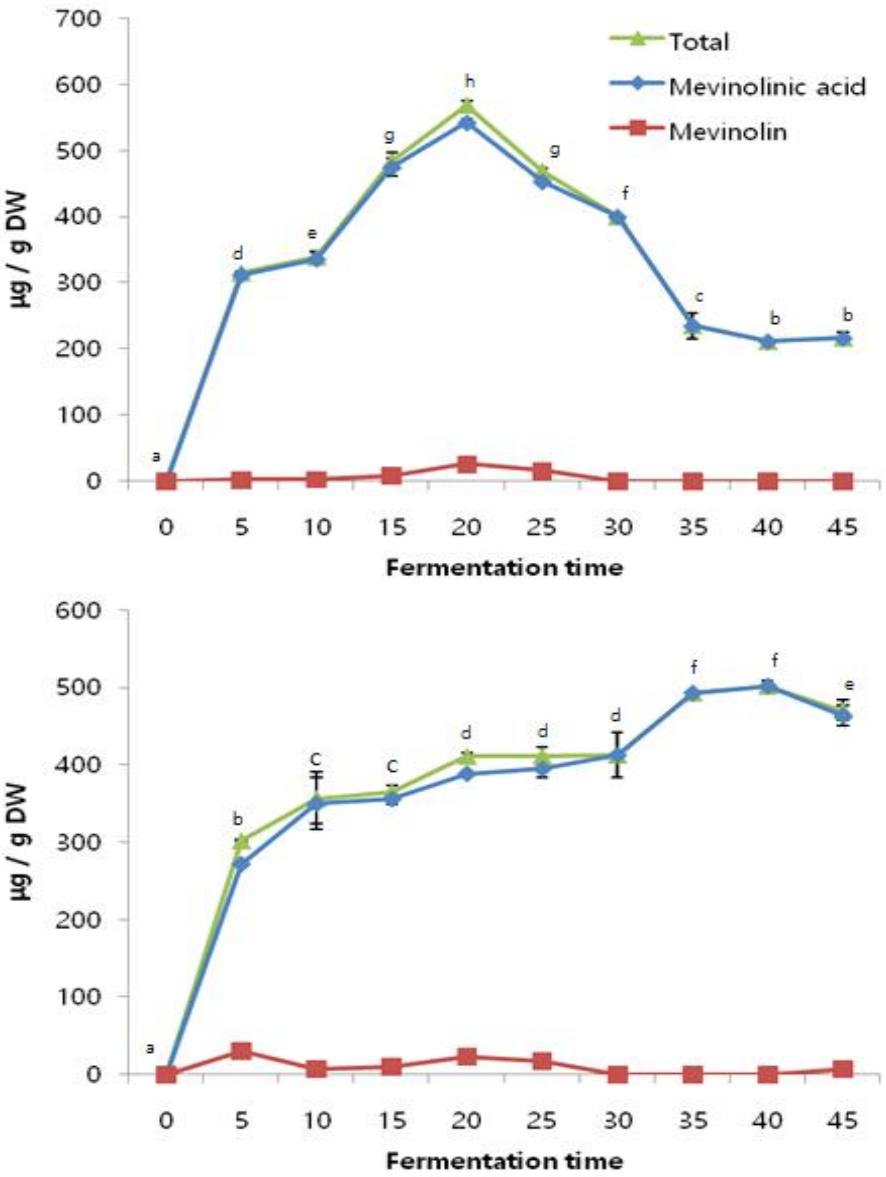


Fig. 9. Effect of fermentation time on the mevinolin production in white soybean (top) and black soybean (bottom) fermented with *Monascus pilosus* 60084.

Different letters indicate significant difference at the $p<0.05$.

Each value is mean±SD(n=3).

Table 4. Contents of mevinolin in soybeans fermented with *Monascus pilosus* 60084

Time (day)	Mevinolin contents (μg/g DW)	
	MFWS	MFBS
0	0.00±0.00[a1)]	0.00±0.00[a]
5	314.92±2.34[a]	302.22±1.66[b]
10	339.31±9.10[a]	357.26±33.29[a]
15	483.35±13.69[a]	365.73±7.56[b]
20	568.18±6.13[a]	411.59±4.69[b]
25	468.90±3.56[a]	412.14±11.13[b]
30	399.88±2.16[a]	413.16±29.07[a]
35	235.24±19.20[a]	493.47±0.96[b]
40	211.56±3.56[a]	502.36±6.41[b]
45	216.44±8.68[a]	470.68±13.11[b]

Each value is mean±SD(n=3).

1) Different letters in the same row indicate significant difference at the $p<0.05$

2) CoQ10 content

Coenzyme Q10 (CoQ10) is a naturally occurring lipid-soluble compound(20) and is a lipid-soluble antioxidant(23). CoQ10 plays an important role in energy production and is an essential carriers of the electron transport system in mitochondria(19). CoQ10 is widely approved as a potent antioxidant dietary supplement linked with immune enhancement, energy boosting, and ease of cardiovascular disease(26).

CoQ10 content from *Monascus*-fermented soybean shown in Fig. 10. The maximum yields of CoQ10 was detected after 20 days of fermentation and was decreased after reaching the maximum yields. The maximum amount of CoQ10 in MFWS was 65.59 ± 9.53 μg/g DW in 20 days. This was increased about 2.2 fold compared with the non-fermented white soybean (30.25 ± 2.34 μg/g DW). In the case of MFBS, the maximum content of CoQ10 was 64.13 ± 2.43 μg/g DW in 25 days and this was increased 2.4 fold than non-fermented black soybean (26.41 ± 4.78 μg/g DW). There was no significant difference between the maximum concentration of CoQ10 in MFWS and MFBS($p > 0.05$). The results were similar to Pyo and Seo(26). They reported the highest CoQ10 content in *Monascus*-fermented soybeans was 0.25 ± 0.01 mg/g in 15 days.

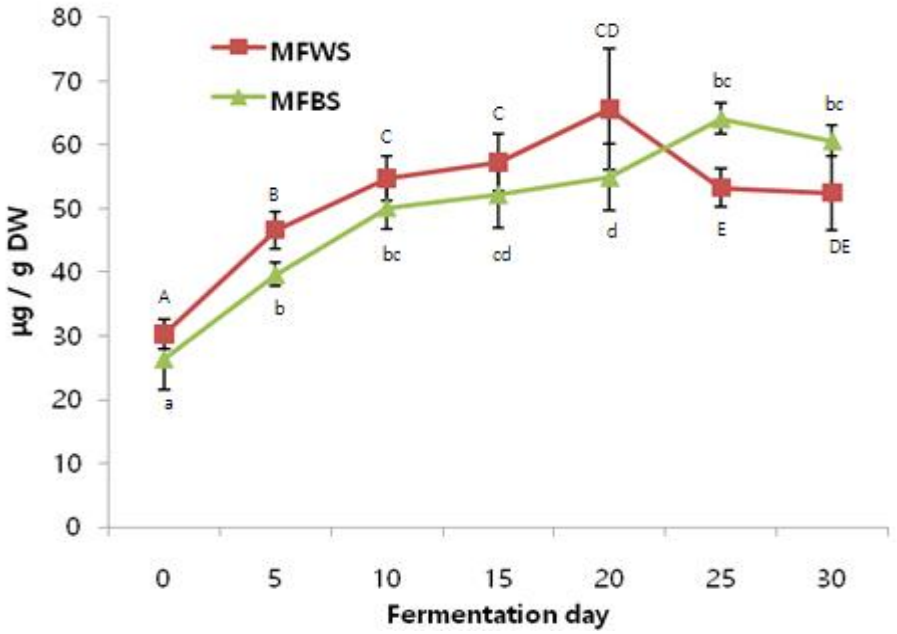


Fig. 10. Effect of soybean substrates on the production of CoQ10 in *Monascus* fermentation.

Different letters indicate significant difference at the $p<0.05$.

Each value is mean±SD (n=3).

Table 5. Contents of CoQ10 in soybeans fermented with *Monascus pilosus* 60084

Time (day)	CoQ10 (μg/ g DW)	
	MFWS	MFBS
0	30.25±2.34[a1)]	26.41±4.78[a]
5	46.65±2.93[a]	39.69±1.90[b]
10	54.70±3.51[a]	50.18±3.46[a]
15	57.24±4.52[a]	52.24±5.26[a]
20	65.59±9.53[a]	54.92±5.27[a]
25	53.23±3.03[a]	64.13±2.43[b]
30	52.45±5.78[a]	60.67±2.47[a]

Each value is mean±SD(n=3).

[1)] Different letters in the same row indicate significant difference at the $p<0.05$.

3) Tocopherol content

Vitamin E is a strong antioxidant that helps protect cells from the free radicals that can induce potentially damaging of the body's metabolism, and the vitamin E consists of α-, β-, ɣ-, and δ-tocopherols and four α-, β-, ɣ-, δ-tocotrienols(27). Among the 4 isomers of tocopherol, α-tocopherol has the most biologically active(27, 30).

As shown in Fig. 11, total-tocopherol concentration of white soybeans fermented with *Monascus pilosus* 60084 were increased with fermentation time until 30 days of incubation. Total-tocopherol concentrations of MFWS were increased after 20 days of fermentation, but there was no significant difference(p>0.05) between each sample. Total-tocopherol concentrations of MFBS also showed the similar trends with the results of MFWS. The highest yield of 283.50 ± 2.74 μg total tocopherols per g dry weight of soybean was obtained after 35 days of fermentation. These results showed a similar to Mau et al(95) who reported that depending on the type of rice, tocopherol concentration of red yeast rice was showed conflicting results. These results indicate that different kinds of grains contain different kinds of nutrition sources and this could affect the growth of *Monascus* sp. In this study, the ranking of tocopherol content was ɣ> δ> α> β. This corresponded to results reported by Murphy et al.(30) who showed the tocopherol ranking (ɣ> δ> α) in 5 kinds of soybean. This suggested that ɣ-tocopherol and δ-tocopherol were main tocopherol both non-fermented and fermented soybeans.

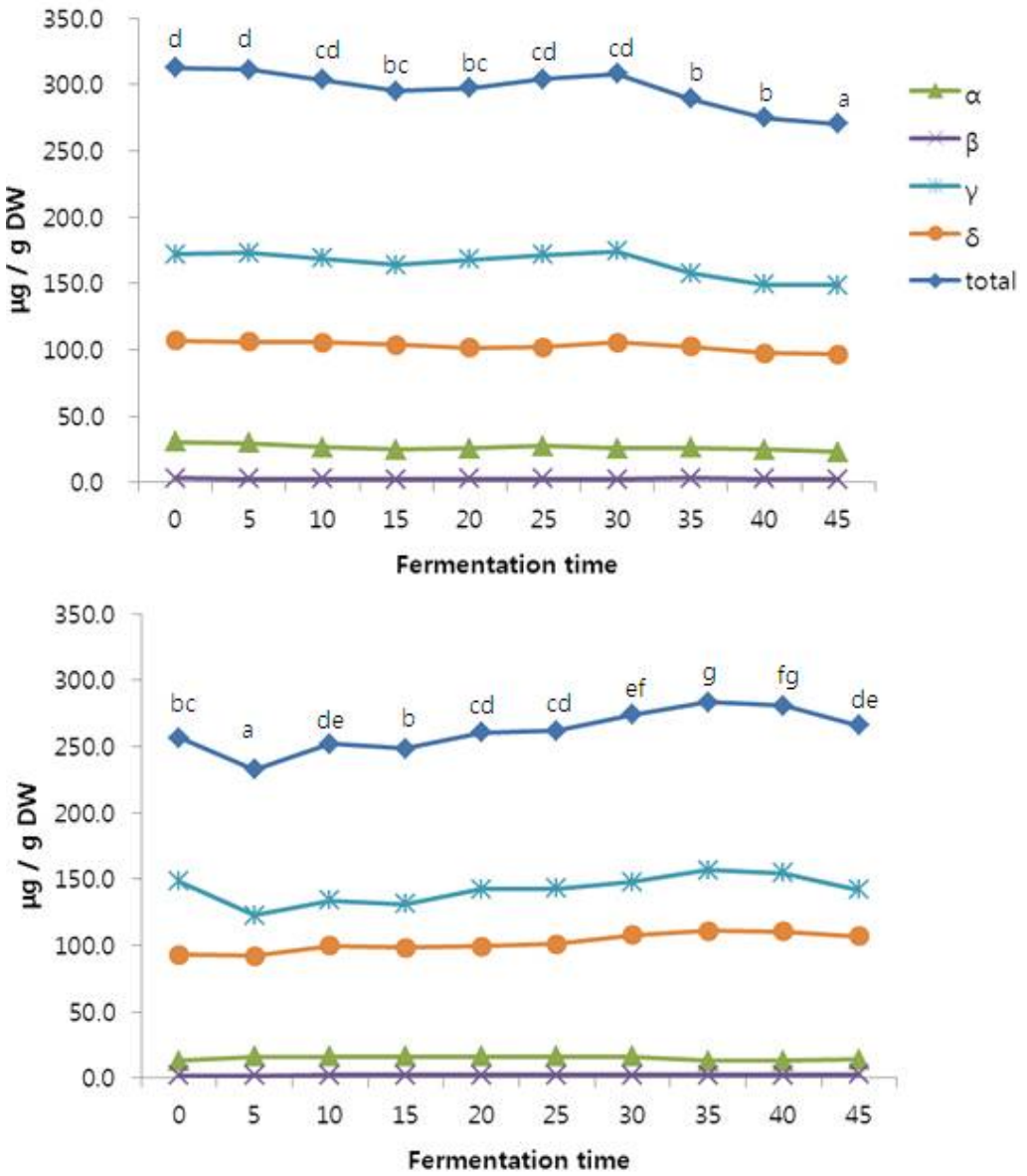


Fig. 11. Effect of fermentation time on the tocopherol concentration in white soybean (top) and black soybean (bottom) fermented with *Monascus pilosus* 60084.

Different letters indicate significant difference at the $p<0.05$.

Each value is mean±SD(n=3).

Table 6. Contents of tocopherol in soybeans fermented with *Monascus pilosus* 60084

Time (day)	T-Tocopherol contents (μg/g)	
	MFWS	MFBS
0	312.87±3.28[a1)]	256.76±8.11[b]
5	311.57±8.22[a]	232.80±2.66[b]
10	303.95±11.41[a]	268.64±3.42[b]
15	295.16±5.37[a]	248.29±5.66[b]
20	297.66±3.62[a]	260.58±6.24[b]
25	304.20±7.26[a]	262.07±2.51[b]
30	308.29±10.91[a]	274.09±3.99[b]
35	289.16±5.93[b]	283.50±2.74[a]
40	274.81±4.85[a]	280.95±6.30[a]
45	270.42±7.53[a]	266.21±5.05[a]

Each value is mean±SD (n=3).

1) Different letters in the same row indicate significant difference at the $p<0.05$

4) Total phenolics contents

The phenolic compounds are widely spread in plant(38). Phenolic compounds contain more than one unit or building block and are divided into phenolic acids, flavonoids, lignans and condensed tannins(40). The phenolic compounds have antioxidant activity(47) and blood pressure decrease effect(48).

The total phenolics compounds was expressed in mg gallic acid equivalent (GAE) per g of dried sample. Fig. 12. shows the total phenolics contents of 80% ethanol extract from *Monascus*-fermented soybeans. The highest total phenolics contents of MFWS (10.49 ± 0.22 mg/g) and MFBS (5.95 ± 0.21 mg/g) were produced after 40 days of fermentation. The highest total phenolics content of MFWS was 1.8 times higher than that of MFBS($p<0.05$). Also, MFWS contained 3.6 times higher phenolics contents compared to non-fermented white soybean. Total phenolics of MFBS had 1.9 times higher contents than that of non-fermented black soybean. These results was similar to the results of Lim et al.(73) who showed that *Monascus*-fermented soybean had 2 times higher total phenolics content compared to non-fermented soybean.

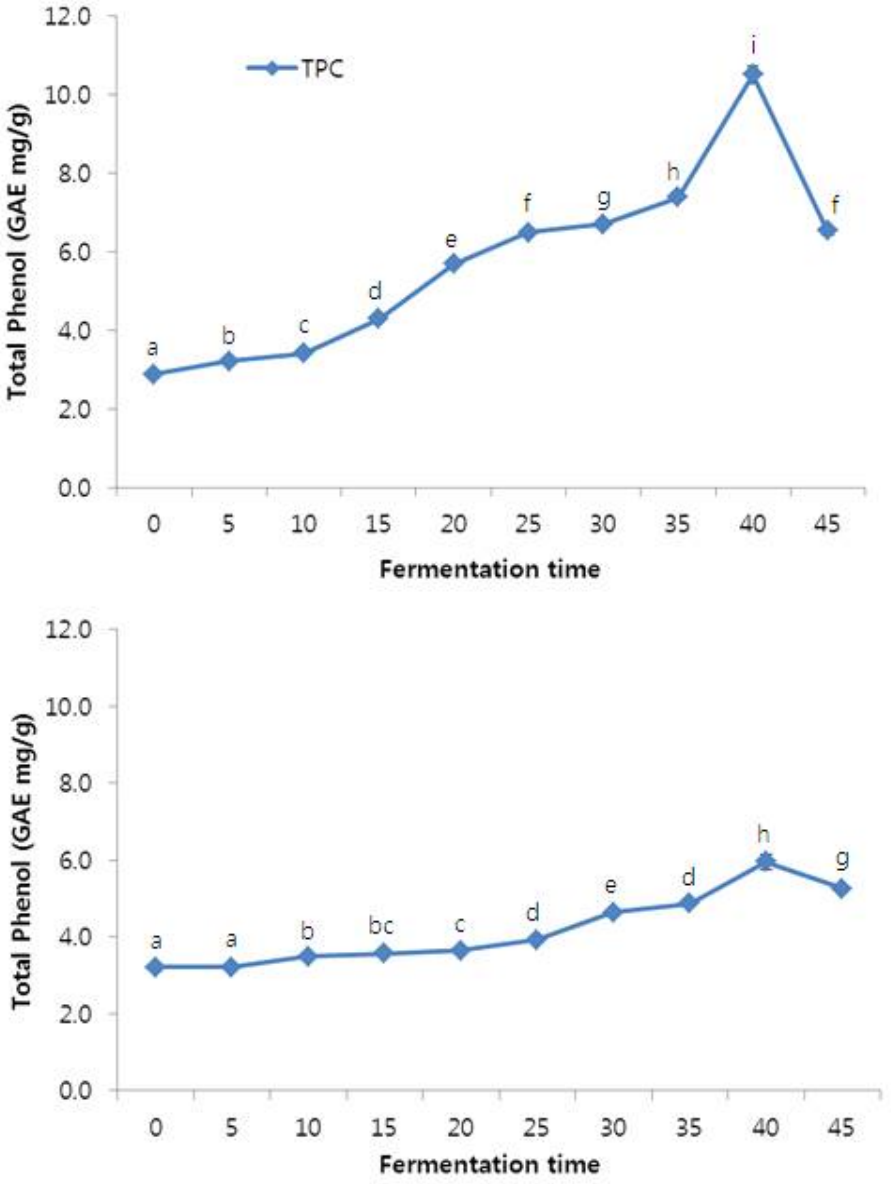


Fig. 12. Effect of fermentation time on the total phenolic contents (TPC, mg GAE/g DW) in white soybean (top) and black soybean (bottom) fermented with *Monascus pilosus* 60084.

Different letters indicate significant difference at the $p<0.05$.

Each value is mean±SD(n=3).

Table 7. Contents of total phenolics in soybeans fermented with *Monascus pilosus* 60084

Time (day)	Total pheonlics contents (GAE mg/ g DW)[1)]	
	MFWS	MFBS
0	2.88±0.06[a2)]	3.20±0.10[b]
5	3.22±0.07[a]	3.21±0.05[a]
10	3.41±0.03[a]	3.48±0.08[a]
15	4.30±0.09[a]	3.55±0.02[b]
20	5.69±0.04[a]	3.64±0.03[b]
25	6.48±0.04[a]	3.92±0.04[b]
30	6.70±0.05[a]	4.63±0.05[b]
35	7.39±0.03[a]	4.86±0.07[b]
40	10.49±0.22[a]	5.95±0.21[b]
45	6.53±0.10[a]	5.25±0.03[b]

Each value is mean±SD(n=3).

1) Expressed as mg gallic acid equivalent (GAE) per g of dried weight.

2) Different letters in the same row indicate significant difference at the $p<0.05$.

5) Total flavonoids contents

Flavonoids are also plant secondary metabolites and abundant in wine, grape fruit juice and green tea(60, 52). Flavonoids exhibit an antioxidant(39) activity and reduce the risk of cardiovascular disease(52).

The total flavonoids compounds was expressed in mg catechin equivalent (CE) per g of dried sample. Fig. 13. shows the total flavonoids contents of 80% ethanol extract from soybeans fermented with *Monascus pilosus* 60084. The highest total flavonoids contents of MFWS and MFBS were 0.57 ± 0.08 mg/g at 35 days and 0.35 ± 0.01 mg/g at 20 days of fermentation, respectively. The total flavonoids contents showed a tendency to decrease after the maximum content which was similar to the total phenolics contents. A total flavonoids content of MFWS was up to 2.5 times compared to non-fermented white soybean, and a total flavonoids content of MFBS was 1.1 times higher than non-fermented black soybean. A similar phenomenon was observed on black soybeans fermented with Bacillus subtilis by Chou's report(49). Chou et al.(49) suggested that the releasing of total phenolics and flavonoids from the soybean during fermentation might lead to increase the contents of total phenolics and flavonoids. The total flavonoids contents was measured very low amount compared to the total phenolics contents.

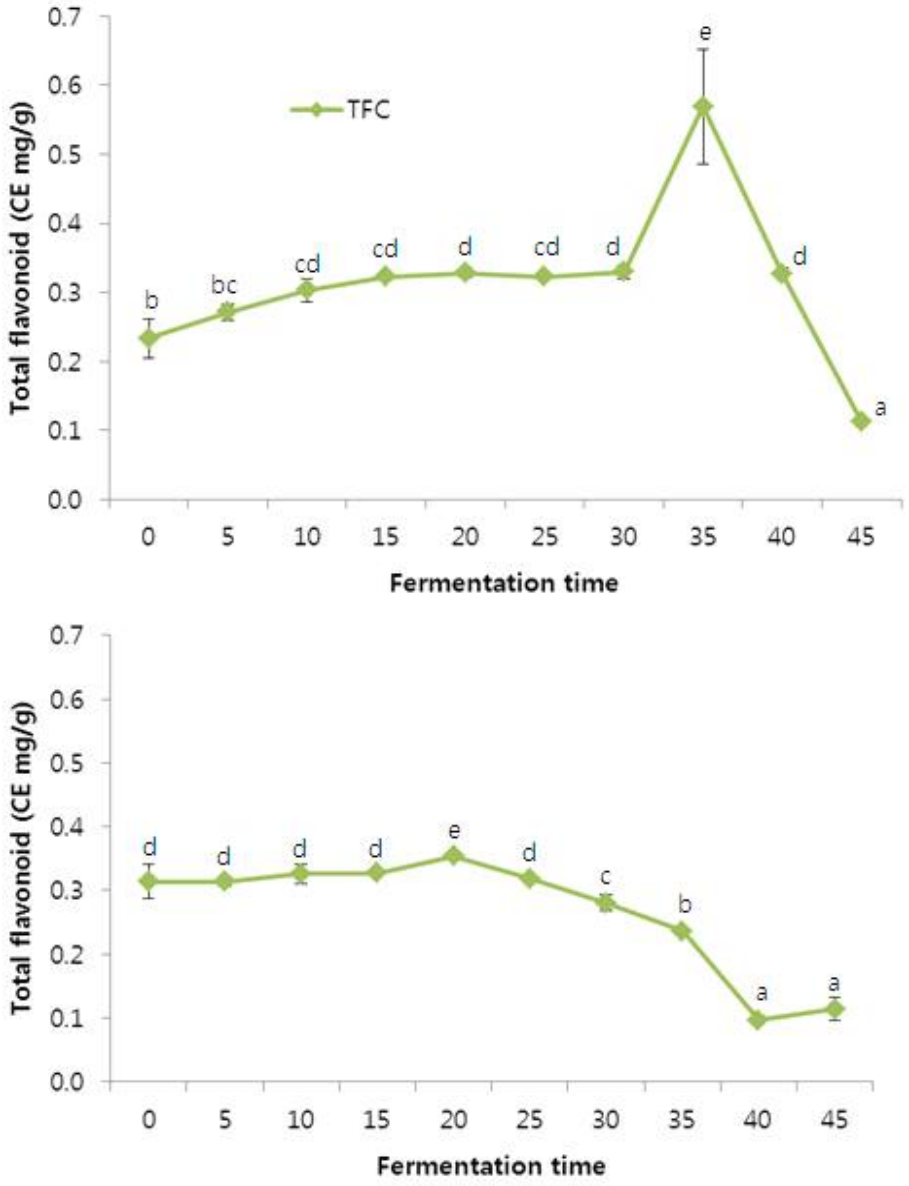


Fig. 13. Effect of fermentation time on the total flavonoid contents (TFC, mg CE/g DW) in white soybean (top) and black soybean (bottom) fermented with *Monascus pilosus* 60084.

Different letters indicate significant difference at the $p<0.05$.

Each value is mean±SD(n=3).

Table 8. Contents of total flavonoid in soybeans fermented with *Monascus pilosus* 60084

Time (day)	Total flavonoids contents (GAE mg/g)[1)]	
	MFWS	MFBS
0	0.23±0.03[a2)]	0.31±0.03[b]
5	0.27±0.01[a]	0.31±0.01[b]
10	0.30±0.02[a]	0.33±0.02[a]
15	0.32±0.01[a]	0.33±0.00[a]
20	0.33±0.01[a]	0.35±0.01[b]
25	0.32±0.00[a]	0.32±0.01[a]
30	0.33±0.01[a]	0.28±0.01[b]
35	0.57±0.08[a]	0.24±0.01[b]
40	0.33±0.01[a]	0.10±0.01[b]
45	0.11±0.01[a]	0.11±0.02[a]

Each value is mean±SD(n=3).

1) Expressed as mg catechin equivalent (CE) per 1g of dried weight.

2) Different letters in the same row indicate significant difference at the $p<0.05$

6) Total carotene content

Carotenoids are widespread natural pigments and found in all kingdoms of the living world(58, 61, 64). The natural extract which contain carotenoids has been used in food coloring for a long time(63) and have functions of the antioxidants, provitamin A(58, 61, 64). Carotenoids also have anticancer effect(65, 66).

The total carotene content was expressed in μg β-carotene equivalent per g of dried sample. Fig. 14. shows the total carotene content of soybeans fermented with *Monascus pilosus* 60084. The highest total carotene contents of MFWS was 8.08 ± 0.44 μg/g at 5 days of fermentation and after that total carotene contents tended to decrease. These results showed a similar to Suntornsuk et al(96) who reported that *Rhodotorula glutinis* DM 28 fermented rice bran showed the highest β-carotene (1.65 mg/kg) contents at 6 days and after that total carotene contents tended to decrease. The total carotene content of MFBS was decreased during initial fermentation but started to increase after 10 days and reached 25.42 ± 0.62 μg/g of total carotene content at 45 days of fermentation.

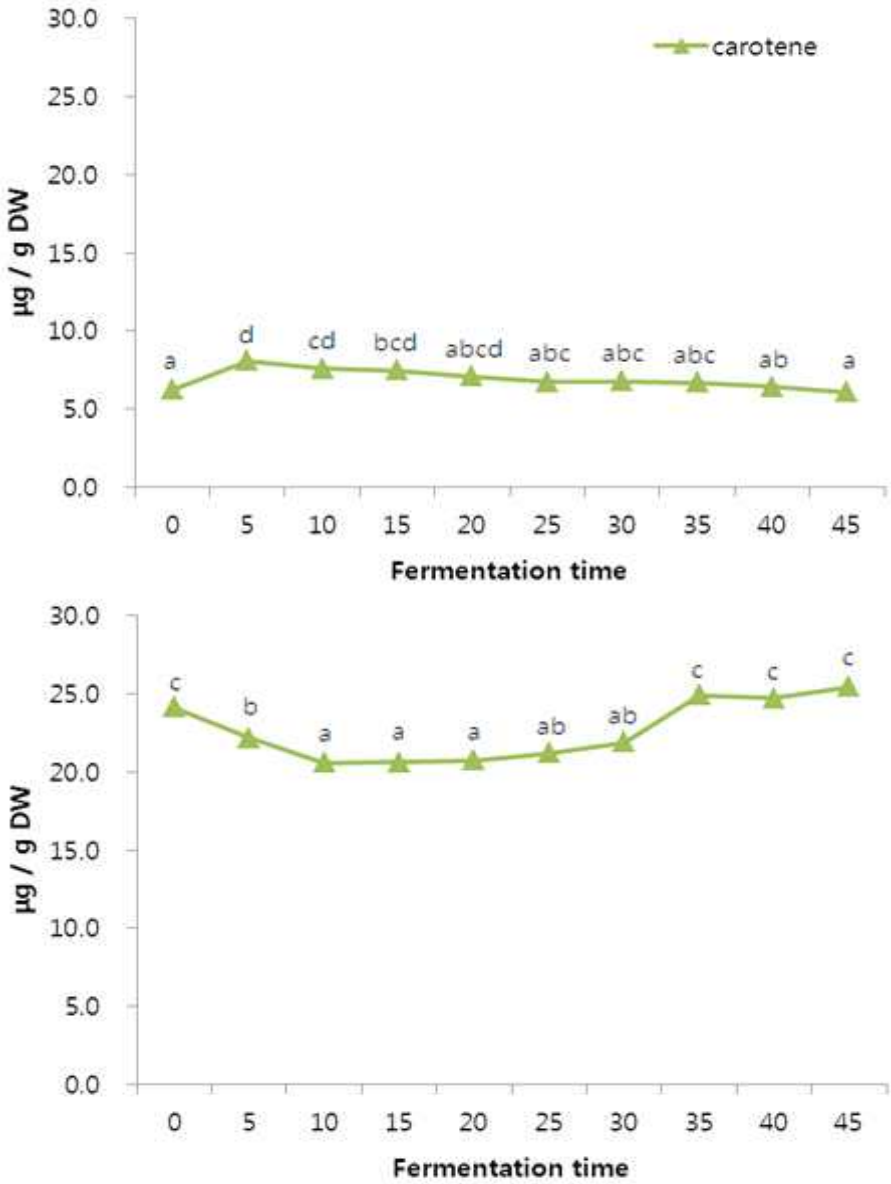


Fig. 14. Effect of fermentation time on the total carotene contents (μg β-carotene/ g DW) in white soybean (top) and black soybean (bottom) fermented with *Monascus pilosus* 60084.

Different letters indicate significant difference at the $p<0.05$.

Each value is mean±SD(n=3).

Table 9. Contents of total carotene in soybeans fermented with *Monascus pilosus* 60084

Time (day)	Carotene contents (μg β-carotene/ g DW)[1)]	
	MFWS	MFBS
0	6.24±0.27[a2)]	24.13±0.44[b]
5	8.08±0.44[a]	22.16±0.41[b]
10	7.58±0.87[a]	20.56±0.44[b]
15	7.49±0.43[a]	20.62±0.22[b]
20	7.10±0.19[a]	20.74±0.16[b]
25	6.76±0.30[a]	21.17±0.32[b]
30	6.79±1.19[a]	21.89±1.90[b]
35	6.72±0.08[a]	24.87±0.70[b]
40	6.45±0.30[a]	24.70±0.53[b]
45	6.11±0.65[a]	25.42±0.62[b]

Each value is mean±SD(n=3).

1) Expressed as μg β-carotene per 1g of dried weight.

2) Different letters in the same row indicate significant difference at the $p<0.05$

7) Poranthocyanidins content

Proanthocyanidins (PAs), a major group of polyphenols, polymers made of elementary flavan-3-ol units(69, 70). PAs are widely distributed in plant foods(69, 47) and have antioxidant(47), anti-cancers and anti-cardiovascular diseases effect(70).

Proanthocyanidins content of 80% ethanol extract from soybean fermented with *Monascus pilosus* 60084 shown in Fig. 15. The proanthocyanidins content of un-fermented black soybean was significantly higher than un-fermented white soybean. This is similar to the studies of moon et al(97) who report that anthocyanidin content of black soybean was higher than yellow soybean. The highest contents of MFWS (15.31 ± 0.86 mg/g) and MFBS (10.72 ± 0.35 mg/g) were obtained at 40 days of fermentation. The highest proanthocyanidins contents of MFWS were 1.4 and 4.8 times higher than those of MFBS and non-fermented white soybean, respectively. The proanthocyanidins content of MFBS had 1.5 times higher compared to non-fermented black soybean. The highest proanthocyanidins contents of MFWS was tended to decrease. The proanthocyanidins content of MFBS was decreased during initial fermentation but started to increase after 10 days and reached 10.72 ± 0.35 mg/g of proanthocyanidins at 40 days of fermentation.

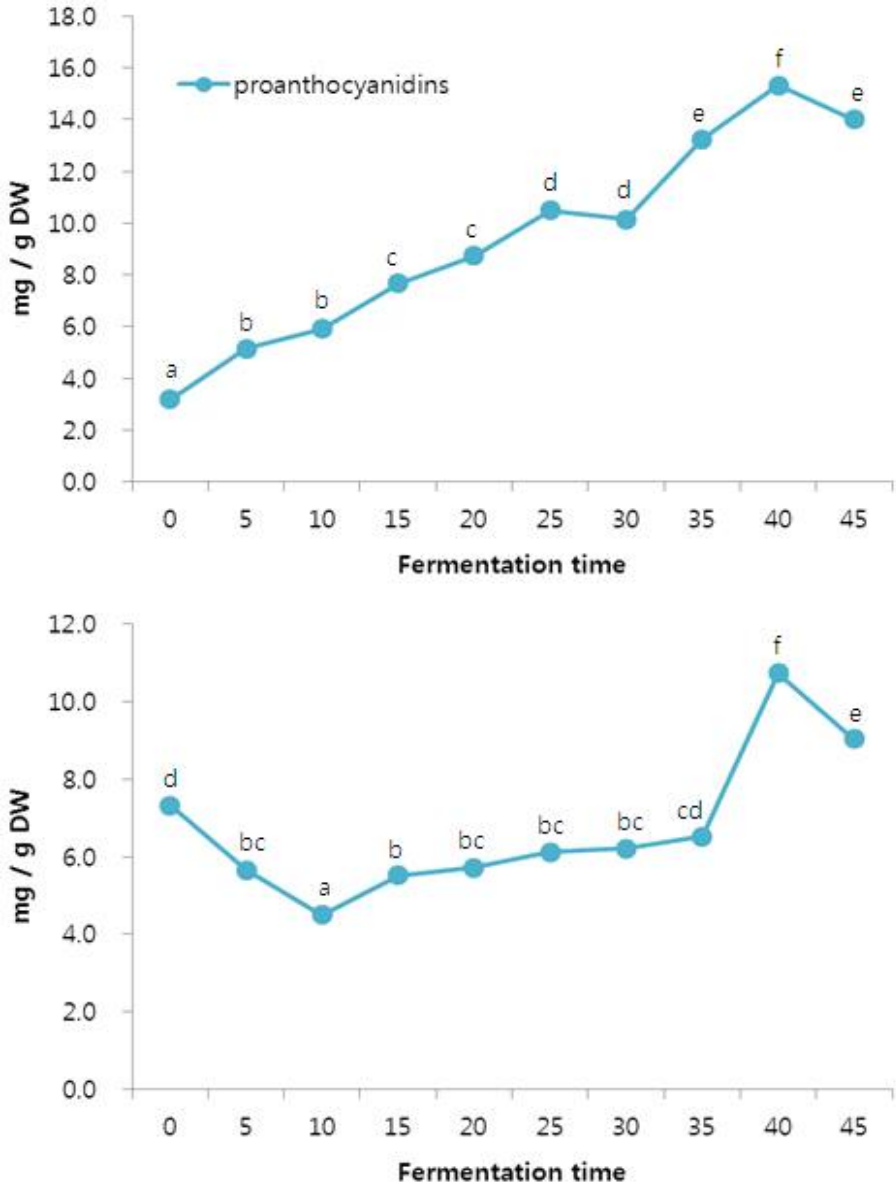


Fig. 15. Effect of fermentation time on the poranthocyanidins contents (mg catechin/ g DW) in white soybean (top) and black soybean (bottom) fermented with *Monascus pilosus* 60084.

Different letters indicate significant difference at the $p<0.05$.

Each value is mean±SD(n=3).

Table 10. Contents of proanthocyanidins in soybeans fermented with *Monascus pilosus* 60084

Time (day)	Proanthocyanidin contents (mg CE/ g DW)[1)]	
	MFWS	MFBS
0	3.20±0.15[a2)]	7.32±0.17[b]
5	5.16±0.66[a]	5.66±0.31[a]
10	5.91±0.30[a]	4.50±0.76[b]
15	7.67±0.23[a]	5.51±0.09[b]
20	8.73±0.23[a]	5.71±0.09[b]
25	10.49±1.14[a]	6.11±0.57[b]
30	10.14±0.94[a]	6.21±0.80[b]
35	13.25±0.23[a]	6.52±0.66[b]
40	15.31± 0.86[a]	10.72±0.35[b]
45	14.00± 1.20[a]	9.03±0.31[b]

Each value is mean±SD(n=3).

1) Expressed as mg catechin equivalent (CE) per 1g of dried weight.

2) Different letters in the same row indicate significant difference at the $p<0.05$

2. Antioxidant potentials of *Monascus*-fermented soybean

1) DPPH radical scavenging effect

DPPH radical scavenging effect is antioxidant activity measuring method that used to measure the antioxidant activity, depending on the extent of the deep purple discoloration(98). The deep purple DPPH radical reduced by bioactive compounds, such as tocopherol, ascorbate, flavonoid compounds and aromatic amines, which have antioxidant activity(98). If DPPH reduced antioxidant compound, purple DPPH radical are decolorized(98). DPPH method is most commonly used as antioxidant activity measuring method of the water-soluble or organic solvent extract of natural products(98, 99).

DPPH radical scavenging effect of *Monascus*-fermented soybean was analyzed and result expressed in mg trolox equivalent per g dry weight (mg TE/g DW). Fig. 16. shows the DPPH radical scavenging effect on 80% ethanol extract of soybean fermented with *Monascus pilosus* 60084. Antioxidant effects of MFWS and MFBS increased with increasing the fermentation time until 40 days. Both MFWS (1.78 ± 0.08 mg TE/g) and MFBS (1.51 ± 0.08 mg TE/g) were exhibited strongest antioxidant activities at 40 days of fermentation. DPPH radical scavenging effects of MFWS and MFBS were 1.9 and 1.5 times higher than non-fermented soybean, respectively. These results were similar to previous studies(95, 100) that the DPPH radical scavenging activity of *Monascus*-fermented substrates had higher than non-fermented one.

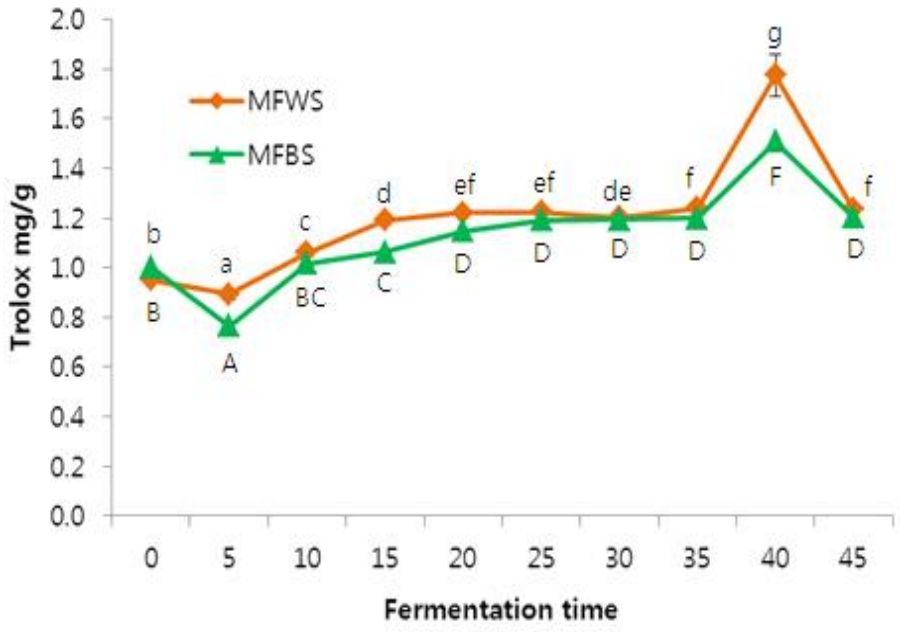


Fig. 16. Effect of fermentation time on the DPPH radical scavenging effects in *Monascus* fermentation.

Different letters indicate significant difference at the $p<0.05$.

Each value is mean±SD(n=3).

Table 11. DPPH radical scavenging effect of white soybean and black soybean fermented with *Monascus pilosus* 60084.

Time (day)	DPPH scavenging activity (Trolox mg/ g)[1)]	
	MFWS	MFBS
0	0.95±0.01[a2)]	1.00±0.02[b]
5	0.89±0.02[a]	0.77±0.04[b]
10	1.06±0.01[a]	1.02±0.02[b]
15	1.19±0.00[a]	1.06±0.03[b]
20	1.22±0.01[a]	1.15±0.01[b]
25	1.22±0.00[a]	1.19±0.00[b]
30	1.20±0.02[a]	1.19±0.01[a]
35	1.24±0.00[a]	1.20±0.01[b]
40	1.78±0.03[a]	1.51±0.08[b]
45	1.24±0.01[a]	1.20±0.02[b]

Each value is mean±SD(n=3).

1) Expressed as mg trolox equivalent (TE) per 1g of dry weight.

2) Different letters in the same row indicate significant difference at the $p<0.05$.

2) ABTS radical scavenging effect

ABTS radical scavenging effect was analyzed and result expressed in trolox equivalent per g dry weight (mg TE/g DW). As shown in Fig. 17, ABTS radical scavenging effects of MFWS and MFBS increased with increasing the fermentation time until 40 days. Both MFWS (3.79 ± 0.04 mg TE/g) and MFBS (2.76 ± 0.14 mg TE/g) were exhibited strongest antioxidant activities at 40 days of fermentation. These result were similar to DPPH radical scavenging activity that showed the highest antioxidant activities at 40 days. The result corresponded to the recent studies(73) that ABTS radical scavenging activity of *Monascus*-fermented soymilk was significantly higher than control soymilk. Pyo(83) suggested that enhanced antioxidant ability of fermented soybeans was derived from the substrate and the mycelia during *Monascus*- fermentation.

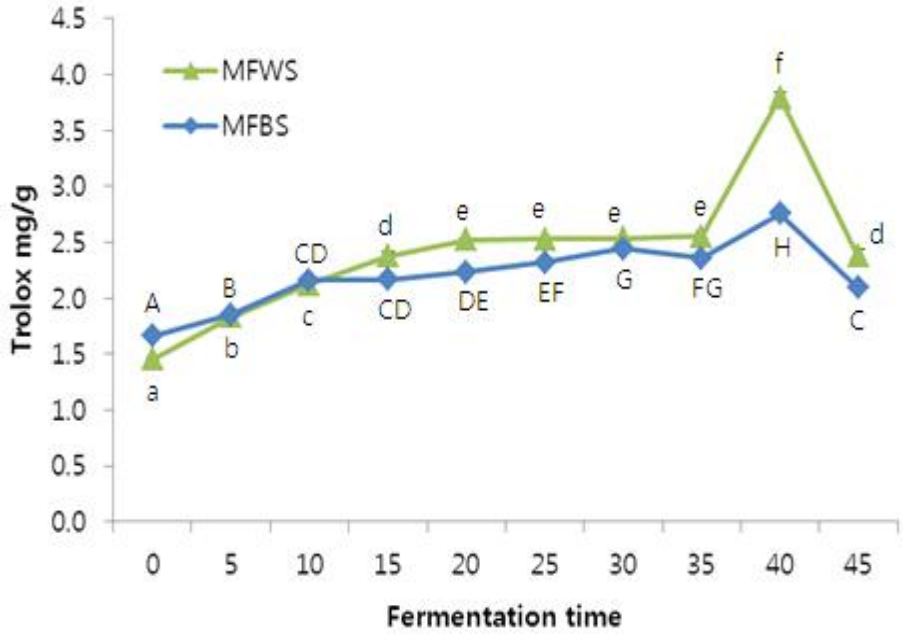


Fig. 17. Effect of fermentation time on the ABTS radical in *Monascus* fermentation.

Different letters indicate significant difference at the $p<0.05$.

Each value is mean±SD(n=3).

Table 12. ABTS radical scavenging effect of white soybean and black soybean fermented with *Monascus pilosus* 60084.

Time (day)	ABTS scavenging activity (Trolox mg/ g)[1)]	
	MFWS	MFBS
0	1.45±0.02[a2)]	1.66±0.04[b]
5	1.83±0.02[a]	1.85±0.05[a]
10	2.12±0.02[a]	2.16±0.02[a]
15	2.38±0.04[a]	2.17±0.05[b]
20	2.52±0.01[a]	2.23±0.03[b]
25	2.53±0.00[a]	2.32±0.00[b]
30	2.53±0.00[a]	2.44±0.03[b]
35	2.55±0.00[a]	2.35±0.02[b]
40	3.79±0.09[a]	2.76±0.14[b]
45	2.37±0.02[a]	2.09±0.07[b]

Each value is mean±SD(n=3).

1) Expressed as mg trolox equivalent (TE) per 1g of dry weight.

2) Different letters in the same row indicate significant difference at the $p<0.05$.

3) Ferric reducing antioxidant power (FRAP) assay

FRAP assay is the antioxidant activity measuring method using the principle that ferric tripyridyltriazine [Fe(III)-TPTZ]2] complex is reduced to ferrous tripyridyltriazine [Fe(II)-TPTZ]2] when the PH lower than 3 (23). FRAP assay was analyzed and result expressed in trolox equivalent per g dry weight (mg TE/g DW). Determination of antioxidant capacity using FRAP assay on the 80% ethanol extract of soybean fermented with *Monascus pilosus* 60084 shown in Fig. 18. The highest reducing powers of MFWS (2.56 ± 0.04 mg TE/g) and MFBS (2.07 ± 0.01 mg TE/g) were observed at 20 days and 30 days of fermentation, respectively. Reducing power of *Monascus*-fermented soybeans increased by a 1.7 to 1.3 fold compared with those of the unfermented products.

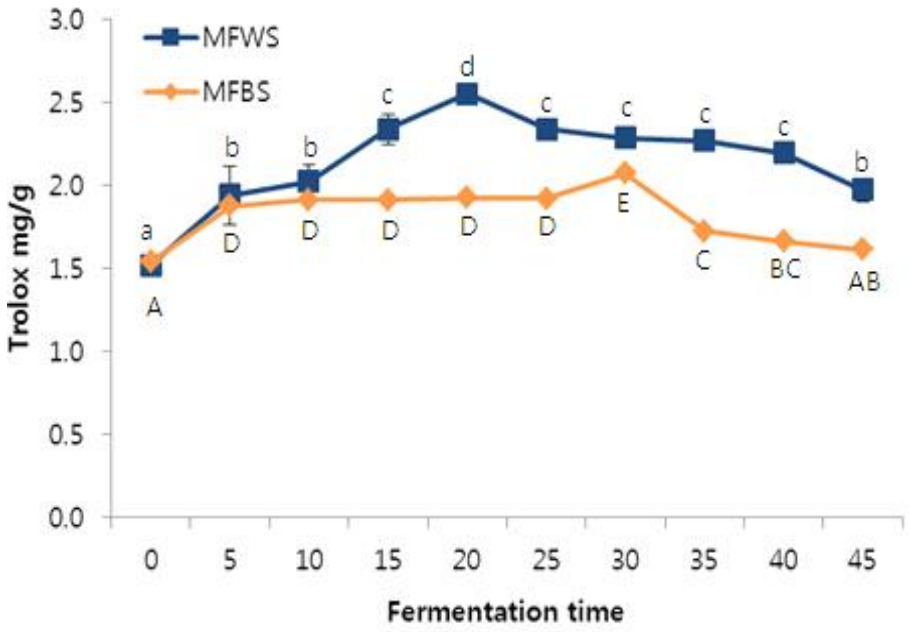


Fig. 18. Effect of fermentation time on the reducing power in *Monascus* fermentation.

Different letters indicate significant difference at the $p<0.05$.

Each value is mean±SD(n=3).

Table 13. Reducing power of white soybean and black soybean fermented with *Monascus pilosus* 60084.

Time (day)	FRAP assay (Trolox mg/ g)[1)]	
	MFWS	MFBS
0	1.52±0.01[a2)]	1.54±0.03[a]
5	1.94±0.18[a]	1.88±0.05[a]
10	2.03±0.10[a]	1.92±0.03[a]
15	2.34±0.09[a]	1.91±0.05[b]
20	2.56±0.04[a]	1.93±0.01[b]
25	2.34±0.03[a]	1.92±0.05[b]
30	2.29±0.05[a]	2.07±0.01[b]
35	2.27±0.05[a]	1.73±0.03[b]
40	2.20±0.04[a]	1.67±0.07[b]
45	1.98±0.07[a]	1.61±0.08[b]

Each value is mean±SD(n=3).

1) Expressed as mg trolox equivalent (TE) per 1g of dry weight.

2) Different letters in the same row indicate significant difference at the $p<0.05$.

3. Correlation between antioxidant components and antioxidant potentials in *Monascus*-fermented soybean.

Correlation between antioxidant components and antioxidant activities in *Monascus*-fermented soybeans was shown in Table 14. Main antioxidant component that gave the greatest impact to the antioxidant activities in *Monascus*-fermented soybeans was toatal phenolic compounds. In particular, strong correlation (r^2 = 0.881) was found in ABTS radical effect. The result indicated that the ABTS and DPPH radical effect might be mostly related to its content of toatal phenolic compounds. The CoQ10 also showed a strong correlation with antioxidant activities, especially strong correlation (r^2 = 0.822) was found in ABTS radical. Total carotene content (r^2 = -0.621) expressed as β-carotene equivalent was showed lower relationship with antioxidant potentials than those of total phenolic and CoQ10. Therefore, it can be concluded that the phenolic compounds and CoQ10 contained in soybean fermented with *Monascus pilosus* 60084 are responsible for its antioxidant potentials.

Table 14. Pearson's correlation coefficients(R2) between antioxidant components and antioxidant potentials of *Monascus*-fermented soybean

	DPPH	ABTS	FRAP
TP	0.847**	0.881**	0.472**
TF	-0.112	0.098	0.516**
Mevinolin	0.232	0.331**	0.418**
CoQ10	0.666**	0.822**	0.701**
T-Tocopherol	0.125	0.054	0.347**
Proanthocyanidin	0.741**	0.734**	0.404**
Carotene	-0.141	-0.220	-0.621**

TP = Total Phenolics contents, TF = Total flavonoids contents, T-Tocopherol = Total tocopherol(α-tocopherol + β-tocopherol + γ-tocopherol + δ-tocopherol).

*: significant difference at the $p<0.05$

**: significant difference at the $p<0.01$

Ⅳ. Conclusion

This study was investigated on the changes of amount of bioactive compounds such as total phenolics, flavonoids, CoQ10, mevinolin, total tocopherols, total carotenes, and proanthocyanidins and the antioxidant activities according to fermentation time of white and black soybean with *Monascus pilosus* 60084. The effect of fermentation time on antioxidant potentials of white soybean (MFWS) and black soybean (MFBS) was evaluated.

1. The highest mevinolin content of MFWS was 568.18 ± 6.13 μg/g at 20 days of fermentation, which is 1.1 times higher than MFBS of 502.36 ± 6.41 μg per g dry weight at 40 days fermentation.

2. The maximum amounts of CoQ10 in MFWS (65.59 ± 9.53 μg) and MFBS (64.13 ± 2.43 μg) were obtained at 20 days and 25 days of fermentation, respectively. They were decreased after reaching the maximum yields.

3. Total-tocopherol concentrations of *Monascus*-fermented soybean tended to decrease during fermentation. A maximum concentration (283.50 ± 2.74 μg/g) of total-tocopherols was observed in MFBS at 35 days of fermentation. The ranking of tocopherol content was γ> δ> α> β. This showed that γ-tocopherol and δ-tocopherol were main tocopherol isomer in both non-fermented and fermented soybeans.

4. The highest total phenolics of MFWS (10.49 ± 0.22 mg GAE/g DW) and MFBS (5.95 ± 0.21 mg GAE/g DW) were produced at 40 days fermentation, respectively. The highest total phenolics content of MFWS was 1.8 times higher than that of MFBS.

5. The highest total flavonoids contents of MFWS (0.57 ± 0.08 mg CE/g DW) and MFBS (0.35 ± 0.01 mg CE/g DW) were obtained at 35 days and 20 days of fermentation, respectively. The total flavonoids contents was measured relatively very low amount compared to the total phenolics contents.

6. The highest total carotene contents of MFWS (8.08 ± 0.44 μg β-carotene equivalent/g DW) and MFBS (25.42 ± 0.62 μg/g DW) were at 5 days and 45 days of fermentation, respectively. The total carotene concentration of MFBS was decreased until 10 days of fermentation, but started to increase after 15 days fermentation.

7. Proanthocyanidins concentration of unfermented black soybean was significantly higher than unfermented white soybean. The highest proanthocyanidins contents of MFWS (15.31 ± 0.86 mg CE/g DW) and MFBS (10.72 ± 0.35 mg CE/g DW) were observed at 40 days of fermentation.

8. Three widely used antioxidant assays (DPPH, ABTS, and FRAP) were used to evaluated the effect of fermentation time on the antioxidant potentials of soybean fermented with *Monascus pilosus* 60084. The total

phenolic showed a strong correlation (r^2 = 0.881) with antioxidant assays. Phenolic compounds in soybean fermented with *Monascus pilosus* 60084 are responsible for antioxidant activities. The results of antioxidant assays used in this study confirmed that the *Monascus*-fermented soybean had significant antioxidant capacities (2.26 mg TE/g DW). In particular, the radical scavenging effect of MFWS and MFBS were increased with increasing fermentation time until 40 days fermentation.

9. A positive and significant correlation existed between antioxidant potentials and CoQ10 (r^2 = 0.822) and total phenolics (r^2 = 0.881) contents of samples, revealing that CoQ10 and total phenolics were the dominant antioxidant components in the soybean fermented with *Monascus pilosus* 60084.

Thus, *Monascus*-fermented soybean may be used as natural and potent dietary antioxidative additives or supplements.

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한글 초록

본 연구는 *Monascus pilosus* 60084로 발효시킨 콩 (백태와 서리태)의 항산화활성 성분과 항산화 활성의 변화를 발효시간에 따라 조사하였다. *Monascus pilosus* 60084를 백태와 서리태에 접종시킨 후 27 ℃에서 45일 동안 발효시키며, 5일 간격으로 발효콩을 수집하여 분석하였다. Mevinolin, CoQ10, 토코페롤의 분석은 HPLC를 이용하였고, 총 페놀, 총 플라보노이드, 총 카로티노이드 및 proanthocyanidins 성분은 분광광도계를 이용하여 분석하였다. 가장 높은 mevinolin 함량(568.18 ㎍/g DW)과 CoQ10(65.59 ㎍/g의 DW)은 27 ℃에서 20일간 발효시킨 *Monascus* 발효 백태에서 관찰되었다. 최대의 총 페놀 함량(10.49 mg GAE/g DW), 총 플라보노이드 함량(0.57 mg CE/g DW), 프로안토시아니딘 함량(20.67 mg CE/ g DW)은 각각 35일과 40일간 발효시킨 *Monascus* 발효 백태에서 측정되었다. 가장 많은 총 카로티노이드의 함량(25.42 μg BCE/g DW)은 45일 동안 발효시킨 *Monascus* 발효 서리태에서 나타났으며, 총 토코페롤 함량(312.87 μg/g DW)은 발효 전의 백태에서 가장 높게 나타났다.

모든 발효콩의 항산화 능력은(0.77-3.79 mg TE/g DW) DPPH, ABTS 라디컬 소거능력과 환원력(reducing power) 시험의 결과로 평가하였다. *Monascus* 균주로 발효시킨 백태와 서리태 시료는 발효시간의 증가에 따라 항산화활성이 증가하는 추세였으며, 항산화활성의 영향인자는 CoQ10 (r^2 = 0.724) 과 총 페놀 함량 (r^2 = 0.557)이 유의적으로 (p<0.01) 가장 높은 상관관계를 보이는 것으로 나타났다. 이상과 같은 결과를 통해 홍국 발효콩에는 mevinolin, CoQ10

이외에도 토코페롤, 페놀화합물, 카로틴류와 프로안토시아니딘 등의 항산화 성분이 함유되어 있다는 것을 확인하였으며, 이들 성분이 홍국발효콩의 항산화활성을 강화하여 천연 항산화제의 사용 가능성을 보여 주었다.

감사의 글

대학원 생활을 마칠 수 있도록 항상 도와준 모두에게 감사를 전합니다.

부족함이 많은 제자를 딸처럼 챙겨주시고 아낌없는 지도와 관심으로 논문이 완성되기까지 지도해 주신 표영희교수님 감사드립니다. 그리고 바쁘신 와중에도 부족한 논문을 꼼꼼히 다듬어주신 이명숙교수님, 나혜경교수님께 감사드립니다. 아울러 학부시절부터 대학원까지 많은 가르침을 주신 안홍석교수님, 한영숙교수님, 이승민교수님, 윤형근교수님, 고성희교수님, 이경연교수님께 감사드립니다. 또한 늘 관심과 조언을 아끼지 않으셨던 중앙기기실의 오현주선생님께도 깊은 감사를 드립니다.

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