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Effect of Dietary Inclusion of Medicinal Herb Extract Mix in a Poultry Ration on the Physico-chemical Quality and Oxidative Stability of Eggs

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ABSTRACT : A mixture of three dietary medicinal herb extracts (MHE, mulberry leaf:Japanese honeysuckle:goldthread = 48.5: 48.5:3.0) was prepared as an additive of hen's feed. One hundred-eight, 28-wk-old Lohmann Brown hens were assigned randomly with three levels of MHE in the diet (0, 0.3, and 1%). Hens were fed for 6 wks and eggs were collected in the 6th week, and stored at 4°C for 14 days to investigate the effect of MHE on the quality and oxidative stability of eggs. Internal quality of the egg including weight, shell color, albumen height, yolk color, shell weight, shell thickness, and Haugh units was not different among the dietary treatments. The oxidation stability of raw and cooked egg was determined by 2-thiobarbituric acid reactive substances (TBARS) value, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and 2,2'-azinobis(3-ethylbenzonthianoline-6-sulfonic acid) ($ABTS^+$) radical reducing ability. Results indicated that TBARS value at day 0 and $ABTS^+$ radical reducing ability of eggs from hens fed MHE were higher than from the control group. However, DPPH radical scavenging activity showed no difference in both raw and cooked samples. Results of the present study indicate that dietary MHE may slightly enhance the oxidative stability of eggs. (**Key Words** : Egg Quality, Dietary Medicinal Herb, Natural Antioxidants, Oxidative Stability)

INTRODUCTION

Antibiotics have been supplemented to animal to improve growth performance and protect animals from the adverse effects of pathogenic and non-pathogenic enteric microorganisms (Dahiya et al., 2006). However, the use of therapeutic antibiotics in animal feed is not approved due to chances of development of antibiotic resistant microbes. Therefore various studies have been conducted to use plants and herbs as alternative of synthetic antibiotics. Herbs are identified to enhance antimicrobial, antiviral, and antioxidative activities and to simulate the endocrine and immune system (Dahiya et al., 2006). In food industry, herbs or their extracts having antioxidative properties are frequently used to improve quality and shelf life of meat products (Vichi et al., 2001), turkey meat (Botsoglou et al., 2007), and egg yolk (Botsoglou et al., 2005). Ahn et al. (2007) also reported grape seed extract and pine bark extract contributed significantly to antimicrobial and antioxidant activities in cooked beef.

Japanese honeysuckle (*Lonicera flos*) has shown a wide spectrum of antibacterial, antiviral (Houghton et al., 1993), antioxidant (Kim et al., 1994), and inhibition of the platelet activating factor (Cheng et al., 1944). It contains anti-complementary polysaccharides and polyphenolic compounds which inhibit the platelet aggregation, thromboxane biosynthesis, and hydrogen peroxide-induced endothelial injury (Chang and Hsu, 1992). Like a number of polyanionic compounds including dextran sulfate, polyanionic polysaccharide, polyhydroxy carboxylates derived from phenolic compounds and flavanoids, tannins selectively inhibit human immunodeficiency virus (HIV) (Chang et al., 1995). Leaves, barks and branches of mulberry (*Morus alba*) have long been used in Chinese medicine to treat fever, protect the liver, improve eyesight, strengthen joints, facilitate discharge of urine and lower blood pressure (Zhishen et al., 1999). Leaves of mulberry species are consumed in Korea and Japan as anti-hyperglycemic nutraceutical for patients with diabetes mellitus because the leaves contain 1-deoxynojirimycin, known to be one of the most potent α-glycosidase inhibitors (Kim et al., 2003). The methanol extract of goldthread

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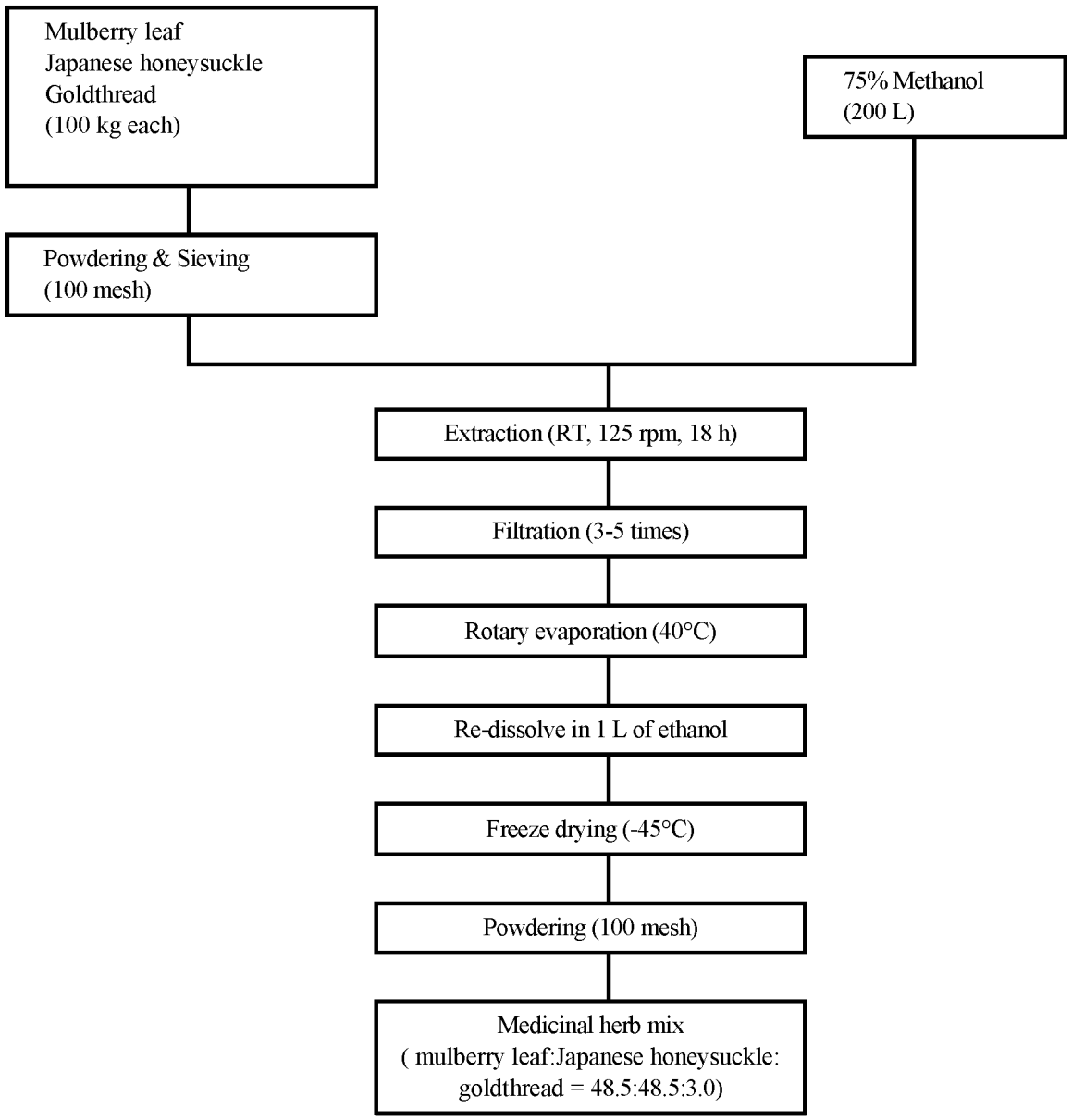

Figure 1. Procedure for medicinal herb extract mix preparation.

(*Coptis chinensis*) showed the very strong antioxidative activity in both methylene blue-sensitized and chlorophyll-sensitized photooxidations of linoleic acid (Jung et al., 1999). Preliminary study in our laboratory indicated that these 3 herbs can be alternatives of synthetic antibiotics due to their sufficient antimicrobial activity.

The objective of this work was to determine the effect of herbs extract mix viz. mulberry leaf, Japanese honeysuckle and god thread in the diet on the quality and oxidative stability of eggs.

MATERIALS AND METHODS

Preparation of medicinal herb extract mix (MHE)

Mulberry leaf, Japanese honeysuckle and goldthread were purchased from Kyung-dong herbal market (Seoul, Korea). Those were chopped and pulverized to pass mesh 100 (2 mm). Medicinal herb was extracted by 75% methanol as described in Figure 1. Briefly, 100 kg of the each powdered medicinal herbs were extracted overnight with 200 L of 75% methanol using a large scale extractor (CoBiotech, Brussels, Belgium) at room temperature. Each extract was filtered 2-3 times with cheesecloth and the filtrate was concentrated by rotary evaporator under vacuum. Each concentrate of mulberry leaf, Japanese honeysuckle, and goldthread was redissolved with 1 L of 75% ethanol and mixed with 48.5:48.5:3.0 ratio, respectively, evaporated, freeze-dried, and used as MHE for broiler's diets (Figure 1). This ratio was decided by the economics and oriental medical doctor's recommendation for

Table 1. Experimental formula for layers

Herb mix ratio (%)	Diets[1]		
	0	0.3	1.0
Ingredients	---------- % ----------		
Yellow corn	52.82	52.82	52.82
Soybean meal	18.05	18.05	18.05
Rapeseed meal	2.00	1.70	1.00
Animal fat	0.80	0.80	0.80
Lysine-HCl	0.46	0.46	0.46
DL-Methionine	0.14	0.14	0.14
Salt	0.25	0.25	0.25
Limestone	9.12	9.12	9.12
Dicalcium phosphate	1.05	1.05	1.05
Mineral premix[2]	0.20	0.20	0.20
Vitamin premix[3]	0.06	0.06	0.06
Choline Cl (50%)	0.05	0.05	0.05
DDGS[4]	15.00	15.00	15.00
Medicinal herb extract mix	0	0.3	1.0
	100.0	100.0	100.0
Analyzed composition (%, as-fed basis)			
Moisture	11.28	11.28	11.28
Crude protein	17.00	17.00	17.00
Crude fat	5.57	5.57	5.57
Crude fiber	3.02	3.02	3.02
Crude ash	13.12	13.12	13.12
Calcium	3.82	3.82	3.82
Phosphorus	0.55	0.55	0.55
TMEn[5] (kcal/kg)	2,780.00	2,780.00	2,780.00
Essential amino acids (%, as-fed basis)			
Lysine	0.87	0.87	0.87
Methionine	0.43	0.43	0.43
Threonine	0.63	0.63	0.63
Tryptophane	0.19	0.19	0.19

[1] Three herb extracts (mulberry leaf:Japanese honeysuckle:goldthread = 48.5:48.5:3.0) were mixed and added with designated ratio to hen's diet.
[2] Provided followings per kg of diet: Cu, 10 mg; Fe, 80 mg; Mn, 80 mg; Zn, 80 mg; I, 0.9 mg; Se, 0.2 mg; Co, 0.5 mg.
[3] Provided followings per kg of diet: Vit. A, 12,000 IU; Vit. D_3, 3,000 IU; tocopherol 15 mg; Vit. K_3, 2 mg; thiamin, 2.0 mg; riboflavin, 6.0 mg; pyridoxin, 2.0 mg; vit. B_{12}, 0.03 mg; folic acid, 1.0 mg; biotin, 0.15 mg; niacin, 45 mg; D-Ca pantothenate, 15 mg; antioxidant, 0.5 mg.
[4] Corn distillers dried grains with solubles from the US.
[5] Calculated values.

future industrial application.

Animals and diets

A total of 108, 28-wk-old Lohman Brown hens were used for this study. The birds were randomly allotted 18 pens with 6 birds per pen (replicate) based on added level of MHE (0, 0.3, and 1%). The birds were fed experimental layer's diets containing MHE (Table 1). The lighting regimen was 17 h of light per day and the birds received feed and water freely during the feeding period for 6 wks. Egg sample collection commenced at 6th week and 5 eggs per each replication (30 eggs per treatment) were collected. The collected eggs were stored at 4°C for 14 days and analysis was performed on the 7th and the 14th day of storage.

Chemicals

1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azinobis(3-ethylbenzonthianoline-6-sulfonicacid) (ABTS), potassium peroxiodisulfate, 2,6-di-tert-butyl-4-methylphenol (BHT) purchased from Sigma-Aldrich Co (St Louis, MO, USA). Trichloroacetic acid (TCA) obtained from Samchun Pure Chemical (Seoul, Korea) and 2-thiobarbituric acid (TBA) from Alfa Aesar (Lancashire, UK).

Egg quality measurement

Egg weight (g), shell color (%), albumen height (mm), yolk color (%), shell weight (g), shell thickness (mm), and Haugh units were determined using QCM$^+$ System (Technical Services and Supplies, York, England). Egg shell hardness was determined using egg shell force gauge model-II (Robotmation Co. LTD, Tokyo, Japan).

2-Thiobarbituric acid reactive substances (TBARS) measurement

Egg yolk (5 g) was mixed for the TBARS measurement (Jo et al., 2002). The mixture was added into 50 μl butylated hydroxytoluene (7.2% BHT) and homogenized (T25B, IKA, Staufen, Germany) in 15 ml deionized distilled water (DDW) at high speed (No. 6) for 20 sec. To the homogenate (1 ml), 2 ml of TBA/TCA solution (20 mM thiobarbituric acid in 15% trichloroacetic acid) was added and heated for 15 min at 90°C. The reaction mixture was centrifuged at 122×*g* for 15 min and the absorbance of supernatant was measured with a spectrophotometer (Beckman, Fullerton, CA, USA) at 532 nm. The lipid oxidation was reported as mg malondialdehyde/kg sample.

Egg yolk and white (each 5 g) was mixed and the mixture was transferred to a conical tube (50 ml) and then was cooked with boiling water for 10 min as cooked egg for TBARS, DPPH radical scavenging activity and reducing activity of ABTS$^+$.

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

Egg yolk and white (each 5 g) was homogenized in DDW (15 ml) with 10 ml of chloroform for removing excess fat from the sample. The sample was then centrifuged at 209×*g* for 15 min and the supernatant was collected for further analysis. The DPPH radical scavenging activity of egg extract was estimated according to the method of Blois (1958). In detail, 1 ml of egg extract with 1 ml of 0.2 mM DPPH (in methanol) was incubated in dark at room temperature for 30 min. Then, the absorbance of the sample was measured at 517 nm using a spectrophotometer. Radical scavenging activity was expressed as percentage

Table 2. Quality characteristics of the eggs from the hen fed medicinal herb extract mix after storage at 4°C for 2 weeks

MHE (%)[1]	ESH (kg)	ESC (%)	EW (g)	ESW (g)	EST (mm)	AH (mm)	HU	YC (%)
0	3.87	35.04	65.09	7.74	0.33	4.36	58.58	8.32
0.3	3.74	35.40	64.78	7.68	0.33	4.41	58.89	8.11
1.0	3.80	35.83	64.32	7.57	0.34	4.29	57.19	8.34
SEM[2]	0.29	1.71	1.65	0.39	0.02	0.35	4.08	0.22

[1] The medicinal herb extract (mulberry leaf:Japanese honeysuckle:goldthread = 48.5:48.5:3.0). [2] Standard errors of the mean (n = 15).
ESH = Egg shell hardness; ESC = Egg shell color; EW = Egg weight; ESW = Egg shell weight.
EST = Egg shell thickness; AH = Albumen height; HU = Haugh units; YC = Yolk color.

Table 3. TBARS values of the raw and cooked egg from the hen fed dietary medicinal herb extract mix during storage at 4°C

MHE (%)[1]	Storage (day)			
	0	7	14	SEM[2]
Raw egg				
0	0.54	0.49	0.50	0.05
0.3	0.56^{a}	0.47^{b}	0.52^{ab}	0.04
1.0	0.53	0.53	0.52	0.07
SEM[2]	0.04	0.04	0.03	
Cooked egg				
0	1.72^{b}	1.81^{bx}	2.46^{a}	0.27
0.3	1.66^{ab}	1.47^{by}	2.30^{a}	0.34
1.0	1.60^{b}	1.59^{by}	2.24^{a}	0.26
SEM[2]	0.29	0.11	0.19	

Means with superscript ^{x,y} letter in a column and ^{a,b} superscripts in a row significantly different ($p \leq 0.05$).
[1] The medicinal herb extract (mulberry leaf:Japanese honeysuckle: goldthread = 48.5:48.5:3.0).
[2] Strandard errors of the mean (n = 15).

according to the following equation:

DPPH radical scavenging activity (%)
= (1-(absorbance of sample/absorbance of blank)×100)

Reducing activity of 2,2'-azinobis(3-ethylbenzonthianoline-6-sulfonicacid) ($ABTS^{+}$) radical cation

The ABTS radical cation ($ABTS^{+}$) reducing activity was measured according to the method described by Erel (2004). ABTS was dissolved in water to a 7 mM concentration. The ABTS radical cation was produced by reacting the ABTS stock solution with 2.45 mM potassium persulfate (final concentration) in the dark at room temperature for 12-16 h to allow the completion of radical generation. This solution was then diluted with ethanol so that its absorbance was adjusted to 0.70±0.02 at 734 nm. The diluted $ABTS^{+}$ solution (3 ml) were added to 20 μl of egg extract prepared by the same method as DPPH radical scavenging activity, and the absorbance was measured at 734 nm using ethanol as a blank. The percentage inhibition was calculated by the following equation:

$$ABTS^{+}\ \text{reducing activity (\%)} = \frac{(\text{absorbance of control} - \text{absorbance of sample})}{\text{absorbance of control}} \times 100$$

Statistical analysis

Significance among treatments was analyzed by general linear model procedure (SPSS 12) and Turkey's multiple range tests was used to differentiate among the mean values at $p<0.05$ level.

RESULTS AND DISCUSSION

Quality

Quality of eggs from hens fed different levels of MHE during 2 wks of cold storage was shown in Table 2. There was no difference found between the treatment and control group. Hong et al. (2001) reported that egg weight, egg shell breaking strength, and egg shell thickness were not influenced by the supplementation of Korean medicinal herb residue into diet of heat stressed laying hens and agreed well with the present result. However, Shon et al. (2004) reported that the supplementation of 0.2% Animunin Powder® (commercialized herb mix) in the diet of hens resulted into higher egg yolk color and Haugh units. In addition, Liu et al. (2008) reported that a dietary supplementation of herb mix to hen did not affect on proximate composition, color and texture of boiled egg.

Oxidative stability

Oxidative stress in animal lead to deteriorate physiological function that include immune functions and growth and reproduction systems (Miller and Brzezinska-Slebodzinska, 1993).

TBARS value of egg yolk did not show any difference among treatments. However, TBARS value of cooked egg increased during storage regardless of treatment and that from hens fed 1% MHE was lower than that of control group at day 7 (Table 3). No difference was found further in other storage weeks. Botsoglou et al. (2005) reported that dietary saffron (*Crocus sativus* L.) at 10 and 20 mg/kg diet for 6 wks had a dose-dependent antioxidative activity in egg yolk adjusted to pH 6.2. Furthermore this activity was greater than the dietary α-tocopheryl acetate at a 200 mg/kg level. The beneficial effect of dietary supplementation with α-tocopheryl acetate had been reported for the subsequently enhancing lipid stability in poultry meat (Ashgar et al., 1989), pork (Monahan et al., 1992), and rabbit meat (Lopez-Bote et al., 1998). Gladine et al. (2007) suggested

Table 4. 2,2'-azinobis(3-ethylbenzonthianoline-6-sulfonicacid) (ABTS) radical cation of raw and cooked egg from the hen fed medicinal herb extract mix during storage at 4°C

MHE (%)[1]	Storage time (day)			
	0	7	14	SEM[2]
Raw egg				
0	31.1^{by}	48.4^{aby}	54.7^{ay}	3.42
0.3	41.3^{bx}	56.9^{ax}	57.5^{ay}	2.59
1.0	48.1^{bx}	64.5^{ax}	64.0^{ax}	3.63
SEM[2]	4.02	2.92	2.57	
Cooked egg				
0	29.8^{ay}	24.0^{ab}	20.8^{b}	2.99
0.3	31.7^{axy}	20.7^{ab}	25.3^{b}	3.05
1.0	33.2^{ax}	25.8^{b}	23.1^{b}	2.66
SEM[2]	1.50	2.36	4.67	

Means with superscript ^{x,y} letter in a column and ^{a,b} superscripts in a row significantly different ($p \leq 0.05$).

[1] The medicinal herb extract (mulberry leaf:Japanese honeysuckle: goldthread = 48.5:48.5:3.0).

[2] Strandard errors of the mean (n = 15).

Table 5. 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of raw egg from the hen fed medicinal herb extract mix during storage at 4°C[1]

MHE (%)[2]	Storage time (day)			
	0	7	14	SEM[3]
Raw egg				
0	37.8	39.0	41.2	2.24
0.3	37.7	41.5	39.2	1.93
1.0	36.8^{b}	40.6^{a}	36.6^{b}	1.41
SEM[3]	1.17	1.52	2.32	

^{a,b} Means with different superscripts within the same row are significantly different at $p \leq 0.05$.

[1] The activity of cooked egg was not detected in all treatments.

[2] The medicinal herb extract (mulberry leaf:Japanese honeysuckle: goldthread = 48.5:48.5:3.0).

[3] Strandard errors of the mean (n = 15).

that malondialdehyde production concentration of liver was decreased as supplemented with 0.5 g/kg diet of four plant extract (rosemary, grape, citrus, marigold) in rat. Botsoglou et al. (2007) recently reported that incorporation of dehydrated rosemary in turkey diet can delay lipid oxidation in the raw and cooked meat during refrigerated storage. Ko et al. (2008) also reported that the incorporation of 0.5% green tea probiotics to diets lowered TBA values in pork. Also, the dietary green tea waste silage (20%, dry matter) to Holstein steers increased their plasma antioxidative activity and concentration of vitamin E (Nishida et al., 2006). These previous results agreed the animal products obtained from dietary herbs can retard their lipid oxidation.

ABTS is an excellent substrate for peroxidases and is frequently used to study for antioxidant properties of natural compounds and electron transfer reactions involving free radicals (Reszka, 2007). Oxidation of ABTS renders a persistent radical cation, $ABTS^{+}$, and intensely absorbs light in visible region of spectrum. Antioxidant properties of a tested compound are usually measured using inhibitory action on the rate of $ABTS^{+}$ appearance, and/or induction and duration of lag period preceding the appearance of the radical. The testing compound quantitatively estimated by absorbance at certain wavelength (Reszka, 2007).

$ABTS^{+}$ reducing activity of raw egg from hens fed MHE was significantly increased with increasing the level of MHE on layer during entire storage (Table 4). However, in cooked eggs, the difference was found only at day 0, which showed that the dietary MHE at 1% level had higher $ABTS^{+}$ reducing activity than that of control. The $ABTS^{+}$ reducing activity showed much lower in cooked egg compared to raw state. Decreasing $ABTS^{+}$ reducing activity in cooked egg might be the occurred by heat treatment. Lopez-Bote et al. (1998) reported that feeding broilers with the oleoresins led to improved oxidative stability. Our previous results showed that the breast meat from broilers fed medicinal herb extract mix had significantly higher total phenol contents compared with control, although the single compound related to antioxidative capacity was not found in the treated meat (Jang et al., 2008). Ashgar et al. (1989) suggested that the effect of these antioxidants in reducing lipid oxidation of the membrane is related to their accumulation on site or the preservative action of thiol-containing enzymes (glutathione reductase and gluthatione peroxidase). It should also be taken in account that the *in vitro* and *in vivo* antioxidant activity of polyphenols from plant extract did not correlate well (Gladine et al., 2007).

Dietary supplementation of the medicinal herb extract mix did not show any effect on DPPH radical scavenging activity of egg (Table 5). Furthermore, the egg was not shown the DPPH scavenging activity after cooking regardless of treatment (Data not shown). The model of scavenging the stable DPPH radical is a widely used method to evaluate antioxidant activities in a relatively short time compared with other methods (Ahn et al., 2007). This method is based on the reduction of ethanolic DPPH-solution in the presence of a hydrogen donating antioxidant, leading to the formation of non-radical form DPPH-H (Blois, 1958). However, Jung et al. (2008) reported that the diet with extract of mixed medicinal herb (0.05%) was more effective in oxidative stability of chicken meat than that of chicken fed basal diet and diet with antibiotics, Albac G150 (Alpharma Inc., Bridgewater, NJ, USA).

Results of the present study indicate that the dietary MHE slightly enhanced the oxidative stability of egg and may provide a beneficial effect for consumer.

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