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ARTICLE

Physicochemical and Functional Characterization of Blue-Shelled Eggs in Korea

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Abstract

The aim of this study was to compare the quality and physicochemical characteristics of blue-shelled eggs (BE) and conventional eggs (CE). Proximate composition, quality, pH value, shell color, collagen content, fatty acid composition, total cholesterol, α-glucosidase inhibition activity, and antioxidation activity were determined. The proximate composition, general qualities, and pH values of CE and BE showed no significant differences, except in moisture composition, weight, and shell thickness. Moisture content and weight of BE were significantly lower than those of CE. However, shell thickness and weight of BE were higher than those of CE ($p<0.05$). Lightness of BE was significantly higher than that of CE (85.20 vs. 58.80), while redness (a*) and yellowness (b*) of BE were lower than those of CE (a*: -4.75 vs. 14.20; b*: 10.45 vs. 30.63). The fatty acid [C18:1n7 (cis-vaccenic acid) and C18:3n6 (gamma-linolenic acid)] contents of BE were significantly higher than those of CE. The total cholesterol contents of BE and CE were similar. DPPH radical scavenging activity of BE was significantly higher than that of CE (40.78 vs. 35.35). Interestingly, α-glucosidase inhibition activity of whole egg and egg yolk in BE (19.27 and 36.06) was significantly higher than that of whole egg and egg yolk in CE (13.95 and 32.46). This result indicated that BE could potentially be used as a functional food material. Further studies are required to evaluate the specific compounds that affect functional activity.

Keywords blue-shelled egg, egg quality, shell thickness, α-glucosidase inhibition activity

Introduction

Shell color is an important characteristics that influences consumer decision while buying eggs (Wang *et al.,* 2009b). Blue-shelled eggs (BE) from laying hens are unique and interesting, differing from the conventional eggs (CE), which may be white-or brown shelled. Wang *et al.* (2009a) reported that some chicken breeds, such as Araucana and Dongxiang, lay BE. In Korea, BE are laid by the blue hen, which is a crossbreed of the Araucana and the White Leghorn. Several studies have been done on the genetical characteristic of Araucana chicken in Korea (Jeong *et al*., 2016; Oh *et al*., 2016). The Araucana chicken in Korea is a relatively new breed, and it is developed in Gyeongbuk, Korea as Gyeongbuk Araucana domestic chicken hybrid breed (Jeong *et al*., 2016). However, there is little report about the physicochemical characteristics of a blue egg from Araucana hens in Korea.

Recently, BE became more popular and expensive for several reasons (Wang *et al.,* 2009a). This is in accordance with the statement from Millet *et al.* (2006) that the public perceives eggs from a 'fancy' source, such as Araucana chicken, to be

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healthier than those from conventional chicken.

Cholesterol in the egg is also a concern for the consumer, because of their high cholesterol content, and limitation of egg consumption (300 mg/d) is recommended (Salma *et al.,* 2007). Cholesterol is naturally produced and deposited by hen due to the preparation for the embryo. Producing the low-cholesterol egg will be beneficial for health of consumers. Many researches have been made to lower the cholesterol content of egg including genetic selection (Kim *et al.,* 2004a).

It is well known that eggs contain many bioactive components and are highly nutritious. For example, eggs possess antidiabetic functions, according to the report by Yu *et al*. (2011) that suggests that egg white protein hydrolysates are potential α-glucosidase inhibitors. Suppressing carbohydrate absorption in the gut by the application of an α-glucosidase inhibitor is considered as an alternative treatment for diabetes (Bhandari *et al.,* 2008). The α-glucosidase and α-amilase are carbohydrate hydrolysing enzyme, interfering these enzymes by inhibitors are considered for decreasing postprandial hyperglycemia (Kim *et al.,* 2004b). Acarbose and voglibose from microorganisms also nojirimycin and 1-deoxynojirimycin from plants are known as α-glucosidase inhibitors (Kim *et al.,* 2004b). The α-glucosidase inhibitor from the egg is needed for alternative source. In addition, it was reported that egg white is rich in antioxidant peptides, making it a potential source of functional food ingredients for formulating bioactive food products (Chamila *et al.,* 2015). Some chronic diseases, such as diabetes mellitus, arthrosclerosis, cancer, and coronary heart disease, are aggravated by free radicals (Yazdi *et al.,* 2012). Antioxidant peptides from eggs can be used for reducing these free radicals (Chamila *et al.,* 2015). Synthetic antioxidants have risk potential for human health, thus many studies focused on natural antioxidants and in addition the bioactive peptides derived from food protein as a natural antioxidant are easily absorbed (Yazdi *et al.,* 2012). Knowing the egg antioxidant value will give benefit information related to the replacement of synthetic antioxidants. One of the methods for determine the antioxidation activity is 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity. Some studies have shown that egg has a good value on DPPH scavenging activity (Chen *et al*., 2012; Lin *et al*., 2013; Liu *et al*., 2015; Yazdi *et al*., 2012). The aim of this study was to compare the physicochemical characteristics, quality, total cholesterol, α-glucosidase inhibition activity, and antioxidation activity of BE with those of CE.

Material and Methods

Eggs

The BE used in this study were obtained from farms in Hwasung-si in Korea. All the crossbred blue hens (Araucana × Ogol × White Leghorn) were kept together in brooding and rearing cages after hatching until 14 wk of age. Two hundred pullets were housed indoors at night. However, they spent some time in free range during the day. They were fed a commercial diet, as well as grass and worms, on the range. Among one hundred of laid BE were randomly selected and stored at 23±1°C before the experiment. CE (Hyline Brown commercial eggs) were obtained from the local market in Hwasung-si.

Proximate composition

The proximate composition of each egg was determined based on standard Association of Official Analytical Chemists (1995) methods. Moisture content was determined from weight loss after drying for 12 h at 105°C in a drying oven (Vision Scientific Co., Ltd., Korea). Crude fat content was determined using a Soxhlet solvent extraction system. The fat content was calculated from the weight loss after lipid extraction with diethyl ether in the Soxhlet apparatus (Soxtec system HT 1043 extraction unit, Foss tecator, Sweden). Crude protein content was determined by the Kjeldahl method as follows: 0.3 g of the sample in oil paper, one Kjeltec catalyst tablet, and 10 mL H_2SO_4 were added into a Kjeldahl tube and digested for 1 h at 420°C. The product was then analyzed using an automatic Kjeldahl analyzer (Kjeltec auto sampler system 1035 analyzer, Foss tecator, Sweden). Crude ash content was determined from weight loss of sample after heating at furnace (30400, Thermolyne, USA) at 550°C for 24 h.

Egg quality

Egg quality was estimated according to the method reported by Liu *et al.* (2009). Weight (g), albumen height (mm), yolk color, Haugh units, and shell thickness (mm) were determined using an Egg Multitester model EMT-5200 (Touhoku Rhythm Co., Ltd., Japan). Shell thickness (mm) was determined using a micrometer (Digimatric Outside Micrometer, Mitutoyo Corporation, Japan)

pH value

The pH value was estimated according to the method reported by Choe *et al.* (2011). pH values were determined with a pH meter (Orion 230 A, Thermo Fisher Sci. Inc.,

USA), after blending 5 g of sample with 20 mL of distilled water for 60 s in a homogenizer (Polytron ® PT-2500 E, Kinematica, Switzerland).

Collagen content

The collagen content was estimated according to the method reported by Kolar (1990). The egg was hydrolyzed in 7 N sulfuric acid at 105°C for 16 h. Then, 1 mL of the hydrolyzed diluted sample was mixed with 0.5 mL of oxidative solution (1.41% chloramine T in collagen buffer solution). The collagen buffer (pH 6.0) was composed of 90 g of sodium acetate trihydrate, 15 g of sodium hydroxide, 30 g of citric acid monohydrate, and 290 mL of 1-propanol per liter. The mixture was incubated in the dark at room temperature for 20 min. The reaction tube was blended and 0.5 mL of reactive color (5 g of 4-methylaminobenzaldehyle with 17.5 mL of 60% sulfuric acid and 32.5 mL of 2-propanol) was added and incubated in a constant-temperature water bath at 60°C for 1 min. After sample incubation, the absorbance of hydroxyproline in the sample was measured at 558 nm using a UV/VIS spectrophotometer (Molecular Device, M2e, USA). A hydroxyproline standard curve was prepared at concentrations of 0.3 μg/mL, 0.6 μg/mL, 1.2 μg/mL, 2.4 μg/mL, and 4.8 μg/mL. Collagen content was calculated using a correction factor of 13.5.

Eggshell color

Eggshell color was estimated according to the method reported by Choe *et al.* (2011). The color measurements were performed using a colorimeter (CR-300 Minolta Co., Japan). The color values (CIE, L^*, a^*, and b^*) of the egg surfaces were measured in triplicate for each egg.

Fatty acid composition measurement

Lipids were extracted according to the method given by Morrison and Smith (1964). Two grams of the freeze-dried extracts of yolk was mixed with 20 μL 0.3% BHA and 20 mL folch solution, which is a mixture of chloroform and methyl alcohol (2:1 vol/vol). After the sample was homogenized, the mixture was filtered using Whatman No.1 filter paper, and the filtered sample was mixed with 0.88% KCl, followed by centrifugation at 1,000×*g* at 15°C for 10 min. The lower phase was concentrated using nitrogen gas. The sample was blended with 14% boron trifluoride (BF_3) in a constant-temperature water bath at 90°C for 1 h, and then dissolved in hexane. The upper layer was filtered using a 0.22-μm filter. Fatty acid was separated and identified using a gas chromatography (Agilent 6890N, Agilent Technologies, USA) system with a Cp-sil BB capillary column (Agilent CP 7489, 100 cm × 0.25 mm × 0.20 μm). The apparatus was programmed at an initial temperature of 150°C, allowing an increase of 7°C/min until the temperature reaches 200°C, which was maintained for 10 min, followed by an increase of 3°C/min until a final temperature of 250°C was reached, which was maintained for 5 min.

Total cholesterol

Freeze-dried extracts (2 g each) of BE and CE yolks were mixed with 20 μL 0.3% BHA and 20 mL Folch solution. After incubating overnight, water and chloroform were removed using nitrogen gas. Sample was blended with 14% BF3 in a constant-temperature water bath at 90°C for 1 h and dissolved in ethanol. Then, 0.02 mL sample was mixed with 3 mL enzyme solution kit (Asan Pharmaceutical, Korea) and incubated at 37°C for 5 min. The cholesterol content was measured spectrophotometrically at 500 nm (SpectraMax M2e, Molecular Devices, USA). The concentration was also expressed as milligram per gram yolk weight.

Ability of eggs to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical

DPPH radical scavenging activity of a sample was estimated according to the method of Blois (1958) with slight modification. In brief, 100 μL of sample solution after extraction was added to 100 μL of methanol (for reference) and to 100 μL of methanolic DPPH solution (0.2 mM). The mixture was vortexed and incubated at room temperature for 30 min. A tube containing 100 μL of distilled water and 100 μL of methanolic DPPH solution (0.2 mM) served as the control. The absorbance of the solution was measured spectrophotometrically at 517 nm. (SpectraMax M2e, Molecular Devices, USA). Trolox was used as a positive control to compare the antioxidation activities of the hydrolysate samples. The percentage of DPPH radical scavenging activity was determined using the following equation:

$$DPPH\ radical\ scavenging\ activity\ (\%) = 1 - \frac{(sample\ O.D. - reference\ O.D.)}{(control\ O.D.)} \times 100$$

*Control = 100 μL DW + 100 μL 0.2 DPPH mM Solution in MeOH

*Reference = 100 μL DW + 100 μL MeOH

α-glucosidase inhibition activity

The α-glucosidase inhibition assay was performed according to a previously described method (Dong *et al.,* 2012) with slight modification. Briefly, 60 μL of the sample solution and 50 μL of 0.1 M phosphate buffer (pH 6.8), containing α-glucosidase solution (0.2 U/mL), was incubated in a 96-well plate at 37°C for 20 min. After pre-incubation, 50 μL of 5 mM p-nitrophenyl-a-D-glucopyranoside solution in 0.1 M phosphate buffer (pH 6.8) was added to each well in the 96-well plate and incubated at 37°C for 30 min. Then, the reaction was stopped by adding 160 μL of 0.2 M Na_2CO_3 into each well, and absorbance readings (O.D) were recorded at 405 nm using a micro-plate reader (Spectra Max M2e, Molecular Devices, USA). The measured values were compared to a control, which contained 60 μL of buffer solution instead of the extract. The α-glucosidase inhibition activity was calculated as follows:

$$\text{Inhibition (\%)} = \frac{(control\ O.D. - sample\ O.D.)}{control\ O.D.} \times 100$$

Statistical analyses

Analyses of variance were conducted using SAS software, and Tukey's test was used to compare differences among mean values. Mean values and pooled standard errors of the mean (SEM) were reported and the significance was defined at $p<0.05$.

Results and Discussion

Proximate composition

The proximate compositions of CE and BE are shown in Table 1, divided into three parts: whole egg, egg yolk, and egg white. Moisture, crude protein, crude fat, and crude ash compositions of CE and BE were 75.89% and 74.89%, 11.99% and 12.05%, 10.46% and 10.77%, and 0.78% and 0.69%, respectively. Our findings are consistent with those of U.S. Department of Agriculture (2016) that reported the following proximate compositions of whole egg: 76.15% moisture, 12.56% protein, and 9.51% fat. The whole egg moisture composition of BE was significantly lower than that of CE. The moisture composition is related to the housing management. In this study, the BE layer hens were kept and maintained in the free range condition, however, they were housed indoor at night. Whereas mostly CE hens were housed indoor and controlled environment. In free range housing system hens are living in more variable circumstances and not fully controlled environment. The egg in the outdoor housing system had more contact with the environment, and the moisture composition in the egg loss may due to the water evaporation. Van Den Brand *et al.* (2004), they reported that indoor housing system had less evaporation. The egg yolk and egg white proximate compositions of CE and BE did not show significant differences.

Table 1. Proximate composition of commercial egg (CE) and blue shelled egg (BE) egg (%)

Items	Proximate composition	CE	BE	SEM
Whole egg	Moisture	75.89[a]	74.89[b]	0.160
	Crude protein	11.99[a]	12.05[a]	0.020
	Crude fat	10.46[a]	10.77[a]	0.118
	Crude ash	0.78[a]	0.69[a]	0.060
Egg yolk	Moisture	51.30[a]	51.71[a]	0.208
	Crude protein	15.97[a]	15.74[a]	0.103
	Crude fat	29.30[a]	28.93[a]	0.308
	Crude ash	1.63[a]	1.64[a]	0.088
Egg white	Moisture	87.87[a]	88.07[a]	0.070
	Crude protein	10.20[a]	10.26[a]	0.064
	Crude fat	0.09[a]	0.08[a]	0.007
	Crude ash	0.44[a]	0.52[a]	0.122

SEM, standard error of the means (n=50).
[a,b]Means within the same row with different superscript letters differ significantly at $p<0.05$.

Table 2. Egg quality of commercial egg (CE) and blue shelled egg (BE)

Items	CE	BE	SEM
Weight (g)	58.58[a]	49.08[b]	0.862
Albumen Height (mm)	5.42[a]	4.96[a]	0.300
Yolk color	7.74[a]	7.58[a]	0.201
Haugh unit	73.04[a]	69.78[a]	2.551
Shell thickness (mm)	0.29[b]	0.32[a]	0.192

SEM, standard error of the means (n=50).
[a,b]Means within the same row with different superscript letters differ significantly at $p<0.05$.

Egg quality

The average values for all monitored egg quality indicators of CE and BE are presented in Table 2. Internal quality of eggs started to decline soon after they were laid. The egg internal quality was greater when measured soon after laid (fresh) compared when measured after storage (Mohiti-Asli *et al.,* 2008). In this study, most of the egg quality indicators, except weight and shell thickness, showed no significant differences between CE and BE. The weight of CE was significantly higher than that of BE (58.8 g vs. 49.08 g). The BE has a lower moisture content

and may this is affected on egg weight. The weight of BE was lower than that of CE may due to the genetic difference of BE. However, the shell of BE was significantly thicker than that of CE (0.32 mm vs. 0.29 mm). The different eggshell thickness might be explained by the amount of dietary Ca and P (Leeson *et al.,* 1993). Although the amount of dietary mineral is not clearly explained in this study, the mineral intake may higher in the BE hens due to free range housing system, because in the range, the hens possibly get an extra mineral intake from grass and worms. The walking activity in the free range housing system also results in more effective mineral metabolism (Van Den Brand *et al.,* 2004). Shell strength and thickness are important parameters for economic consideration, since the thickness of the shell is related to microbial contamination and breakage during processing or storage (Biladeau and Keneer, 2009). Based on the shell thickness, BE may be more resistant to microbial contamination than CE, resulting in longer shelf life. Our findings agree well with those reported by Wang *et al*. (2009a), who reported the weight, albumen height, yolk color, Haugh unit, and shell thickness of BE (Dongxiang) as 46.42 g, 5.34 mm, 7.98, 76.15, and 0.33 mm, respectively. Moreover, the egg quality can be affected by different housing systems: outdoor and cage housing system (Van Den Brand *et al.,* 2004). In Korea, BE production is not largely prevalent yet, thus necessitating further studies regarding housing, egg laying, and egg collection for blue hens.

pH value and collagen content

pH values were measured on whole egg, egg yolk, and egg white of CE and BE (Table 3). The pH values of CE and BE were not significantly different: 7.56 and 7.59, whole egg; 6.28 and 6.34, egg yolk; and 9.24 and 9.17, egg white, respectively. The pH values of whole eggs in this study are consistent with those reported by Wong *et al.* (1996), who observed pH values ranging from 7.54 to 7.87. Furthermore, they reported that the pH values can be influenced by eggshell coating, which controls the rate of carbon dioxide transfer through the shell.

Collagen is mostly found in the skin, cartilage, and connective tissues (Khiari *et al.,* 2014). However, eggs also contain collagen; it has been observed in the whole egg, egg yolk, and egg white (Yamauchi *et al.,* 2016). Collagen content in BE and CE in the whole egg was found to be 1.01 mg/mL and 1.10 mg/mL; in the egg yolk, 1.57 mg/mL and 1.66 mg/mL; and in the egg white, 0.63 mg/mL and 0.68 mg/mL, respectively (Table 3). The collagen contents in whole egg and egg yolk of BE were significantly higher than those of CE. The collagen content in BE was higher may due to the higher plant intake (grass) in the free range housing system. In agreement with Yamauchi *et al.* (2016) study that showed higher collagen content in the egg which treated with the plant processing product diet supplementation. Recently, the demand for the collagen has increased due to their beneficial effects on skin, as collagen is believed to prevent the formation of wrinkles on the skin (Khiari *et al.,* 2014). Our results showed that BE has a higher collagen content than CE, and, thus, BE could have better effect on the skin than CE.

Table 3. pH value and the collagen content (mg/ml) of commercial egg (CE) and blue shelled egg (BE)

	Items	CE	BE	SEM
pH	Whole egg	7.56[a]	7.59[a]	0.021
	Egg yolk	6.28[a]	6.34[a]	0.040
	Egg white	9.24[a]	9.17[a]	0.045
Collagen Contents	Whole egg	1.010[b]	1.10[a]	0.011
	Egg yolk	1.57[b]	1.66[a]	0.016
	Egg white	0.63[b]	0.68[a]	0.007

SEM, standard error of the means (n=50).
[a,b]Means within the same row with different superscript letters differ significantly at $p<0.05$.

Table 4. Shell color of commercial egg (CE) and blue shelled egg (BE)

Items	CE	BE	SEM
*L**	58.80[b]	85.20[a]	0.080
*a**	14.20[a]	-4.75[b]	0.128
*b**	30.63[a]	10.45[b]	0.211

SEM, standard error of the means (n=50).
[a,b]Means within the same row with different superscript letters differ significantly at $p<0.05$.

Eggshell color

Eggshell color influence the consumer's decision to purchase the egg, making it an important characteristic (Wong *et al.,* 1996). The visual appraisal is the first test that consumers conduct, while making a decision to purchase products. As shown in Table 4, the color values (*L**, *a**, and *b**) were significantly different between BE and CE. The *L** value of the shell color of BE was higher than that of CE, possibly due to bright blue color of BE compared to the dull brown color of CE. The *a** and *b** values were also significantly different. The *a** values of CE and BE were 14.20 and -4.75, respectively, indicating that CE was towards red, while BE was towards green. The towards red color in CE which had a brown eggshell color due to

Table 5. Fatty acid composition and total cholesterol content of commercial egg (CE) and blue shelled egg (BE) (%)

Fatty acid	Name	CE	BE	SEM
C14:0	Myristic acid	0.29[a]	0.27[a]	0.015
C16:0	Palmitic acid	27.29[a]	27.20[a]	0.240
C16:1n7	Palmitoleic acid	2.02[a]	1.21[b]	0.085
C18:0	Stearic acid	12.39[a]	13.08[a]	0.211
C18:1n9	Oleic acid	35.77[a]	33.46[b]	0.413
C18:1n7	cis-vaccenic acid	0.20[b]	0.93[a]	0.199
C18:2n6	Linoleic acid	12.37[a]	13.98[a]	0.598
C18:3n6	Gamma-Linolenic acid	0.10[b]	0.12[a]	0.003
C18:3n3	Linolenic acid	0.20[a]	0.19[a]	0.024
C20:1n9	Eicosenoic acid	1.40[a]	1.58[a]	0.127
C20:4n6	Arachidonic acid	4.32[a]	4.05[a]	0.140
C20:5n3	Eicosapentaenoic acid (EPA)	2.07[a]	2.45[a]	0.324
C22:4n6	Adrenic acid	0.36[a]	0.34[a]	0.014
C22:6n3	Docosahexaenoic acid (DHA)	1.29[a]	1.13[a]	0.069
	SFA	39.98[a]	40.55[a]	0.191
	USFA	60.02[a]	59.45[a]	0.191
	MUFA	39.31[a]	37.19[a]	0.733
	PUFA	20.71[a]	22.26[a]	0.811
	w3/w6	0.21[a]	0.20[a]	0.020

SEM, standard error of the means (n=50).
[a,b]Means within the same row with different superscript letters differ significantly at $p<0.05$.

protoporphyrin and uroporphyrin content as a red color pigment (Lukanov *et al.,* 2015). The *b** values of CE and BE were 30.63 and 10.45, respectively, indicating that CE was towards yellow, while BE was towards blue. It is well known that the pigments in BE are biliverdin IX and biliverdin zinc chelate (Lukanov *et al.,* 2015).

Fatty acid composition and total cholesterol content

The fatty acid compositions of CE and BE are shown in Table 5. Several fatty acid contents in BE, such as C18:1n7 cis-vaccenic acid (0.93 vs. 0.20) and C18:3n6 gamma-linoleic acid (0.12 vs. 0.10), were significantly higher than those in CE. The difference of chemical composition including fatty acid on a chicken egg can be affected by many factors such as breeds (Simmons and Somes, 1985), different dietary supplementation (Altuntas and Aydin, 2014), and also housing system (Matt *et al.,* 2009). The C18:3n6 fatty acid was higher in BE due to the higher intake of the plants as a feed supplementation in the free range, agree with those of reported by Altuntas and Aydin (2014) the C18:3n6 fatty acid in the egg was higher in the plant supplementation. Field *et al.* (2009) reported that consuming vaccenic acid is beneficial for health as it could possibly reduce tumor growth and prevent myocardial infarction (heart attack). Gamma-linoleic acid (GLA) content of BE was significantly higher than that of CE. Fan and Chapkin (1998) demonstrated that dietary GLA reduces the possibility of a cardiovascular disease, as GLA is the fatty acid source of dihomo gamma linoleic acid (DGLA), which becomes prostaglandin of the 1-series (PGE1), known to suppress plaque formation in the blood vessels. They also reported that the GLA could potentially suppress tumor growth and metastasis. Another study reported that the compositions of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) in the egg are 32.2, 48.9, and 19.2%, respectively (Grobas *et al.,* 2001). In this study, the SFA and PUFA contents were higher than those reported in the study of Grobas *et al.* (2001), while the MUFA content was lower. The concentrations of the different fatty acids in the egg depend on breeds and dietary supplements provided for the chickens, as reported by Millet *et al.* (2006). The total SFA, PUFA, and MUFA compositions in BE and CE showed no significant differences.

The high cholesterol content is generally an unwanted characteristic of eggs (Millet *et al.,* 2006), as cholesterol is associated with the risk of coronary heart disease (Castelli *et al.,* 1977; Mannien *et al.,*1992; Naviglio *et al.,* 2012). Krauss *et al.* (2000) recommend a dietary cholesterol intake for all individuals to be <300 mg/d, and USDA (2016) reported that the cholesterol content of the jumbo egg (63

g edible portion) was 234 mg. Therefore, consuming one egg per day is acceptable. Cholesterol is naturally produced and deposited by hen due to the preparation for the embryo. Many studies have been done to reduce cholesterol content in egg including dietary treatment (Park *et al.,* 2005), storage treatment (Mazalli and Bragagnolo, 2009) and also by mixing the egg yolk with another compound (Valcare *et al.,* 2002). Producing the high cholesterol content will increasing the risk of coronary heart disease, thus the cholesterol content in BE and CE was measured. In agreement with Salma *et al.* (2007) that genetic selection is one of the efforts for reducing egg cholesterol content.

The cholesterol content in the yolks of BE and CE were measured. The total cholesterol content in the egg yolk of CE and BE are not significantly different (19.72 mg/g and 18.93 mg/g, respectively). This result contradicts the findings of Somes *et al.* (1977) and Simmons and Somes (1985), who reported that BE (Araucana) has higher cholesterol content than CE. The cholesterol contents of yolks of BE and CE, reported by Somes *et al.* (1977), were 21.8 mg/g and 20.4 mg/g, respectively. Simmons and Somes (1985) reported that BE has 22.55 mg/g cholesterol content and CE (White Leghorn), 21.56 mg/g. In their study, the cholesterol content of BE was higher than that in our findings. Wang *et al.* (2015) reported that the cholesterol content of eggs from chickens provided with a different dietary treatment was 15.7 mg/g yolk. Another study, Wang *et al.* (2009a) reported that the cholesterol content of BE (Dongxiang) was significantly lower when the chickens were raised in an outdoor housing system (8.64 mg/g), as compared to that noted in eggs from chickens raised in a cage housing system (10.32 mg/g). The total cholesterol content was higher in this study, as compared to that reported by other studies (Wang *et al.,* 2009a; Wang *et al.,* 2015). This difference may due to the differences in breed and treatment of the hens, even though they all produced BE. Cunningham (1977) has reported that the differences in total cholesterol content of egg may vary due to multiple factors: differences in breed, experimental conditions, and extraction methods influencing the yolk cholesterol concentration of the egg. The cholesterol contents of CE and BE in this study were not significantly different, possibly because the BE hen, obtained from crossbreeding conventional chicken with Ogol chicken and Araucana, was genetically influenced by the conventional hen.

DPPH radical scavenging activity

The DPPH scavenging activity is one of the methods to determine the antioxidation activity. Table 6 shows that the DPPH scavenging activity of BE (40.78%) was significantly higher than that of CE (35.35%). This result is similar to that of Lin *et al.* (2013), who reported that the DPPH scavenging activity derived from egg white is 44.92%. Another study reported that the DPPH scavenging activity of egg white protein in their study was 39.51% (Liu *et al.,* 2015). DPPH scavenging activity of BE in our study showed a higher value (40.78%), as compared to the reports of Liu *et al.* (2015). Another study reported that the DPPH radical scavenging activity of the egg white lysozyme hydrolysates by trypsin, papain, and trypsin-papain were 37.2%, 50.4%, and 64.2% respectively (Yazdi *et al.,* 2012). In our study, BE showed a higher DPPH scavenging activity than that of trypsin egg white lysozyme hydrolysate. However, the activity of BE was lower than that of the papain and trypsin-papain hydrolysates from eggs reported by Yazdi *et al.* (2012). The smaller size of hydrolysates after degradation by enzyme may result in a higher antioxidation activity. Chen *et al.* (2012) reported that the DPPH scavenging activity of the egg white protein hydrolysates by papain after 3 h of hydrolysis was 73.14%, which was higher than that in our findings.

Table 6. DPPH radical scavenging activity of commercial egg (CE) and blue shelled egg (BE) (%)

Items	CE	BE	SEM
Egg yolk	35.35[b]	40.78[a]	0.365
Egg White	n.t [1)]	n.t	n.t

SEM, standard error of the means (n=50).
[a,b]Means within the same row with different superscript letters differ significantly at $p<0.05$.
[1)] n.t, Not Tested.

Table 7. α-glucosidase inhibition activity of commercial egg (CE) and blue shelled egg (BE) (%)

Items	CE[1)]	BE[1)]	SEM
Whole egg	13.95[b]	19.27[a]	0.406
Egg yolk	32.46[b]	36.06[a]	0.787
Egg white	n.d[2)]	n.d	n.d

SEM, standard error of the means (n=50).
[a,b]Means within the same row with different superscript letters differ significantly at $p<0.05$.
[1)]All samples used were at 90 mg/mL concentration.
[2)]n.d, Not detected.

α-glucosidase inhibition activity

The α-glucosidase inhibition activity was measured for the whole egg, egg yolk, and egg white. Table 7 shows that in the 90 mg/mL concentration, the α-glucosidase inhibi-

tion activity values of BE were significantly higher than those of CE (19.27 vs. 13.95 in the whole egg and 36.06 vs. 32.46 in the egg yolk). No α-glucosidase inhibition activity was detected in the egg white. α-glucosidase is one of the enzymes that hydrolyzes carbohydrates into glucose (Kim *et al.,* 2004b). Preventing absorption of glucose could be beneficial for diabetics. This activity is one of the therapeutic parameters for diabetics (Kim *et al.,* 2004b). One of the antidiabetic agents for commercial use is acarbose, which showed 45.8% of α-glucosidase inhibition activity at a concentration of 1.2 mg/mL (Dong *et al.,* 2012). Acarbose is obtained from the fermentation process of a microorganism *Actinoplanes utahensis* (Wang *et al.,* 2012). Another study reported that trilobatin, derived from *Lithocarpus polystachyus* Rehd. at a concentration of 0.8 mg/mL, has an 80% higher α-glucosidase inhibition activity than acarbose at the same concentration (Dong *et al.,* 2012).

Yu *et al.* (2011) reported that the α-glucosidase inhibition activities of the bioactive peptides (RVPSLM and TPSPR) derived from egg white protein were comparable to that of acarbose. The IC_{50} values of the peptides RVPSLM and TPSPR were 23.07 μmol L^{-1} and 40.02 μmol L^{-1}, respectively, and the IC_{50} value of acarbose was 60.8 μmol L^{-1} (Yu *et al.,* 2011). The α-glucosidase inhibition activity from the bioactive peptide derived from BE egg yolk protein is higher than that noted for CE. In this study, BE was shown to have a significantly higher α-glucosidase inhibition activity than CE. Nevertheless, further studies regarding bioactive peptides in BE, which can function as antidiabetics, are necessary.

Conclusion

In general, the egg quality between BE and CE showed no significant differences except egg weight. Although the CE was heavier than BE, BE had a higher antioxidant activity and α-glucosidase inhibition activity. The difference of characteristics between the two types of eggs may be due to the differences in the breeds of chicken. Further studies are necessary to identify the stability of those functions for the egg industry and consumers.

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References

1. Altuntaş, A. and Aydin, R. (2014) Fatty acid composition of egg yolk from chickens fed a diet including Marigold (*Tagetes erecta* L.). *J. Lipids* **2014**, 1-4.
2. AOAC (1995) Official methods of analysis of AOAC. 16th Ed. Association of Official Analytical Chemists. Washington D.C.
3. Bhandari, M. R., Jong, A. N., Hong, G., and Kawabata, J. (2008) Alpha-glucosidase and alpha-amylase inhibitory activities of Nepalese medicinal herb Pakhanbhed (*Bergenia ciliata* Haw.). *Food Chem.* **106,** 247-252.
4. Biladeau, A. M. and Keener, K. M. (2009) The effects of edible coatings on chicken egg quality under refrigerated storage. *Poultry Sci.* **88,** 1266-1274.
5. Blois, M. S. (1958) Antioxidant determination by the use of a stable free radical. *Nature* **181,** 1199-1200.
6. Castelli, W. P., Doyle, J. T., Gordon, T., Hames, C. G., Hjortland, M. C., Hulley, S. B., Kagan, A., and Zukel, W. J. (1977) HDL cholesterol and other lipids in coronary heart disease the cooperative lipoprotein phenotyping study. *Circulation* **55,** 767-772.
7. Chamila, N., Nandika, B., and Jianping, W. (2015) Purification and characterization of antioxidant peptides from enzymatically hydrolyzed chicken egg white. *Food Chem.* **188,** 467-472.
8. Chen, C., Chi, Y. J., Zhao. M. W., and Lv, L. (2012) Purification and identification of antioxidant peptides from egg white protein hydrolysate. *Amino Acid.* **43,** 457-466.
9. Choe, J. H., Jang, A., Lee, E. S., Choi, J. H., Choi, Y. S., Han, D. J., Kim, H. Y., Lee, M. A., Shim, S Y., Kim, C. J. (2011) Oxidative and color stability of cooked ground pork containing lotus leaf (*Nelumbo nucifera*) and barley leaf (*Hordeum vulgare*) powder during refrigerated storage. *Meat Sci.* **87,** 12-18.
10. Cunningham, F. E. (1977) Composition of Araucana eggs. *Poultry Sci.* **56,** 463-467.
11. Dong, H. Q., Li, M., Zhu, F., Liu, F. L., and Huang, J. B. (2012) Inhibitory potential of trilobatin from *Lithocarpus polystachyus* Rehd against α-glucosidase and a-amylase linked to type 2 diabetes. *Food Chem.* **130,** 261-266.
12. Fan, Y. Y. and Chapkin, R. S. (1998) Importance of dietary γ-linoleic acid in human and nutrition. *J. Nutr.* **128,** 1411-1414.
13. Field, C. J., Blewett, H. H., Proctor, S., and Vine, D. (2009) Human health benefits of vaccenic acid. *Appl. Physiol. Nutr. Metab.* **34,** 979-991.
14. Grobas, S., Méndez, J., Lázaro, R., de Blas, C., and Mateos, G. G. (2001) Influence of source and percentage of fat added to diet on performance and fatty acid composition of egg yolks of two strains of laying hens. *Poultry Sci.* **80,** 1171-1179.

15. Jeong, H., Kim, K., Caetano-Anollés, K., Kim, H., Kim, B. K., Yi, J. K., and Oh, D. Y. (2016) Whole genome sequencing of Gyeongbuk Araucana, a newly developed blue-egg laying chicken breed, reveals its origin and genetic characteristics. *Sci. Rep.* **6,** 1-9.
16. Khiari, Z., Ndagijimana, M., and Betti, M. (2014) Low molecular weight bioactive peptides derived from the enzymatic hydrolysis of collagen after isoelectric solubilization/precipitation process of turkey by-products. *Poultry Sci.* **93,** 2347-2362.
17. Kim, J. H., Hong, S. T., Lee, H. S., & Kim, H. J. (2004a). Oral administration of pravastatin reduces egg cholesterol but not plasma cholesterol in laying hens. *Poultry Sci.* **83,** 1539-1543.
18. Kim, Y. M., Wang, M. H., and Rhee, H. I. (2004b) A novel α-glucosidase inhibitor from pine bark. *Carbohyd. Res*. **339,** 715-717.
19. Kolar, K. (1990) Colorimetric determination of hydroxyproline as measure of collagen content in meat and meat products: NMKL collaborative study. *J. Assoc. Off. Anal. Chem.* **73,** 55-60.
20. Krauss, R, M., Eckel, R, H., Howard, B., Appel, L, J., Daniels, S, R., Deckelbaum, R, J., Erdman, Jr, J, W., Etherton, P, K., Goldberg, I, J., Kotchen, T, A., Lichtenstein, A, H., Mitch, W, E., Mullis, R., Robinson, K., Rosett J, W., St. Jeor, S., Suttie, J., Tribble, D, L., Bazzarre, T, L. (2000) AHA dietary guidelines: revision 2000: a statement for healthcare professionals from the nutrition committee of the american heart association. *Circulation* **102,** 2284-2299.
21. Leeson, S., Summers, J. D., and Caston, L. (1993) Response of brown-egg strain layers to dietary calcium or phosphorus. *Poultry Sci*. **72,** 1510-1514.
22. Lin, S., Jin, Y., Liu, M., Yang, Y., Zhang, M., Guo, Y., Liu, J., Yin, Y., and Jones, G. (2013) Research on the preparation of antioxidant peptides derived from egg white with assisting of high-intensity pulsed electric field. *Food Chem.* **139,** 300-306.
23. Liu, J., Jin, Y., Lin, S., Jones, G. S., and Chen, F. (2015) Purification and identification of novel antioxidant peptides from egg white protein and their antioxidant activities. *Food Chem.* **175,** 258-266.
24. Liu, X. D., Jang, A., Kim, D. H., Lee, B. D., Lee, M., and Jo, C. (2009) Effect of combination of chitosan coating and irradiation on physicochemical and functional properties of chicken egg during room-temperature storage. *Radiat. Phys. Chem.* **78,** 589-591.
25. Lukanov, H., Genchev, A., and Phaflov, A. (2015) Colour traits of chicken eggs with different eggshell pigmentation. *Trakia J. Sci*. **2,** 149-158.
26. Manninen, V., Tenkanen, L., Koskinen, P., Huttunen, J. K., Manttari, M., Heinonen, O. P., and Frick, M. H. (1992) Joint effects of serum triglyceride and LDL cholesterol and HDL cholesterol concentrations on coronary heart disease risk in the Helsinki heart study, implications for treatment. *Circulation* **85,** 37-45.
27. Matt, D., Veromann, E., and Luik, A. (2009) Effect of housing systems on biochemical composition of chicken eggs. *Agron. Res.* **7,** 662-667.
28. Mazalli, M. R and Bragagnolo, N. (2009) Increase of cholesterol oxidation and decrease of pufa as a result of thermal processing and storage in eggs enriched with n-3 fatty acids. *J. Agric. Food Chem.* **57,** 5028-5034
29. Millet, S., De Ceulaer, K., Van Paemel, M., Raes, K., De Smet, S., and Janssens, G.P.J. (2006) Lipid profile in eggs of Araucana hens compared with Lohmann Selected Leghorn and ISA Brown hens given diets with different fat sources. *Br. Poult. Sci*. **47,** 294-300.
30. Mohiti-Asli, M., Shariatmadari, F., Lotfollahian, H., and Mazuji, M. T. (2008) Effects of supplementing layer hen diets with selenium and vitamin E on egg quality, lipid oxidation and fatty acid composition during storage. *Can. J. Anim. Sci*. **88,** 475-483.
31. Morrison, W. R. and Smith, L. M. (1964) Preparation of fatty acid methyl esters and dimethyl acetals from lipids with boron trifluoride-methanol. *J. Lipid Res.* **5,** 600-608.
32. Naviglio, D., Gallo, M., Grottaglie, L. L., Scala, C., Ferrara, L., and Santini, A. (2012) Determination of cholesterol in Italian chicken eggs. *Food Chem.* **132,** 701-708.
33. Oh, D., Son, B., Mun, S., Oh, M. H., Oh, S., Ha, J., and Han, K. (2016) Whole genome re-sequencing of three domesticated chicken breeds. *Zool. Sci.* **33,** 73-77.
34. Park, B. S., Kang, H. K., and Jang, A. (2005) Influence of feeding β-Cyclodextrin to laying hens on the egg production and cholesterol content of egg yolk. *Asian-Aust. J. Anim. Sci.* **18,** 835-840.
35. Salma, U., Miah, A. G., Tareq, K. M. A., Maki, T., and Tsujii, H. (2007) Effect of dietary *Rhodobacter capsulatus* on egg-yolk cholesterol and laying hen performance. *Poultry Sci.* **86**, 714-719.
36. Simmons, III. and Somes, J. R. (1985) Chemical characteristics of Araucana chicken eggs. *Poultry Sci.* **64,** 1246-1268.
37. Somes, R. G., Francis, P. V., and Tlustohowicz, J. J. (1977) Protein and cholesterol content of Araucana chicken eggs. *Poultry Sci.* **56,** 1636-1640.
38. USDA (2016) USDA National Nutrient Database for Standard Reference. Basic Report 01123, Egg, whole, raw, fresh. Report Date:June 15, 2016 00:17 EDT. The National Agricultural Library.
39. Valcarce, G., Nusblat, A., Florin-Christensen, J., and Nudel, B. C. (2002) Bioconversion of egg cholesterol to pro-vitamin D-sterols with Tetrahymena Thermophila. *J. Food Sci.* **67,** 2045-2409.
40. Van Den Brand, H., Parmentier, H. K., and Kemp, B. (2004) Effects of housing system (outdoor vs. cages) and age of laying hens on egg characteristics. *Br. Poult. Sci.* **45,** 745-752.
41. Wang, C. L., Chiang, C. J., Chao, Y. P., Yu, B., and Lee, T. T. (2015) Effect of cordyceps militaris waster medium on production performance, egg traits and egg yolk cholesterol of laying hens. *J. Poult. Sci.* **52,** 188-196.
42. Wang, X. L., Zheng, J. X., Ning, Z. H., Qu, L. J., Xu, G. Y., and Yang, N. (2009a) Laying performance and egg quality of

blue-shelled layers as affected by different housing systems. *Poultry Sci.* **88,** 1485-1492.
43. Wang, X. T., Zhao, C. J., Li, J. Y., Xu, G. Y., Lian, L. S., Wu, C. X., and Deng, X. M. (2009b) Comparison of the total amount of eggshell pigments in Dongxiang brown-shelled eggs and Dongxiang blue-shelled eggs. *Poultry Sci.* **88,** 1735-1739.
44. Wang, Y. J., Liu, L. L., Wang, Y. S., Xue, Y. P., Zheng, Y. G., and Shen, Y. C. (2012) Actinoplanes utahensis ZJB-08196 fed-batch fermentation at elevated osmolality for enhancing acarbose production. *Biores. Technol.* **103,** 337-342.
45. Wong, Y. C., Heralds, T. J., and Hachmeister, K. A. (1996) Evaluation of mechanical and barrier properties of protein coatings on shell eggs. *Poultry Sci.* **75,** 417-422.
46. Yamauchi, K., Matsumoto, Y., and Yamauchi, K. (2016) Egg collagen content is increased by a diet supplemented with wood charcoal powder containing wood vinegar liquid. *Br. Poultry Sci.* ***27,*** 1-11.
47. Yazdi, M. M., Asodeh, A., and Chamani, J. (2012) A novel antioxidant and antimicrobial peptide from hen egg white lysozyme hydrolysates. *J. Funct. Foods* **4,** 278-286.
48. Yu, Z., Yin, Y., Zhao, W., Yu, Y., Liu, B., Liu, J., and Chen, F. (2011) Novel peptides derived from egg white protein inhibiting alpha-glucosidase. *Food Chem.* **129,** 1376-1382.