JAST (Journal of Animal Science and Technology) TITLE PAGE

Upload this completed form to website with submission

ARTICLE INFORMATION	Fill in information in each box below
Article Type	Research article
Article Title (within 20 words without abbreviations)	Comprehensive comparison of egg yolk of three Korean native chicken varieties: Physicochemical quality, antioxidant activity, and flavor-related substances
Running Title (within 10 words)	Korean Native Chicken Egg Yolk Quality and Flavor
Author	Yousung Jung[1], Dongwook Kim[1], Soomin Oh[1], Sangrok Lee[1], Hee-Jeong Lee[1], Hyo-Jun Choo[2], Aera Jang[1,*]
Affiliation	[1]Department of Applied Animal Science, College of Animal Life Sciences, Kangwon National University, Chuncheon 24341, Korea [2]Poultry Research Institute, National Institute of Animal Science, Pyeongchang 25342, Korea
ORCID (for more information, please visit https://orcid.org)	Yousung Jung (https://orcid.org/0000-0003-2095-2394) Dongwook Kim (https://orcid.org/0000-0002-5496-1961) Soomin Oh (https://orcid.org/0009-0006-2403-4813) Sangrok Lee (https://orcid.org/0009-0001-6476-0127) Hee-Jeong Lee (https://orcid.org/0000-0003-3806-482X) Hyo-Jun Choo (https://orcid.org/0000-0002-7747-5077) Aera Jang (https://orcid.org/0000-0003-1789-8956)
Competing interests	No potential conflict of interest relevant to this article was reported.
Funding sources State funding sources (grants, funding sources, equipment, and supplies). Include name and number of grant if available.	This work was carried out with the support of "Cooperative Research Program for Agriculture Science and Technology Development" (Project No. PJ016205) of the Rural Development Administration, Republic of Korea.
Acknowledgements	Not applicable.
Availability of data and material	Upon reasonable request, the datasets of this study can be available from the corresponding author.
Authors' contributions Please specify the authors' role using this form.	Conceptualization: Jung Y, Kim D, Oh S, Lee S, Lee HJ, Choo HJ, Jang A Data curation: Jung Y, Kim D, Jang A Formal analysis: Jung Y, Kim D, Oh S, Lee S, Lee HJ, Jang A Methodology: Jung Y, Kim D, Oh S, Lee S, Lee HJ, Choo HJ, Jang A Software: Jung Y, Choo HJ, Jang A Validation: Jung Y, Choo HJ, Jang A Investigation: Jung Y, Kim D, Oh S, Lee S, Lee HJ, Jang A Writing - original draft: Jung Y Writing - review & editing: Jung Y, Kim D, Oh S, Lee S, Lee HJ, Choo HJ, Jang A
Ethics approval and consent to participate	This article does not require IRB/IACUC approval because there are no human and animal participants.

CORRESPONDING AUTHOR CONTACT INFORMATION

For the corresponding author (responsible for correspondence, proofreading, and reprints)	Fill in information in each box below
First name, middle initial, last name	Aera Jang
Email address – this is where your proofs will be sent	ajang@kangwon.ac.kr

Secondary Email address	ajang@naver.com
Address	Department of Applied Animal Science, College of Animal Life Sciences, Kangwon National University, Chuncheon 24341, Korea
Cell phone number	+82-10-9017-8643
Office phone number	+82-33-250-8643
Fax number	+82-33-259-5574

Comprehensive comparison of egg yolk of three Korean native chicken varieties: Physicochemical quality, antioxidant activity, and flavor-related substances

Yousung Jung[1], Dongwook Kim[1], Soomin Oh[1], Sangrok Lee[1], Hee-Jeong Lee[1], Hyo-Jun Choo[2], and Aera Jang[1,*]

[1]Department of Applied Animal Science, College of Animal Life Sciences, Kangwon National University, Chuncheon 24341, Korea

[2]Poultry Research Institute, National Institute of Animal Science, Pyeongchang 25342, Korea

[*]Corresponding author : Aera Jang

Tel: +82-33-250-8643

Fax: +82-33-259-5574

Email: ajang@kangwon.ac.kr

Abstract

The conservation of native chicken breeds is essential for promoting sustainable farming practice and preserving local genetic resources. Herein, we investigated the physicochemical quality, antioxidant activity, and flavor-related compounds of egg yolk from three Korean native chicken (KNC) breeds, namely blue-shelled egg (BSE), Woorimatdag No. 1 and 2 chicken egg (WRMD1E and WRMD2E), and commercial egg (CE). The physicochemical characteristics of chicken eggs, such as Haugh unit, yolk color, and eggshell characteristics, along with yolk traits including moisture, crude fat, pH, and cholesterol levels, were found to vary depending on the species ($p < 0.05$). The yolk of WRMD2E and CE exhibited significantly higher levels of monounsaturated- and polyunsaturated fatty acids, respectively, compared to those of other groups. Umami-related free amino acids (Glu + Asp) were significantly higher in CE yolk than in KNC egg yolk. Multivariate analysis identified potential bio-volatile markers, including hexane, 3-ethyl-, hexadecane, and nonane, 2,5-dimethyl-, which could effectively differentiate between the yolk of various KNCs and CE. Notably, WRMD2E yolk exhibited substantial antioxidant power, as demonstrated by DPPH, ABTS, and ferric reducing antioxidant power assay. The overall results indicate that eggs from these native lines match the quality of a commercial egg product in many characteristics, with some superior traits, such as antioxidant capacity. The results of this study offer comprehensive insights into the egg yolk characteristics of KNC, particularly Woorimatdag chicken, providing fundamental data to enhance the utilization of KNC eggs in food applications while supporting efforts toward the conservation of native chicken biodiversity.

Keywords: genetic diversity, chicken egg, egg quality, fatty acid composition, free amino acid, volatile organic compounds.

Introduction

Commercial laying hens are selected for their intensive egg production performance and account for the major egg proportion in Korea. However, increasing dependence on commercial hens for egg production increasingly threatens the diversity of genetic resources [1, 2]. Several studies have been undertaken to develop indigenous chicken breeds to protect and promote their genetic variability in Korea. Therefore, the National Institute of Animal Science in Korea conducted a restoration project (Golden Seed Project) to preserve Korean native chicken (KNC) and developed intrinsic KNC breeds. Consequently, Woorimatdag No. 1 breed was developed in 2008 via three-way crossbreeding between Black Cornish and Brown KNC males with Rhode Island Red females, which exhibit distinctive taste and texture compared to traditional KNC [3]. However, this breed exhibits low growth performance, making it challenging to meet market demands. Therefore, Woorimatdag No. 2 breed was developed in 2010 by cross-mating between Black Cornish/Brown Cornish males and Brown KNC/Rhode Island Red females, aiming to generate cost-effective KNC for commercial purposes [4]. Blue hen, a crossbreed of White Leghorn and Araucana, is reared in Korea as the Gyeong-buk Araucana domestic breed [5]. This breed has been garnering popularity owing to its unique blue color eggs [6]. To date, several efforts have been made to identify the meat quality in these KNC breeds [4, 7]; however, detailed information on the distinct egg qualities of KNC remains limited.

Chicken egg quality is determined by both external and internal characteristics. External characteristics include traits that influence consumer acceptability, such as eggshell shape, strength, color, and weight. Internal quality, on the other hand, is assessed through various parameters, including the Haugh unit, yolk color, and yolk-to-albumen ratio. In addition, chicken eggs are rich in essential nutrients, including proteins, lipids, minerals, and vitamins, which are beneficial for human health. Egg yolk, which accounts for 28%–29% of total egg weight, comprises

approximately 16% and 32% of proteins and lipids, respectively [8]. Egg yolk is extensively used as a functional ingredient in many food, cosmetic, and pharmaceutical industries because it exhibits various multifunctional characteristics [9]. Furthermore, it provides high–value biological components, including α-tocopherol, tryptophan, phospholipids, and peptides, contributing to its antioxidant activity [10].

Consumers are not only interested in the quality and components of eggs and their products but also in the organoleptic qualities, including taste and aroma. Egg yolk plays a major role in the formation of egg flavor, as it contains a significant amount of flavor precursors, including lipids and proteins compared to albumen. Flavor encompasses a complex reaction between taste- and aroma-related compounds, including free amino acids, fatty acids, and volatile compounds [11]. Volatile compounds, responsible for the aroma characteristics, are primarily formed via the oxidative decomposition of unsaturated fatty acids and the Strecker reaction of amino acids [12]. Recent evidence suggests that breeding and genetics can affect amino and fatty acid profiles of egg yolk [13]. Furthermore, differences in nutritional and genetic conditions resulted in the distinct chemical composition of eggs [1]. These variations can subsequently lead to differences in the aroma and flavor of egg yolk. To date, several studies have been carried out to explore flavor-related compounds in the egg yolk of indigenous chickens extensively raised in China (volatile compounds), Japan (free amino acids), and Portugal (fatty acid composition) [2, 13–15]. Nevertheless, a comprehensive study on egg and yolk quality and egg yolk flavor characteristics from novel KNC breeds, particularly Woorimatdag breeds, are very scarce.

Therefore, this study was designed to provide a basic understanding of the quality and flavor profiles of KNC eggs by evaluating both egg quality and yolk characteristics, including antioxidant activity and non-volatile and volatile flavor compounds, in three KNC breeds: blue-shelled egg (BSE), Woorimatdag No. 1 egg (WRMD1E), and Woorimatdag No. 2 egg (WRMD2E).

Materials and methods

Birds and sampling

Commercial eggs (CE, Hyline Brown, 54-wk-old) and KNC eggs were provided by the National Institute of Animal Science in Korea. BSE, WRMD1E, and WRMD2E were obtained from blue hens (Araucana × Ogol × White Leghorn, 54-wk-old), Woorimatdag No. 1 chicken (56-wk-old), and Woorimatdag No. 2 chicken (56-wk-old), respectively. The birds were raised in free range during the day and housed indoors at night. All birds were fed a commercial diet (metabolizable energy, 2,500 kcal/kg; crude protein, 15.0%; calcium, 3.60%; phosphorus, 0.48%). Fifty eggs produced within two days were randomly collected from each bird group, resulting in a total of 200 eggs. The eggs were transported to the laboratory at 4°C and analyzed immediately upon arrival. Briefly, 30 eggs were subjected to determine egg quality, then yolk were separated using egg separator, and two yolk were randomly pooled to prepare 15 egg yolk samples for subsequent analysis of yolk physicochemical traits, antioxidant activity, and non-volatile flavor compounds. Another 20 eggs were boiled at 100°C for 15 min. Thereafter, yolk were separated and randomly pooled to make 10 boiled yolk samples to analyze volatile compounds profiles. The egg specimens used in this study conformed to the same criteria (52–60 g) of the [Egg Weight Standard] established by the Korea Institute for Animal Products Quality Evaluation.

Egg quality traits of KNC eggs

An electronic digital caliper (AD5763150, A&D Company, Tokyo, Japan) was used to measure egg length (mm). Yolk color and Haugh unit (HU) were determined using an egg multi-tester (EMT-5200, Robotmation, Tokyo, Japan). The yolk: albumen (Y/A) ratio was calculated by dividing the yolk weight by the albumen weight. A colorimeter (Chroma Meter CR-400 instrument,

Minolta, Osaka, Japan) was used to determine eggshell color [lightness (CIE L*), redness (CIE a*), and yellowness (CIE b*)] after calibration (Y = 93.60, x = 0.3134, and y = 0.3194). A TA1 texture analyzer (Lloyd Instruments, Berwyn, IL, USA) equipped with a 10-mm diameter probe (load cell, 500 N; crosshead speed, 50 mm/min) was used to measure eggshell strength (kgf) at the small end of the egg. Eggshell weight (g) and thickness (mm) were determined as previously described with slight modifications [16]. The eggshells were washed carefully, dried (50°C for 24 h) and cooled (20°C for 30 min) before weighing. A micrometer (103-109 Outside Micrometer 0-25 mm, Mitutoyo, Japan) was used to measure eggshell thickness as the average value of the large end, small end, and equatorial regions of the shell.

Physicochemical characteristics of KNC egg yolk

Proximate composition

Moisture, crude protein, crude fat, and crude ash composition was evaluated according to the AOAC official methods [17].

pH

The pH was analyzed by calibrated (pH 4.1 and 7.0) pH meter (Orion 230A, Thermo Fisher Scientific, Waltham, MA, USA).

Cholesterol content

The extraction of cholesterol was conducted as previously described [7]. Gas chromatography (7890N, Agilent Technologies, Santa Clara, CA, USA) equipped with a HP-5 column (30 m × 0.33 mm × 0.25 mm, Agilent Technologies) was used. Analytical condition was as follows; carrier gas: helium, flow rate: 1.0 mL/min, split ratio: 1:12.5, injector temperatures: 250°C, and flame

ionization detector temperatures: 300°C. The oven temperature program was set as follows: an initial temperature of 190°C was maintained for 2 min, increased at 20°C/min to reach 230°C, where it was held for 3 min. Subsequently, the temperature was raised at 40°C/min to 270°C and maintained for 25 min. The cholesterol content (mg/ g) was obtained by the ratio of target area to the internal standard area.

Antioxidant activity of KNC egg yolk

Sample preparation

The egg yolk extract was prepared as previously described with modifications [10]. A 2.0 g lyophilized egg yolk with 8 mL 80% methanol was vortexed (2 min), which was further extracted by sonicator (10 min) and then centrifuged (4°C, 3,000 × g, 20 min). The supernatant (4 mL) was combined with 400 μL 10% trichloroacetic acid (w/v) and centrifuged (4°C, 3,000 × g, 20 min) to obtain the supernatant for antioxidant assays. Trolox was used to establish the standard curve in four antioxidant assays.

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

The DPPH radical scavenging activity was evaluated as previously described [18]. A 100 μL sample and 0.2 mM DPPH radicals (in methanol) were mixed and a mixture was incubated (25°C for 30 min). The absorbance was measured at 517 nm through spectrophotometer (SpectraMax M2, Molecular Devices, CA, USA) and the result was obtained by following equation.

$$(1)\ DPPH\ radical\ scavenging\ activity\ (\%) = 1 - \frac{Sample\ O.D. - Reference\ O.D.}{Control\ O.D.} \times 100$$

2,2-Azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity

The ABTS radical scavenging activity was determined as previously described [19]. Equal volumes of 5.9 mM potassium persulfate and 14 mM $ABTS^+$ solution was mixed to create $ABTS^+$ radical stocks (23°C for 12 h), which was subsequently diluted to achieve an absorbance of 0.700 ± 0.02 at 735 nm. A 50 μL sample was mixed with 950 μL $ABTS^+$ radical solution (30°C for 30 min). The absorbance was measured at 735 nm and the result was obtained by following equation.

$$(2)\ ABTS\ radical\ scavenging\ activity\ (\%) = \frac{Control\ O.D. - Sample\ O.D.}{Control\ O.D.} \times 100$$

Oxygen radical absorbance capacity (ORAC) assay

The ORAC was evaluated as previously described with slight modification [20]. A 25 μL sample and 150 μL 80 nM fluorescein were mixed and then incubated (37°C for 15 min). Subsequently, 25 μL 50 mM 2,2'-azobis (2-amidinopropane) hydrochloride was added to generate peroxyl radicals. The change in the absorbance was monitored every minute at an excitation wavelength of 480 nm and an emission wavelength of 520 nm at 37°C. The results are expressed as mmol Trolox equivalent (TE)/ g dry matter (DM).

Ferric reducing antioxidant power (FRAP) assay

FRAP was evaluated according to previously described [18]. The working solution was prepared by combining 300 mM acetate buffer (composed of 1.55 g sodium acetate trihydrate and 8 mL acetic acid, diluted to 500 mL, and adjusted to pH 3.6), 10 mM 2,4,6-tripyridyl-S-triazine in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ solution in a ratio of 10:1:1 (v/v/v). A 25 μL extracted sample and 175 μL FRAP working solution were mixed and incubated (37°C for 30 min). The absorbance was measured at 590 nm and the result was expressed as mmol TE/g DM.

Flavor-related substances of KNC egg yolk

Fatty acid composition

For determination of fatty acid composition, lipid extraction and derivatization were conducted as previously described [7]. An Agilent 7890N gas chromatograph (Agilent Technologies) with an Omegawax 250 capillary column (30 m × 0.25 mm × 0.25 μm, Supelco, Bellefonte, PA, USA) was utilized. The temperature for injection port and flame ionization detector was set to 250°C and 260°C, respectively. The carrier gas was helium with a flow rate of 1.2 mL/min and a split ratio of 1:100. The oven was initially set to 150°C and held for 2 min, then heated from 150°C to 220°C at a rate of 4°C/min and maintained for 30 min. According to their standards (PUFA No. 2-Animal Source, Supelco), fatty acids were identified.

Free amino acid (FAA)

Extraction of FAA was conducted as previously described [11]. An amino acid analyzer (S433, SYKAM, Germany) was used and the analytical condition was as follows: eluting solution: lithium citrate buffer (pH 2.9, 4.2, and 8.0), column: lithium-form resin (150 mm × 4.6 mm), column and reaction temperature: 37°C and 110°C, respectively, flow rate and analysis time: 0.45 mL/min and 120 min, respectively. The FAA contents were obtained from their respective standards.

Volatile organic compound (VOC)

A pilot study was conducted to establish the analytical condition for detecting hydrogen sulfide and trimethylamine in egg yolk, as these compounds are significant VOC responsible for undesirable egg flavor. Interestingly, they were not detected in fresh egg yolk but identified in boiled samples. Therefore, we decided to analyze the VOC profiles of egg yolk using only boiled egg yolk. The extraction of volatiles was conducted using the headspace solid-phase micro-extraction as previously outlined [7]. An Agilent 8890 gas chromatograph with an Agilent 5977B

mass spectrometer and a DB-5MS column (30 m × 0.25 mm × 0.25 μm, Agilent Technologies) were used. The analysis was operated under spitless conditions (250°C for 5 min) with helium as carrier gas and 1.3 mL/min as flow rate. The oven was initially set to 40°C for 5 min, then heated at a rate of 5°C/min to achieve 250°C and maintained for 5 min. The interface temperature was set to 280°C. The mass spectrometer operated in electron impact mode with an electron energy of 70 eV and a scan range of 30–300 m/z (scan rate: 4.37 scans/s, gain factor:1, resulting EM voltage:1140 V). The temperature of the MS source and quadrupole was set at 230°C and 150°C, respectively. The identification of VOC and its aroma description was performed as previously described [7]. All identified VOCs and their corresponding aroma descriptors are provided as Supplementary Tables S2 and S3.

Statistical analysis

The data are presented as mean values with the standard error of the mean (SEM) based on the following replicates: 30 replicates for egg quality traits; 15 replicates for proximate composition, pH, and fatty acid composition of yolk; 10 replicates for cholesterol content, antioxidant activity, and VOCs of yolk; and 5 replicates for FAA content of yolk. The results were processed statistically through SAS software (version 9.4, SAS Institute Inc., Cary, NC, USA), using one-way analysis of variance and Duncan's range test ($p < 0.05$). Multivariate analysis was performed using hierarchical cluster analysis (HCA), partial least squares-discriminant analysis (PLS-DA), and its variable importance in projection (VIP) scores. The MetaboAnalyst 5.0 software (https://www.metaboanalyst.ca/) was utilized for this purpose using log-transformation and auto-scaling.

Results and discussion

Egg quality traits of KNC eggs

Egg weight and length

Significant differences were noted in the egg quality characteristics among the various KNC breeds (Fig. 1 and Table S1). As shown in Fig. 1A, the egg weigh of WRMD1E (59.38 g) and WRMD2E (58.30 g) was significantly heavier than that of CE (54.64 g) and BSE (55.05 g). Regarding egg length, WRMD1E exhibited the longest egg length (57.45), followed by WRMD2E (56.33), BSE (54.96), and CE (53.23), with statistical differences ($p < 0.05$).

Yolk color and Haugh unit

As illustrated in Fig. 1B, WRMD2E had the darkest yolk color (9.15), followed by WRMD1E (8.62), CE (8.23), and BSE (7.93), with significant differences ($p < 0.05$). HU is a measure used to evaluate internal egg quality by adjusting albumen height with egg weight and a higher HU indicates better quality and freshness [15]. A significantly lower HU was observed in BSE (66.20) compared to the other groups, with no significant differences observed between CE (80.33), WRMD1E (77.15), and WRMD2E (76.61). Similar findings were reported by Lordelo et al. [2] for Portuguese indigenous chicken varieties and commercial breeds. This phenomenon might be attributed to differences in bird genetics and environmental factors [21]. Many factors influencing the HU of eggs have been identified, including the hen's age, strain or breed, dietary components, and potential diseases [22].

Yolk: albumen ratio

As demonstrated in Fig. 1C, the Y/A ratio of KNC eggs (0.48–0.59) was significantly higher than that of CE (0.37), which is consistent with an earlier observation [16]. Among the KNC eggs, BSE exhibited a significantly higher Y/A ratio (0.59), followed by WRMD2E (0.54) and

WRMD1E (0.48) ($p < 0.05$). Haunshi et al [23]. reported that a heavier egg weight is associated with a higher Y/A ratio. However, in this study, the smaller-sized BSE exhibited a significantly higher Y/A ratio compared to WRMD1E and WRMD2E. This result suggests that egg size does not always correlate with Y/A ratio. These contradictory findings could be due to differences in the genetic backgrounds of the chicken groups, which affect both the yolk and albumen composition. The literature has demonstrated that the compositional traits of eggs (e.g., albumen and yolk) are correlated with the heritability of the chicken breed [13, 14]. Consistent with our findings, Tharrington et al. [24] found that as egg weight decreases, the proportion of yolk increases while the proportion of albumen decreases, leading to a higher Y:A ratio.

Eggshell color

Eggshell color is an important factor affecting consumer purchasing decisions [6]. BSE exhibited the highest L*-value (82.99), followed by WRMD2E (72.71), WRMD1E (69.53), and CE (56.30), with significant differences ($p < 0.05$) (Fig. 1D). Commercial brown-egg lines have been selectively bred for dark-brown shells over many years, resulting in darker shell colors compared to native chicken lines. The bright color of native chicken eggs might be due to their distinct genetic background, considering the relatively high heritability of shell color [2]. CE exhibited a significantly higher a*-value (20.74) than KNC eggs (-4.56–13.65). The relatively redder hues in CB eggs compared to KNC eggs are attributed to the red pigments in CB, such as protoporphyrin and uroporphyrin [5]. CE exhibited a significantly higher b*-value (30.84) among groups, while both WRMD1E (27.90) and WRMD2E (26.82) resulted in similar b*-value, surpassing those of BSE (13.38) ($p < 0.05$). The b*-value of eggshells positively correlates with the composition of several minerals, including Ca, Cu, Zn, and Al, present in eggshells [25].

Eggshell strength, thickness, and weight

As observed in Fig. 1E, CE exhibited significantly higher eggshell strength (5.64 kgf) than the other groups, whereas WRMD2E exhibited significantly greater eggshell strength (4.92 kgf) than BSE (3.72 kgf) and WRMD1E (3.53 kgf). CE exhibited a significantly thicker eggshell (0.43 mm) than KNC eggs (0.38–0.40 mm), which is supported by an earlier study [16]. Eggshell strength increased with an increase in eggshell thickness; however, BSE exhibited significantly lower eggshell strength than WRMD2E, although the eggshell thickness was similar. This finding suggests that eggshell strength might be influenced by factors other than just its thickness. It is well known that various factors, including eggshell thickness, mineral composition, and microstructure, can affect the mechanical endurance of eggshells [26]. Next, the eggshell weight of CE (5.69 g) was significantly heavier than that of KNC eggs, which was also reported in an earlier study [16]. Furthermore, WRMD2E had a significantly higher eggshell weight (5.33 g) than BSE (4.99 g) and WRMD1E (5.02 g), which was consistent with the trend observed for eggshell strength.

Physicochemical characteristics of KNC egg yolk

Proximate composition

Moisture, crude protein, fat, and ash of egg yolk were 48.60%–50.76%, 16.03%–16.21%, 30.30%–31.51%, and 1.65%–1.86%, respectively (Table 1). These values are consistent with an earlier observation on CE and BSE yolk [5]. WRMD1E yolk showed significantly higher moisture than the others. In contrast, WRMD2E exhibited significantly lower yolk moisture than BSE. Compared to WRMD1E, crude fat and ash were significantly higher in BSE and WRMD2E, respectively. Our finding is slightly different from that of Lai et al. [27], who reported that crude fat and ash contents of hen egg yolk are 31.8%–35.5% and 1.1%, respectively.

pH

WRMD1E exhibited a significantly higher pH value (6.29) than the other groups (6.12–6.21), while BSE exhibited a significantly higher pH value than CE and WRMD2E (Table 1). These results are inconsistent with a previous study, in which no significant differences in pH value were noted for CE and BSE [5]. Differences in the pH value of egg yolk from different chicken breeds may be due to their genetic diversity [28].

Cholesterol content

The cholesterol content of egg yolk was 9.06–10.06 mg/g (Table 1); this is consistent with earlier studies on egg yolk cholesterol content [6, 29]. WRMD1E yolk exhibited significantly higher cholesterol levels than BSE and WRMD2E yolk. However, no significant differences in yolk cholesterol levels were observed between CE and BSE, BSE and WRMD2E, and CE and WRMD1E. KNC eggs generally contain a higher cholesterol content because of their larger Y/A ratio and heavier yolk than conventional hen eggs [16]. Nevertheless, not all KNC egg yolk exhibited high cholesterol content per gram compared with CE yolk. The overall amount of cholesterol in eggs often depends on multiple factors, such as breed differences and experimental and extraction conditions [5].

Antioxidant activity of KNC egg yolk

Antioxidant capacity determination using a single method is challenging owing to the complexity of food [10]; therefore, we conducted four different assays (Fig. 2). Regarding DPPH scavenging activity, WRMD2E yolk (60.67%) exhibited significantly higher levels than in the other samples. Furthermore, CE yolk (57.47%) showed significantly higher levels than WRMD1E

yolk (54.27%), but did not differ significantly from BSE (55.84%). Previous studies revealed the DPPH scavenging activity of CE yolk (29–35%) and BSE yolk (40.78%) [5, 10]. However, these values are lower than our findings, which might be attributed to lyophilization affecting the accumulation of antioxidant components in egg yolk. Moving on to ABTS assay, the ABTS scavenging activity of egg yolk was 23.6%–26.3%; this is higher than that reported in previous studies on 18- and 30-wk-old commercial hen egg yolk (8.1%–13.0%) [10, 30]. Lyophilization might have contributed to this difference, as discussed above. WRMD1E and WRMD2E yolk exhibited significantly higher ABTS scavenging activities than CE and BSE yolk. The mean DPPH scavenging activity (57.1%) was approximately 2.3 times higher than the ABTS scavenging activity (24.8%). This difference might be due to the distinct mechanisms of the DPPH and ABTS assays, with the involvement of organic and aqueous solvents, respectively [31]. We assumed that this results could be attributed to potential antioxidant candidates in the egg yolk with fat-soluble properties, such as phospholipids, carotenoids, and α-tocopherol.

ORAC is a common analytical method in which a biologically relevant radical source is used and both inhibition degree and time of peroxyl radical-induced oxidation within a single quantity are considered [32]. WRMD1E yolk exhibited significantly higher ORAC value compared with CE and BSE yolk. However, the ORAC value of WRMD2E yolk did not significantly differ from that of the other groups. Next, the FRAP assay evaluates the ability of phenolics to convert ferric to ferrous based on the electron-donating activities of antioxidants [31]. WRMD2E yolk exhibited a significantly higher FRAP score than the other groups. Compared with WRMD1E yolk, both CE and BSE yolk exhibited significantly lower FRAP scores. These differences might be due to the presence of phosvitin, an antioxidant material in egg yolk that generally functions by chelating iron ions [33]. Interestingly, WRMD2E yolk exhibited substantial antioxidant capacity. Similarly, Liu et al. [34] discovered a substantial antioxidant capacity of Tibetan indigenous chicken egg

yolk; this might be due to the abundance of antioxidant-associated candidate proteins, such as PIT 54 and glutathione-peroxidase 3. In the present study, the specific antioxidant-related compounds in KNC egg yolk were not investigated, suggesting the need for further research.

Flavor-related substances of KNC egg yolk

Fatty acid composition

C18:1n9, C16:0, and C18:2n6 were identified as the predominant fatty acids, constituting approximately 84.5% of the total abundance (Table 2). This is similar to the findings reported in previous studies on egg yolk from commercial laying hens and Portuguese chicken [2, 29, 30]. C16:0 levels were significantly higher in CE yolk (32.46%) compared to the others (28.22%–30.01%) and significantly lower in WRMD2E yolk than in BSE yolk. Nevertheless, WRMD2E exhibited the highest yolk C18:1n9 levels (43.94%), followed by BSE (41.12%), WRMD1E (39.19%), and CE yolk (35.73%), with significant differences ($p < 0.05$). It can be suggested that WRMD2E exhibits a richer flavor, as C18:1n9 is one of the key compounds associated with organoleptic properties and contributes to the enhancement of egg yolk flavor [11]. Gao et al. [13] found a strong relationship between egg yolk flavor and the presence of C18:1n9 and C20:4n6, both of which amplify positive milky flavor. In contrast, C18:2n6 levels were the highest in CE yolk (18.03%), followed by WRMD1E (15.13%), BSE (13.93%), and WRMD2E yolk (10.79%), with significant differences ($p < 0.05$). C18:3n6 was identified as trace amounts (0.04%–0.12%), which is consistent with an earlier study [5]. C18:3n6 levels were the highest in WRMD1E yolk, followed by WRMD2E, BSE, and CE yolk ($p < 0.05$). Dietary C18:3n6 may reduce cardiovascular disease risk by converting to C20:3n6, which produces prostaglandin E1 and suppresses plaque formation [5]. C20:4n6 levels were significantly lower in CE yolk (1.73%) than in KNC egg yolk (2.23%–2.33%). KNC egg yolk may positively contribute to the organoleptic character of eggs

because C20:4n6 is associated with the umami-related taste and milky flavor as discussed above [7, 13]. C22:6n3 accounted for 0.08%–0.36% of total fatty acid abundance, which is relatively lower than the levels previously reported for CE/BSE and Portuguese indigenous chickens [2, 5]. C22:6n3 levels were significantly higher in WRMD2E than in other groups. The proportions of total saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), and polyunsaturated fatty acid (PUFA) were 38.25%–39.88%, 40.00%–47.65%, and 13.88%–20.12%, respectively. These values are slightly different from those of egg yolk from Portuguese chickens and hybrid hens, which might be due to genetic differences [2]. Total unsaturated fatty acid composition was significantly higher in KNC egg yolk than in CE yolk. Furthermore, PUFA and MUFA levels were markedly higher in CE yolk and WRMD2E yolk, respectively, than in the other groups. This might be due to the high proportions of C18:2n6 and C18:1n9, respectively. The ratio of PUFA/SFA (≥0.4–0.5 is recommended) is an indicator for evaluating the nutritional quality of meat fat [19]. The egg yolk generally matched the recommended ratio (0.44–0.51), except for WRMD2E yolk (0.36). In contrast, WRMD2E yolk exhibited a significantly higher MUFA/SFA ratio than the other groups. Consequently, we confirmed that differences in the individual and total egg yolk fatty acids are affected by the genetic diversity of chickens. This outcome is supported by Lordelo et al. [15], who identified various fatty acids, including C18:1n9 (34.9%–41.4%) and PUFA (19.6%–30.2%), in hen eggs. They also reported that egg fatty acids can be influenced by different housing systems, breeds, feed, and specialty eggs.

Multivariate analysis of fatty acid composition

The yolk fatty acid profile of WRMD2E was clearly distinct from those of CE and BSE yolk, with some overlap with WRMD1E yolk, while CE, BSE, and WRMD1E yolk formed a slightly

407 overlapping cluster (Fig. 3A). This suggests that different chicken breeds considerably influenced
408 the fatty acid profile, though not to the extent of distinctly separating the clusters.
409
410 *Free amino acid*
411 Twenty-one FAA were identified in egg yolk, with concentrations of 1902.81–1961.62 μg/g;
412 however, the difference was insignificant. (Table 3). This range was lower than that reported in
413 previous studies on white-shelled eggs and 25-wk-old Lohmann hen eggs [35, 36] but higher than
414 that on Japanese chicken eggs [14]. FAA indicate several taste attributes, such as bitter, sweet,
415 salty, and umami, which are crucially responsible for egg yolk taste. Glutamic acid, leucine, and
416 lysine were identified as the most abundant FAA, which was consistent with previous works on
417 white/brown Leghorn and Japanese indigenous breeds [14, 37]. Aspartic and glutamic acids are
418 associated with strong umami taste, which are presented as umami score [11]. CE yolk had a
419 significantly higher umami score than WRMD2E yolk; however, no significant difference was
420 observed among KNC egg yolk. In particular, aspartic acid content was significantly higher in CE
421 yolk than in KNC egg yolk, whereas no significant difference in glutamic acid content was
422 observed. Goto et al. [14] reported a significantly higher aspartic acid content in egg yolk from
423 commercial brown layer than in that from Nagoya chickens. They also reported that different
424 chicken breeds affect glutamic acid content, which differs from our findings. Sulfur-containing
425 FAA, including methionine and cysteine, can produce meat-like sweet flavor. A significantly
426 higher level of cysteine was observed in BSE yolk compared to the other groups; however, no
427 significant differences were observed for methionine. Phenylalanine and tyrosine can enhance the
428 savory taste in the presence of acidic FAA [11]. Phenylalanine content was not significantly
429 different between CE and KNC egg yolk; however, tyrosine content was significantly higher in
430 CE yolk than in BSE yolk. Essential FAA accounted for approximately 43% of the total FAA; this

is slightly lower than that reported previously (46–47%) [35]. Although no significant difference in total essential FAA contents was confirmed, chicken breeds significantly affected the content of several essential FAA, such as threonine and valine.

As aromatic amino acids, both tyrosine and tryptophan can generate high antioxidant activities, as their structural characteristics are associated with phenolic acids and indole groups, respectively [35]. Tryptophan levels were significantly higher in BSE and WRMD1E yolk (26.49–26.58 μg/g) than in WRMD2E yolk (24.46 μg/g). Interestingly, the three types of antioxidant activities (DPPH, ABTS, and FRAP assay) of WRMD2E yolk were significantly higher than those of CE yolk (Fig. 2), even though no significant differences in tyrosine and tryptophan contents were observed between CE and WRMD2E yolk. Furthermore, the three types of antioxidant activities (ABTS, ORAC, and FRAP assay) of WRMD1E yolk were significantly higher than those of CE and BSE yolk. However, no statistically significant difference was observed in tyrosine and tryptophan contents in WRMD1E yolk compared to CE and BSE yolk ($p > 0.05$). In this study, the antioxidant activities of egg yolk might be affected not only by aromatic amino acids but also by various factors in egg yolk acting as antioxidants, such as vitamin A, vitamin E, carotenoids, and phospholipids [36].

Multivariate analysis of free amino acid

The FAA profiles of BSE and WRMD2E yolk were clearly distinct from those of CE and WRMD1E yolk, as well as from each other, while CE and WRMD1E yolk clusters exhibited a slight overlap (Fig. 3B). This suggests that genetic differences in blue hen and Woorimatdag No. 2 chicken have a significant effect on their egg yolk FAA profiles. It has been reported that chicken breeds influence the distribution of FAA, potentially leading to variations in taste sensor traits in egg yolk [14].

Volatile organic compound

A total of 123 VOCs were identified and categorized as acids, alcohols, aldehydes, esters, hydrocarbons, ketones, and sulfur compounds (Table S2). Although the number of detected VOCs in WRMD2E yolk was similar to CE and WRMD1E, and higher than BSE, the total amount was significantly lower in WRMD2E than the other samples (Table 4 and Fig. 4A). This could be attributed to the increased antioxidant properties of WRMD2E yolk (Fig. 2), which play a crucial role in inhibiting oxidative decomposition of fatty acids, thereby influencing the formation of several VOCs, such as aldehydes and hydrocarbons [38]. Several VOC, such as 1-octen-3-ol, decanal, nonanal, octanal, D-limonene, and 2-pentylfuran, were also identified from heated egg yolk [39]. Acids normally originated from lipid oxidation or secondary degradation of oxidative products, such as hexanal [11]. Herein, total acids and octadecanoic acid were significantly higher in WRMD2E than in BSE and WRMD1E yolk. Further, total alcohols were significantly higher in WRMD2E yolk than in WRMD1E yolk. In contrast, total aldehydes were significantly higher in WRMD1E yolk, followed by BSE, CE, and WRMD2E yolk ($p < 0.05$). Lipid oxidation is responsible for the formation of aldehydes, thereby modifying egg flavor [40]. Propanal, 2,2-dimethyl- was the predominant aldehyde, accounting for 78.5%–97.3% of total abundances. 1-Octen-3-ol levels were significantly higher in CE and WRMD2E yolk than in WRMD1E yolk. The 1-octen-3-ol is a product of the enzymatic or non-enzymatic decomposition of omega-6 fatty acids, imparting mushroom odors, which are also present in fresh egg yolk [41]. Nonanal levels were significantly higher in the WRMD2E yolk than in BSE and WRMD1E yolk. Moreover, octanal content was significantly higher in CE, WRMD1E, and WRMD2E yolk than in BSE yolk. However, decanal was significantly lower in WRMD1E yolk than in CE and WRMD2E yolk. Octanal and nonanal belong to the pleasant aroma group [11]. In addition to decanal, they can also

be generated by the oxidation of omega-9 fatty acids present in egg yolk [39]. Hexadecanal (cardboard-like off-odor compound), was isolated in trace amount from all egg yolk. It was significantly higher in WRMD2E yolk than the other groups. The Strecker degradation of yolk amino acids is responsible for generating benzeneacetaldehyde and 2-pentylfuran from boiled egg yolk [39]. Benzeneacetaldehyde levels were significantly higher in BSE and WRMD2E yolk than CE and WRMD1E yolk. Generally, no significant differences in 2-pentylfuran levels were observed between treatments, except for WRMD1E yolk, which had significantly lower levels than CE and WRMD2E yolk. Ren et al. [41] have reported that 1-octen-3-ol and 2-pentylfuran are the key off-odor volatiles isolated from thermal egg yolk at different temperatures.

Total ester content was significantly higher in WRMD1E yolk than the others. The most abundant ester was arsenous acid, tris(trimethylsilyl) ester (comprising as 74.9%–90.8% of the total ester), with WRMD1E yolk exhibiting significantly higher content, followed by WRMD2E, BSE, and CE yolk ($p < 0.05$). Differences in aldehydes and esters can impact on the formation of distinctive boiled egg yolk flavors, as they have low odor thresholds [42]. The VOC classified as hydrocarbons accounted for approximately 56% of the total identified VOC, comprising 53.78%–61.67% of total VOC concentration in yolk samples. The total hydrocarbon content was significantly lower in WRMD2E yolk than the other groups. Indole was only detected in CE ($p < 0.05$). Indole is partially responsible for the unpleasant pastoral odor that may not be preferred by some consumers [43]. As evidenced by Jung et al. [11], 2-ketones are important aroma contributors to meat. 2,3-Butanedione, characterized by its butter, caramel, and oily aroma, was significantly higher in BSE yolk than in WRMD1E yolk. (+)-2-Bornanone and 2-octanone levels were significantly higher in WRMD2E yolk than in CE and BSE yolk. Carbon disulfide content was significantly higher in WRMD1E than that in remaining samples. Disulfide species can impart a meaty aroma with an exceptional low odor threshold [42]. Excess hydrogen sulfide could originate

503 from the decay process in boiled eggs, in which protein matter decomposes or is metabolized by
504 microorganisms, thereby producing them as a key odorant for rotten egg flavor [44]. Herein,
505 hydrogen sulfide levels were significantly higher in CE yolk (0.262 AU×10^6), followed by
506 WRMD1E (0.167 AU×10^6), WRMD2E (0.102 AU×10^6), and BSE (not detected), with significant
507 differences ($p < 0.05$). Trimethylamine is a nitrogenous compound with an ammonia and fish odor
508 that contributes to the flavor of decaying boiled eggs. Herein, trimethylamine levels were not
509 significantly different among the groups (0.092–0.158 AU×10^6). Consequently, our study reveals
510 different VOC profiles in KNC egg yolk, suggesting that individual VOCs and their classes were
511 influenced by genetic differences, might affect the distinct flavor of egg yolk.
512
513 *Multivariate analysis of volatile organic compound*
514 To describe the similarities and differences of VOC, HCA was performed based on the Euclidian
515 distance measure and Ward clustering algorithm [45]. In HCA, a larger distance between the
516 sample clusters indicates a more significant difference in the VOC profiles. In the third stage of
517 the hierarchy, CE and BSE clustered together, while WRMD1E and WRMD2E formed a cluster
518 in the next stage of the hierarchy (Fig. 4B). Subsequently, both CE and BSE yolk and both
519 WRMD1E and WRMD2E yolk exhibited similar VOC profiles and were named Group 1 and
520 Group 2, respectively. These results suggest that the VOC profiles of Group 1 are markedly
521 different from that of Group 2 [45]. To validate the clustering and identify the key markers
522 distinguishing the different samples, the PLS-DA was performed (Fig. 4C). In the PLS-DA model,
523 the sum of components 1 and 2 was 23.1% (correlation coefficient: 0.99; cross-validation
524 correlation coefficient: 0.86). Based on the X-axis, CE and BSE were positioned below the zero
525 point, whereas WRMD1E and WRMD2E were nearly above the zero point. This result suggests
526 that the VOC profiles between CE and BSE, and WRMD1E and WRMD2E are relatively similar

and distinctly different between Groups 1 and 2. VOC with high VIP scores (≥1.0) are considered markers for differentiating treatments [11]. Nineteen important VOCs (VIP≥1.4) were screened, comprising 13 hydrocarbons, 3 aldehydes, 2 alcohols, and 1 ester (Fig. 4D). Among them, ten VOCs, including hexane, 3-ethyl- and nonane, 2,5-dimethyl-, were higher in Group 1 than in Group 2. In contrast, five VOCs, including hexadecane and octanal, were found at higher levels in Group 2 than in Group 1. As mentioned above, almost all compounds in Group 1 or Group 2 belonged to hydrocarbons, whereas some were alcohol and aldehyde. This suggests that the differences in the VOC profiles of egg yolk might be due to lipid degradation, as lipid oxidation is a major pathway responsible for hydrocarbons, alcohols, and aldehydes formation [11].

Conclusions

This study clearly indicated the impact of genetic differences of KNC on the physicochemical quality, antioxidant activity, and flavor characteristics of egg yolk, with a particular focus on Woorimatdag chicken lines. The egg quality tratis, including HU, yolk color, Y/A ratio, and eggshell traits, were significantly dependent on their genetic differences. Interestingly, Woorimatdag chicken egg yolk exhibited substantial antioxidant activities, as demonstrated by DPPH, ABTS, and FRAP assays for WRMD2E and the ORAC assay for WRMD1E compared to yolk of CE and BSE. In terms of taste and aroma, KNC egg yolk had higher proportions of C18:1n9, C20:4n6, and C18:3n6 compared to CE yolk; however, it contained less umami-related aspartic acid than CE yolk. The primary VOC in boiled egg yolk included 1-octen-3-ol, benzeneacetaldehyde, decanal, nonanal, carbon disulfide, 2-pentylfuran, and trimethylamine. The proportion and abundance of VOCs were species-dependent: WRMD2E yolk contained fewer aldehydes and hydrocarbons compared to the other groups, whereas WRMD1E yolk exhibited higher levels of esters and aldehydes. In conclusion, eggs from KNC lines indicated comparable

quality to commercial eggs, with WRMD2E yolk showing notable strengths in antioxidant capacity and organoleptic fatty acids. Several VOCs with high VIP scores, such as hexane, 3-ethyl-, and hexadecane, may warrant further investigation as potential volatile-markers for discriminating boiled egg yolk of various chicken breeds. Our findings contribute to a deeper understanding of KNC egg characteristics, which could support the sustainable use and conservation of native chicken biodiversity.

Abbreviations: ABTS, 2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid); BSE, blue-shelled egg; CE, commercial egg; DPPH, 1,1-diphenyl-2-pricrylhydrazyl; DM, dry matter; FAA, free amino acid; FRAP, ferric reducing antioxidant power; HCA, hierarchical cluster analysis; HU, Haugh unit; KNC, Korean native chicken; MUFA, monounsaturated fatty acid; ORAC, oxygen radical absorption capacity; PLS-DA, partial least squares-discriminant analysis; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; TE, Trolox equivalent; UFA, unsaturated fatty acid; VIP, variable importance in projection; VOC, volatile organic compound; WRMD1E, Woorimatdag No. 1 chicken egg; WRMD2E, Woorimatdag No. 2 chicken egg; Y/A, yolk-to-albumen.

Author Contributions

Conceptualization: Jung Y, Kim D, Oh S, Lee S, Lee HJ, Choo HJ, Jang A. **Data curation**: Jung Y, Kim D, Jang A. **Formal analysis**: Jung Y, Kim D, Oh S, Lee S, Lee HJ, Jang A. **Methodology**: Jung Y, Kim D, Oh S, Lee S, Lee HJ, Choo HJ, Jang A. **Software**: Jung Y, Choo HJ, Jang A. **Validation**: Jung Y, Choo HJ, Jang A. **Investigation**: Jung Y, Kim D, Oh S, Lee S, Lee HJ, Jang A. **Writing - original draft**: Jung Y. **Writing - review & editing**: Jung Y, Kim D, Oh S, Lee S, Lee HJ, Choo HJ, Jang A.

Declaration of Competing Interest

The authors have no conflicts of interest on this paper to declare.

Acknowledgments

This work was carried out with the support of "Cooperative Research Program for Agriculture Science and Technology Development" (Project No. PJ016205) of the Rural Development Administration, Republic of Korea.

Supplementary material

Supplementary data to this article is uploaded separately

References

1. González Ariza A, Arando Arbulu A, Navas González FJ, Ruíz Morales FDA, León Jurado JM, Barba Capote CJ, et al. Sensory preference and professional profile affinity definition of endangered native breed eggs compared to commercial laying lineages' eggs. Animals. 2019;9(11):920.

2. Lordelo M, Cid J, Cordovil CM, Alves SP, Bessa RJ, Carolino I. A comparison between the quality of eggs from indigenous chicken breeds and that from commercial layers. Poult Sci. 2020;99(3):1768-1776.

3. Jin S, Jayasena DD, Jo C, Lee JH. The breeding history and commercial development of the Korean native chicken. World's Poult Sci J. 2017;73(1):163-174.

4. Jung Y, Oh S, Lee S, Lee HJ, Choo HJ, Jo C, et al. Characterization of meat quality, storage stability, flavor-related compounds, and their relationship in Korean Woorimatdag No. 2 chicken breast meat during cold storage. Poult Sci. 2025;104(1):104566.

5. Sujiwo J, Kim D, Yoon JY, Kim H, Kim JS, Lee SK, Jang A. Physicochemical and functional characterization of blue-shelled eggs in Korea. Korean J Food Sci Anim Resour. 2017;37(2):181.

6. Wang XL, Zheng JX, Ning ZH, Qu LJ, Xu GY, Yang N. Laying performance and egg quality of blue-shelled layers as affected by different housing systems. Poult Sci. 2009;88(7):1485-1492.

7. Jung Y, Oh S, Kim D, Lee S, Lee HJ, Shin DJ, et al. Effect of cinnamon powder on quality attributes and off-flavor in fried chicken drumsticks made from long-term thawed Korean native chicken. Poult Sci. 2024;103(5):103583.

8. Zhao Y, Feng F, Yang Y, Xiong C, Xu M, Tu Y. Gelation behavior of egg yolk under physical and chemical induction: A review. Food Chem. 2021;355:129569.

9. Laca A, Paredes B, Rendueles M, Díaz M. Egg yolk plasma: Separation, characteristics and future prospects. LWT-Food Sci Technol. 2015;62(1):7-10.

10. Shang H, Zhang H, Guo Y, Wu H, Zhang N. Effects of inulin supplementation in laying hens diet on the antioxidant capacity of refrigerated stored eggs. Int J Biol Macromol. 2020;153:1047-1057.

11. Jung Y, Kim HJ, Kim D, Joo B, Jhoo JW, Jang A. Physicochemical properties and volatile organic compounds of dairy beef round subjected to various cooking methods. Food Sci Anim Resour. 2023;43(5):767.

12. Plagemann I, Zelena K, Krings U, Berger RG. Volatile flavours in raw egg yolk of hens fed on different diets. J Sci Food Agric. 2011;91(11):2061-2065.

13. Gao LB, Obianwuna UE, Zhang HJ, Qiu K, Wu SG, Qi GH, et al. A comparison between the egg yolk flavor of indigenous 2 breeds and commercial laying hens based on sensory evaluation, artificial sensors, and GC-MS. Foods. 2022;11(24):4027.

14. Goto T, Ohya K, Takaya M. Genotype affects free amino acids of egg yolk and albumen in Japanese indigenous breeds and commercial Brown layer chickens. Poult Sci. 2022;101(2):101582.

15. Lordelo M, Fernandes E, Bessa RJB, Alves SP. Quality of eggs from different laying hen production systems, from indigenous breeds and specialty eggs. Poult Sci. 2017;96(5):1485-1491.

16. Suk YO, Park C. Effect of breed and age of hens on the yolk to albumen ratio in two different genetic stocks. Poult Sci. 2001;80(7):855-858.

17. AOAC [Association of Official Analytical Chemists] International. Official methods of analysis of AOAC International. 19th ed Gaithersburg, MD: AOAC International. 2012.

18. Cho J, Barido FH, Kim HJ, Kim HJ, Kim D, Shin DJ, Jang, A. Effect of calamansi pulp ethanol extracts on the meat quality and biogenic amine formation of pork patty during refrigerated storage. Food Sci Anim Resour. 2023;43(1):25.

19. Kim HJ, Kim HJ, Jang A. Nutritional and antioxidative properties of black goat meat cuts. Asian-Australas J Anim Sci. 2019;32(9):1423.

20. Gillespie KM, Chae JM, Ainsworth EA. Rapid measurement of total antioxidant capacity in plants. Nat Protoc. 2007;2(4):867-870.

21. Ukwu HO, Ezihe CO, Asaa SK, Anyogo ME. Effect of egg weight on external and internal egg quality traits of Isa Brown egg layer chickens in Nigeria. J Anim Sci Vet Med. 2017;2(4):126-132.

22. Roberts JR. Factors affecting egg internal quality and egg shell quality in laying hens. J Poult Sci. 2004;41(3):161-177.

23. Haunshi S, Niranjan M, Shanmugam M, Padhi MK, Reddy MR, Sunitha R, et al. Characterization of two Indian native chicken breeds for production, egg and semen quality, and welfare traits. Poult Sci. 2011;90(2):314-320.

24. Tharrington JB, Curtis PA, Jones FT, Anderson KE. Comparison of physical quality and composition of eggs from historic strains of single comb White Leghorn chickens. Poult Sci. 1999;78(4):591-594.

25. Drabik K, Karwowska M, Wengerska K, Próchniak T, Adamczuk A, Batkowska J. The variability of quality traits of table eggs and eggshell mineral composition depending on hens' breed and eggshell color. Animals. 2021;11(5):1204.

26. Ketta M, Tůmová E. Relationship between eggshell thickness and other eggshell measurements in eggs from litter and cages. Ital J Anim Sci. 2018;17(1):234-239.

27. Lai KM, Chuang YS, Chou YC, Hsu YC, Cheng YC, Shi CY, et al. Changes in physicochemical properties of egg white and yolk proteins from duck shell eggs due to hydrostatic pressure treatment. Poult Sci. 2010;89(4):729-737.

28. Anggita AD, Wahyuni E, Maharani D. The quality and chemical composition of eggs derived from Kampung Unggul Balitbangtan (KUB) crossed with Merawang and Murung Panggang local chickens. 7th Int Conf Food Agric Nat Resour (IC-FANRES 2022). 2023;58-70. Indonesia.

29. Aydin R, Dogan I. Fatty acid profile and cholesterol content of egg yolk from chickens fed diets supplemented with purslane (Portulaca oleracea L.). J Sci Food Agric. 2010;90(10):1759-1763.

30. Jung S, Han BH, Nam K, Ahn DU, Lee JH, Jo C. Effect of dietary supplementation of gallic acid and linoleic acid mixture or their synthetic salt on egg quality. Food Chem. 2011;129(3):822-829.

31. Karadag A, Ozcelik B, Saner S. Review of methods to determine antioxidant capacities. Food Anal Methods. 2009;2:41-60.

32. Prior RL, Hoang HA, Gu L, Wu X, Bacchiocca M, Howard L, et al. Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity (ORACFL)) of plasma and other biological and food samples. J Agric Food Chem. 2003;51(11):3273-3279.

33. Kovacs-Nolan J, Phillips M, Mine Y. Advances in the value of eggs and egg components for human health. J Agric Food Chem. 2005;53(22):8421-8431

34. Liu Y, Sheng L, Ma M, Jin Y. Proteome-based identification of chicken egg yolk proteins associated with antioxidant activity on the Qinghai-Tibetan Plateau. Int J Biol Macromol. 2020;150:1093-1103.

35. Nimalaratne C, Lopes-Lutz D, Schieber A, Wu J. Free aromatic amino acids in egg yolk show antioxidant properties. Food Chem. 2011;129(1):155-161.

36. Wang J, Zhang T, Wan C, Lai Z, Li J, Chen L, Li M. The effect of theabrownins on the amino acid composition and antioxidant properties of hen eggs. Poult Sci. 2023;102(11):102717.

37. Nimalaratne C, Schieber A, Wu J. Effects of storage and cooking on the antioxidant capacity of laying hen eggs. Food Chem. 2016;194:111-116.

38. Luo Y, Wang B, Liu C, Su R, Hou Y, Yao D, et al. Meat quality, fatty acids, volatile compounds, and antioxidant properties of lambs fed pasture versus mixed diet. Food Sci Nutr. 2019;7(9):2796-2805.

39. Xiang X, Liu YY, Liu Y, Wang XY, Jin YG. Changes in structure and flavor of egg yolk gel induced by lipid migration under heating. Food Hydrocoll. 2020;98:105257.

40. Harlina PW, Yuliana T, Shahzad R, Ma M. Study on the development and functional characteristics of salted egg with liquid smoke. Food Sci Anim Resour. 2023;43(3):471.

41. Ren L, Ma J, Xu W, Lv Y, Tong Q. Stability of low density lipoprotein particles affect the formation of off-flavor in thermal egg yolk. Food Res Int. 2022;154:111029.

679 42. Rasinska E, Rutkowska J, Czarniecka-Skubina E, Tambor K. Effects of cooking methods on changes in fatty acids
680 contents, lipid oxidation and volatile compounds of rabbit meat. LWT-Food Sci Technol. 2019;110:64-70.

681 43. Almela E, Jordan MJ, Martinez C, Sotomayor JA, Bedia M, Banon S. Ewe's diet (pasture vs grain-based feed)
682 affects volatile profile of cooked meat from light lamb. J Agric Food Chem. 2010;58(17):9641-9646.

683 44. Jo SH, Kim KH, Kim YH, Lee MH, Ahn JH, Szulejko JE, et al. Study of odor from boiled eggs over time using
684 gas chromatography. Microchem J. 2013;110:517-529.

685 45. Wei M, Liu X, Xie P, Lei Y, Yu H, Han A, et al. Characterization of volatile profiles and correlated contributing
686 compounds in pan-fried steaks from different Chinese yellow cattle breeds through GC-Q-orbitrap, e-nose, and
687 sensory evaluation. Molecules. 2022;27(11):3593.

688

Tables

Table 1. Proximate composition, pH value, and cholesterol contents of egg yolk of three Korean native chicken breeds

Items	CE	BSE	WRMD1E	WRMD2E	SEM
Proximate composition					
Moisture (%)	49.21^{bc}	49.82^{b}	50.76^{a}	48.60^{c}	0.313
Crude protein (%)	16.21	16.10	16.03	16.20	0.328
Crude fat (%)	30.75^{ab}	31.51^{a}	30.30^{b}	30.94^{ab}	0.174
Crude ash (%)	1.73^{ab}	1.78^{ab}	1.65^{b}	1.86^{a}	0.058
pH	6.16^{c}	6.21^{b}	6.29^{a}	6.12^{c}	0.015
Cholesterol (mg/ g)	9.80^{ab}	9.40^{bc}	10.06^{a}	9.06^{c}	0.165

$^{a-c}$Means within same row with different superscript letters differ significantly ($p < 0.05$). CE, commercial egg (Control); BSE, blue-shelled egg; WRMD1E, Woorimatdag No. 1 chicken egg; WRMD2E, Woorimatdag No. 2 chicken egg.

Table 2. Fatty acid composition of egg yolk of three Korean native chicken breeds

Fatty acid (%)	CE	BSE	WRMD1E	WRMD2E	SEM
C14:0 (myristic acid)	0.54^{ab}	0.53^{ab}	0.59^{a}	0.47^{b}	0.029
C16:0 (palmitic acid)	32.46^{a}	30.01^{b}	29.25^{bc}	28.22^{c}	0.375
C16:1n7 (palmitoleic acid)	2.97^{a}	2.51^{b}	2.98^{a}	2.55^{b}	0.102
C18:0 (stearic acid)	6.88^{d}	7.70^{c}	8.47^{b}	9.78^{a}	0.124
C18:1n9 (oleic acid)	35.73^{d}	41.12^{b}	39.19^{c}	43.94^{a}	0.461
C18:1n7 (vaccenic acid)	1.10^{a}	1.02^{a}	1.02^{a}	0.88^{b}	0.029
C18:2n6 (linoleic acid)	18.03^{a}	13.93^{c}	15.13^{b}	10.79^{d}	0.397
C18:3n6 (r-linolenic acid)	0.04^{d}	0.08^{c}	0.12^{a}	0.10^{b}	0.007
C18:3n3 (a-linolenic acid)	0.19^{b}	0.25^{a}	0.30^{a}	0.17^{b}	0.022
C20:1n9 (eicosenoic acid)	0.19^{c}	0.28^{b}	0.35^{a}	0.28^{b}	0.016
C20:4n6 (arachidonic acid)	1.73^{b}	2.33^{a}	2.23^{a}	2.33^{a}	0.075
C22:4n6 (adrenic acid)	0.05^{c}	0.07^{bc}	0.15^{a}	0.13^{ab}	0.024
C22:6n3 (docosahexaenoic acid)	0.08^{c}	0.17^{bc}	0.22^{b}	0.36^{a}	0.044
SFA	39.88^{a}	38.25^{b}	38.31^{b}	38.47^{b}	0.349
UFA	60.12^{b}	61.75^{a}	61.69^{a}	61.53^{a}	0.349
MUFA	40.00^{d}	44.92^{b}	43.54^{c}	47.65^{a}	0.438
PUFA	20.12^{a}	16.83^{c}	18.15^{b}	13.88^{d}	0.422
MUFA/SFA	1.00^{c}	1.18^{b}	1.14^{b}	1.24^{a}	0.018
PUFA/SFA	0.51^{a}	0.44^{b}	0.47^{ab}	0.36^{c}	0.013

$^{a-d}$Means within same row with different superscript letters differ significantly ($p < 0.05$).
SFA, saturated fatty acid; UFA, unsaturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid. CE, commercial egg (Control); BSE, blue-shelled egg; WRMD1E, Woorimatdag No. 1 chicken egg; WRMD2E, Woorimatdag No. 2 chicken egg.

Table 3. Free amino acid contents of egg yolk of three Korean native chicken breeds

FAA (μg/g)	CE	BSE	WRMD1E	WRMD2E	SEM
Aspartic acid[3,4,6]	113.51[a]	105.62[b]	100.55[bc]	94.85[c]	2.615
Threonine[1,5]	117.46[a]	121.99[a]	107.60[b]	118.68[a]	3.115
Serine[1,6]	119.83[a]	115.95[ab]	109.56[b]	120.90[a]	2.605
Asparagine[6]	73.72[a]	64.13[b]	65.37[b]	67.08[b]	1.758
Glutamic acid[3,4]	292.36	283.84	297.21	282.32	6.455
Glutamine[6]	104.12[a]	89.04[b]	99.39[a]	94.26[ab]	3.219
Glycine[1,6]	39.75	43.23	40.64	41.80	1.667
Alanine[1,6]	69.66	68.95	64.97	68.72	1.577
Valine[2,5]	106.54[a]	98.05[b]	101.57[ab]	96.40[b]	2.487
Cysteine[6]	4.56[bc]	6.18[a]	4.32[c]	5.23[b]	0.222
Methionine[5]	47.00	47.30	46.81	48.22	1.374
Isoleucine[2,5]	86.35	79.71	84.92	84.94	2.382
Leucine[2,5]	174.91	161.10	175.20	172.72	4.382
Tyrosine[2,6]	138.37[a]	124.63[b]	133.69[ab]	132.07[ab]	3.389
Phenylalanine[2,5]	104.22	98.76	105.18	103.39	2.374
Tryptophan[5]	26.25[ab]	26.58[a]	26.49[a]	24.46[b]	0.628
Ammonia	16.73[b]	15.80[b]	20.08[a]	16.35[b]	0.314
Ornithine	3.62[c]	5.86[a]	4.34[b]	3.93[bc]	0.149
Lysine[2,5]	157.51	165.18	152.98	163.51	4.430
Histidine[2,3,5]	30.49	31.41	29.96	32.85	0.976
Arginine[2,6]	133.06[b]	148.77[a]	130.53[b]	146.47[a]	4.345
Sweet FAA[1]	346.70[a]	350.11[a]	322.76[b]	350.10[a]	7.565
Bitter FAA[2]	931.45	907.59	914.02	932.36	21.036
Acidic FAA[3]	436.36	420.87	427.71	410.02	9.124
Umami FAA[4]	405.86[a]	389.46[ab]	397.75[ab]	377.17[b]	8.406
Essential FAA[5]	850.74	830.06	830.70	845.17	18.413
Non-Essential FAA[6]	1090.53	1051.92	1047.68	1062.74	22.968
Total FAA	1961.62	1903.65	1902.81	1920.58	40.696

[a-c]Means within same row with different superscript letters differ significantly ($p < 0.05$).
[1]Sweet FAA; [2]Bitter FAA; [3]Acidic FAA; [4]Umami FAA; [5]Essential FAA; [6]Non-Esstional FAA; FAA, free amino acid. CE, commercial egg (Control); BSE, blue-shelled egg; WRMD1E, Woorimatdag No. 1 chicken egg; WRMD2E, Woorimatdag No. 2 chicken egg.

705 **Table 4. Major volatile organic compounds of boiled egg yolk of three Korean native chicken breeds**

VOC (AU×10^6)	m/z	LRI	CE	BSE	WRMD1E	WRMD2E	SEM
Acid							
Octadecanoic acid	73.0	2161	0.021^{ab}	ND^{b}	0.004^{b}	0.054^{a}	0.0141
Subtotal*			0.147^{ab}	0.039^{b}	0.044^{b}	0.292^{a}	0.0767
Alcohol							
1-Octen-3-ol	57.0	970	0.443^{a}	0.411^{ab}	0.266^{b}	0.529^{a}	0.0611
2-Propanol, 1-(2-butoxy-1-methylethoxy)-	59.0	1247	ND^{b}	ND^{b}	0.004^{b}	0.027^{a}	0.0034
1H-Tetrazol-5-amine	43.1	571	0.110^{bc}	0.278^{a}	0.206^{ab}	0.019^{c}	0.0358
Subtotal*			0.705^{ab}	0.756^{ab}	0.606^{b}	1.025^{a}	0.1044
Aldehyde							
Benzeneacetaldehyde	91.1	1044	0.021^{c}	0.047^{b}	0.025^{c}	0.061^{a}	0.0045
Decanal	57.0	1206	0.022^{a}	0.016^{bc}	0.014^{c}	0.020^{ab}	0.0016
Hexadecanal	57.0	1817	0.006^{b}	0.002^{b}	0.005^{b}	0.013^{a}	0.0019
Nonanal	57.0	1111	0.129^{ab}	0.099^{b}	0.051^{c}	0.159^{a}	0.0140
Octanal	41.1	998	0.025^{b}	ND^{c}	0.039^{ab}	0.055^{a}	0.0070
Propanal, 2,2-dimethyl-	57.1	554	4.306^{c}	5.836^{b}	6.705^{a}	1.389^{d}	0.1738
Subtotal*			5.153^{c}	6.062^{b}	6.894^{a}	1.769^{d}	0.2101
Ester							
Arsenous acid, tris(trimethylsilyl) ester	207.0	700	5.695^{d}	6.629^{c}	9.687^{a}	7.790^{b}	0.2920
Borinic acid, diethyl-, methyl ester	43.1	585	1.013^{b}	1.981^{a}	1.861^{a}	0.483^{c}	0.0795
Subtotal*			7.019^{c}	8.853^{b}	12.115^{a}	8.582^{b}	0.3174
Hydrocarbon							
2-Propen-1-amine	57.0	551	0.167^{a}	0.223^{a}	ND^{b}	0.037^{b}	0.0353

Benzene, 1,2,3-trimethyl-	105.0	1016	ND[b]	ND[b]	0.010[a]	0.018[a]	0.0033
Benzene, 1,2,4-trimethyl-	105.0	981	0.004[b]	0.007[b]	0.023[ab]	0.037[a]	0.0064
Benzene, 1,3-bis(1,1-dimethylethyl)-	175.1	1258	0.129[a]	0.110[a]	0.062[b]	0.038[c]	0.0083
Butane, 1-isocyano-	41.1	537	0.030[b]	0.044[a]	0.029[bc]	0.015[c]	0.0049
Cyclopentane, 1,2-dimethyl-, cis-	43.0	724	0.017[a]	0.013[a]	ND[b]	ND[b]	0.0021
D-Limonene	68.0	1026	ND	ND	0.005	0.006	0.0019
Hexadecane	71.1	1600	ND[c]	ND[c]	0.013[b]	0.035[a]	0.0023
Hexane, 3,3-dimethyl-	43.0	751	0.033[ab]	0.040[a]	0.026[b]	ND[c]	0.0035
Hexane, 3-ethyl-	43.1	768	0.681[a]	0.688[a]	0.441[b]	0.289[c]	0.0323
Hexane, 3-ethyl-2,5-dimethyl-	57.1	887	0.012[a]	0.015[a]	0.009[ab]	0.004[b]	0.0023
Indole	117.0	1298	0.077[a]	ND[b]	ND[b]	ND[b]	0.0217
Nonane	57.0	894	0.016[ab]	0.023[a]	0.007[b]	0.007[b]	0.0037
Nonane, 2,5-dimethyl-	57.0	1012	0.113[a]	0.120[a]	0.057[b]	0.053[b]	0.0068
Pentane, 2,3,3,4-tetramethyl-	43.1	875	0.005[ab]	0.007[a]	0.002[bc]	ND[c]	0.0013
Subtotal*			23.108[a]	25.773[a]	25.291[a]	15.038[b]	1.1627
Ketone							
(+)-2-Bornanone	95.0	1148	ND[b]	ND[b]	ND[b]	0.003[a]	ND9
2,3-Butanedione	43.1	581	0.046[ab]	0.068[a]	ND[b]	0.035[ab]	0.0191
2-Octanone	43.1	985	ND[b]	ND[b]	0.005[ab]	0.007[a]	0.0018
Subtotal*			0.046	0.068	0.005	0.045	0.0191
Sulfur compound							
Carbon disulfide	76.0	546	0.267[b]	0.088[c]	0.527[a]	0.192[bc]	0.0446
Hydrogen sulfide	34.0	1099	0.262[a]	ND[d]	0.167[b]	0.102[c]	0.0202
Subtotal*			1.037[ab]	0.223[ab]	1.122[a]	0.320[b]	0.1252
Others							

2-Pentylfuran	81.0	983	0.099[a]	0.087[ab]	0.067[b]	0.099[a]	0.0069
Trimethylamine	58.0	1099	0.158	0.137	0.145	0.092	0.0379
Subtotal[*]			0.257[b]	0.223[b]	0.950[a]	0.190[b]	0.1980
Total[*]			37.471[b]	42.000[ab]	47.027[a]	27.262[c]	1.4740

706 [a-d]Means within same row with different superscript letters differ significantly ($p < 0.05$).
707 LRI, linear retention index; m/z, mass to charge; VOC, volatile organic compound; ND, not detected. CE, commercial egg (Control); BSE,
708 blue-shelled egg; WRMD1E, Woorimatdag No. 1 chicken egg; WRMD2E, Woorimatdag No. 2 chicken egg. [*]Subtotal and total amounts of
709 VOCs are given as the sum of each detected compounds listed in Supplementary Table S2.
710

Figures

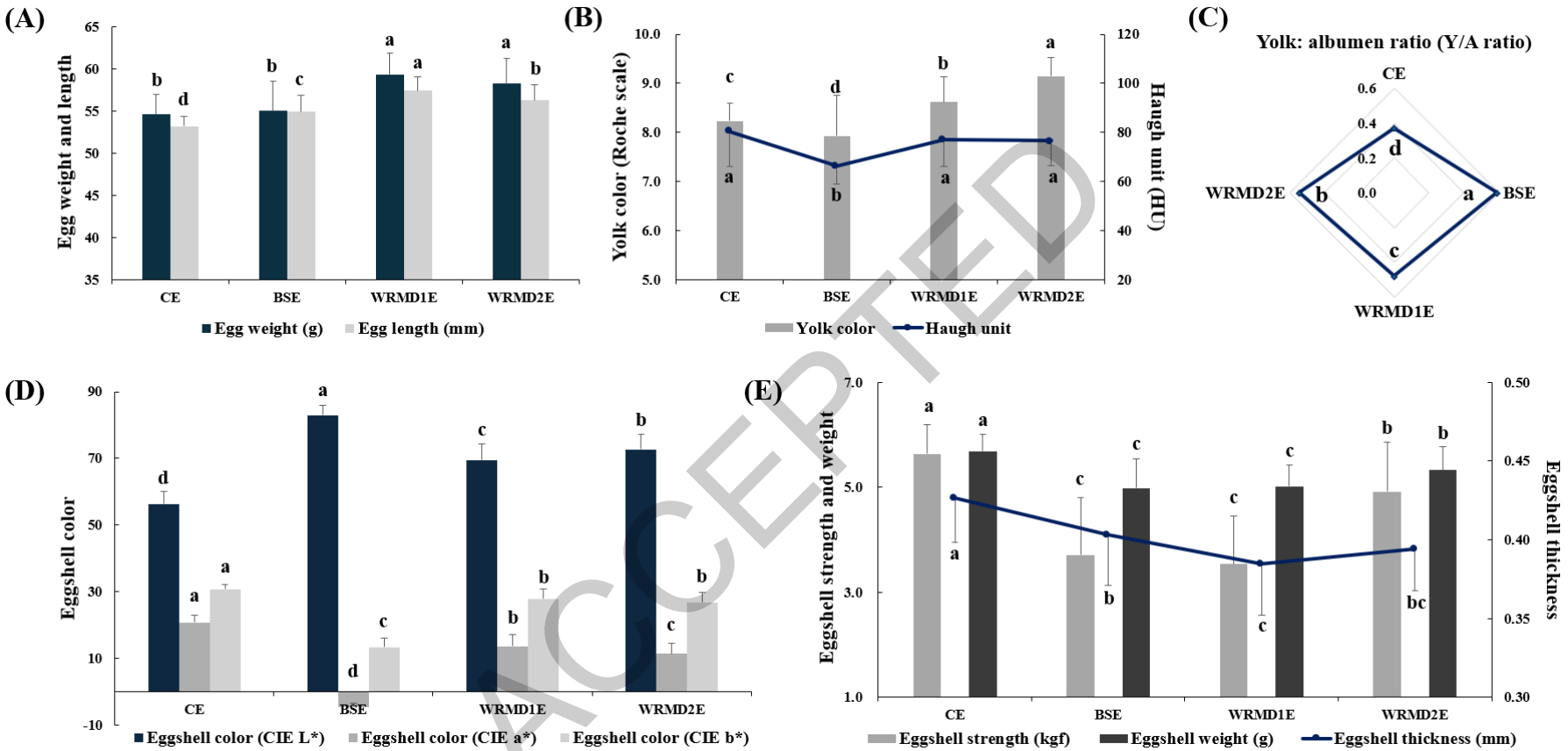


Figure 1. Internal and external quality properties of eggs of three Korean native chicken breeds. [a-d]Means value on the bar or dot with different small letters among treatments differ significantly ($p < 0.05$). (A), egg weight and length; (B), yolk color and Haugh unit; (C), yolk: albumen ratio; (D), eggshell color; (E), eggshell strength, weight, and thickness. CE, commercial egg (Control); BSE, blue-shelled egg; WRMD1E, Woorimatdag No. 1 chicken egg; WRMD2E, Woorimatdag No. 2 chicken egg.

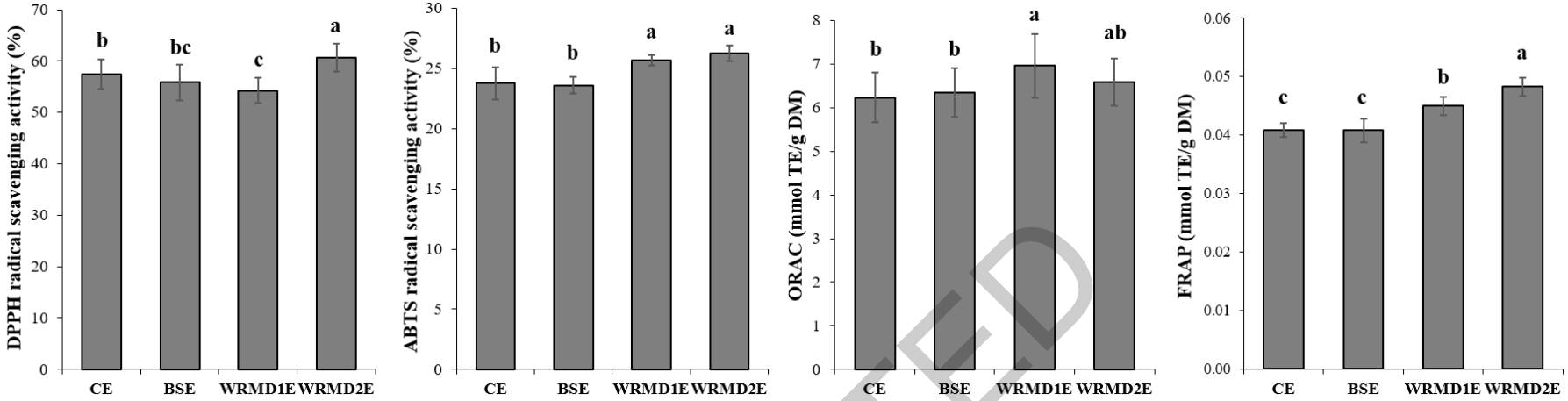


Figure 2. Antioxidant activity of egg yolk of three Korean native chicken breeds. [a-c]Means value on the bar with different small letters among treatments differ significantly ($p < 0.05$). DPPH, 2,2-diphenyl-1-picrylhydrazyl; ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); ORAC, oxygen radical absorbance capacity; FRAP, ferric reducing antioxidant power; TE, Trolox equivalent; DM, dry matter. CE, commercial egg (Control); BSE, blue-shelled egg; WRMD1E, Woorimatdag No. 1 chicken egg; WRMD2E, Woorimatdag No. 2 chicken egg.

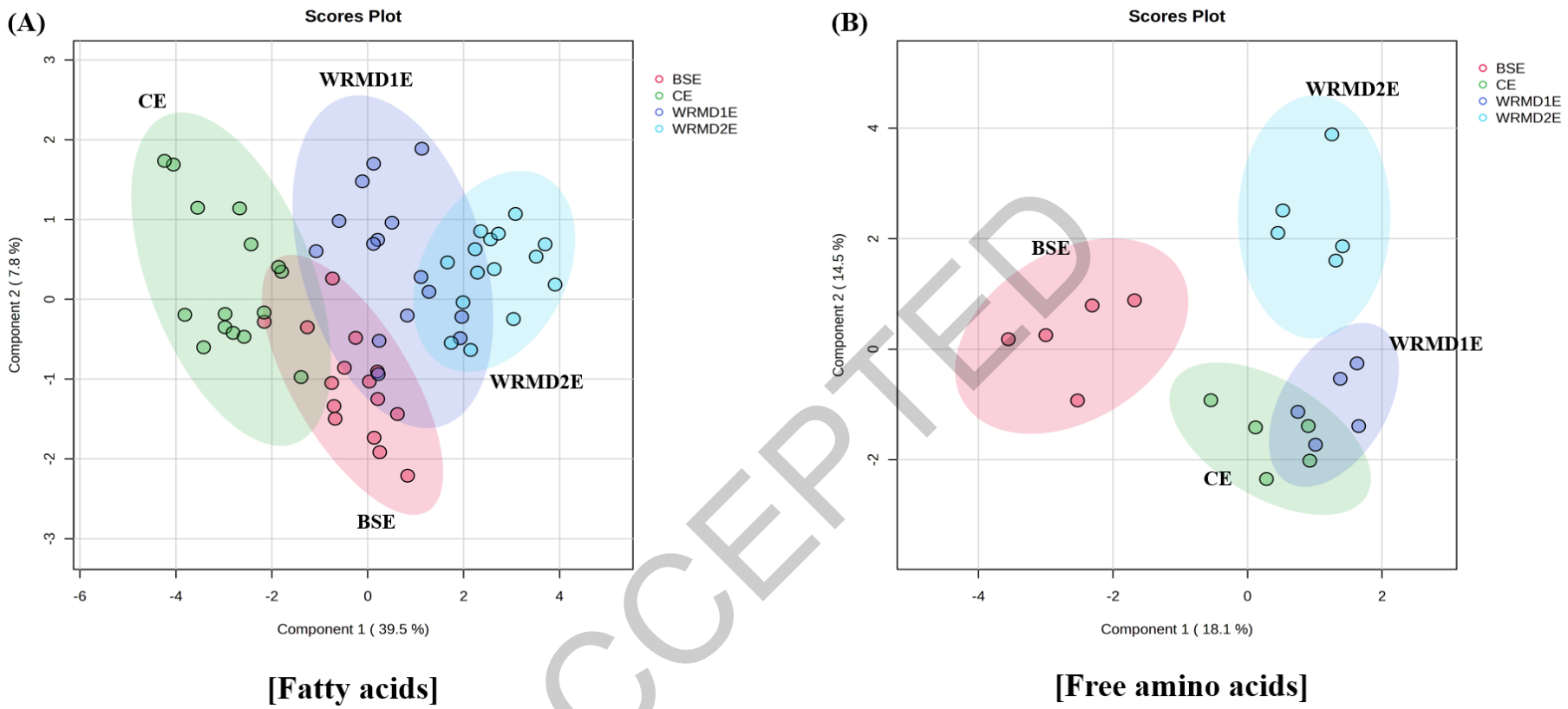
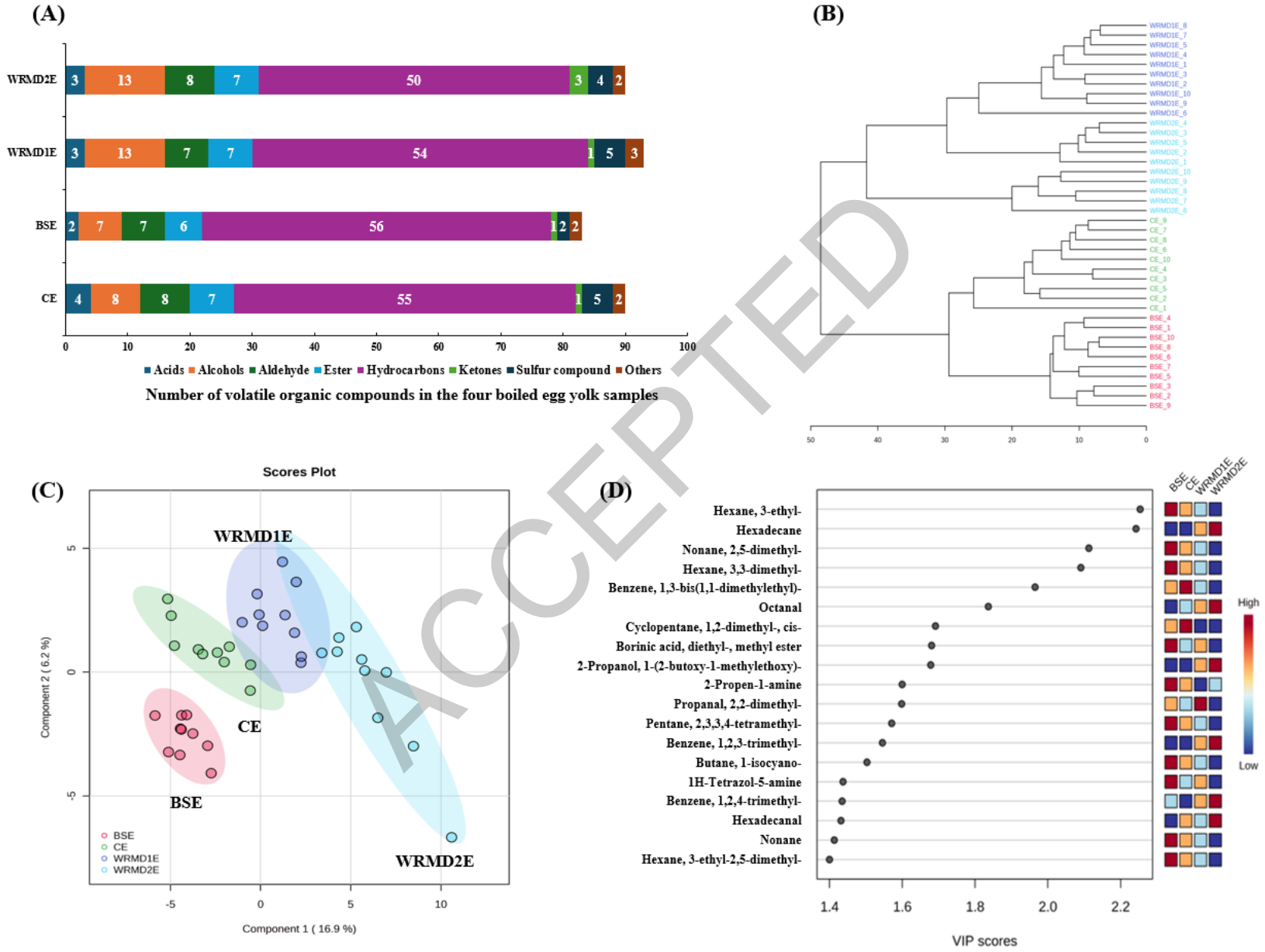


Figure 3. Non-volatile flavor compounds (fatty acids and free amino acids) of egg yolk of three Korean native chicken breeds. (A), partial least squares-discriminant analysis (PLS-DA) plot of fatty acid composition; (B), PLS-DA plot of free amino acids. CE, commercial egg (Control); BSE, blue-shelled egg; WRMD1E, Woorimatdag No. 1 chicken egg; WRMD2E, Woorimatdag No. 2 chicken egg.



730

731 **Figure 4. Volatile organic compounds of boiled egg yolk of three Korean native chicken breeds.** (A), number of VOCs in each chemical
732 class; (B), hierarchical cluster analysis (HCA) of VOCs; (C), partial least squares-discriminant analysis (PLS-DA) of VOCs; (D), variable
733 importance in projection (VIP) scores (≥1.4) of PLS-DA; VOC, volatile organic compound. CE, commercial egg (Control); BSE, blue-shelled
734 egg; WRMD1E, Woorimatdag No. 1 chicken egg; WRMD2E, Woorimatdag No. 2 chicken egg.
735