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ARTICLE

Comparative Study on Histological Structures of the Vitelline Membrane of Hen and Duck Egg Observed by Cryo-Scanning Electron Microscopy

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The histological structures of the vitelline membranes (VM) of hen and duck eggs were observed by cryo-scanning electron microscopy (cryo-SEM), and the chemical characteristics were also compared. The outer layer surface (OLS) of duck egg VM showed networks constructed by fibrils and sheets (0.1−5.2 μm in width), and that of hen egg presented networks formed only by sheets (2−6 μm in width). Thicker fibrils (0.5−1.5 μm in width) with different arrangement were observed on the inner layer surface (ILS) of duck egg VM as compared to those (0.3−0.7 μm in width) of hen egg VM. Upon separation, the outer surface of the outer layer (OSOL) and the inner surface of the inner layer (ISIL) of hen and duck egg VMs were quite similar to fresh VM except that the OSOL of duck egg VM showed networks constructed only by sheets. Thin fibrils interlaced above a bumpy or flat structure were observed at the exposed surface of the outer layer (ESOL) of hen and duck egg VMs. The exposed surfaces of inner layers (ESIL) of hen and duck egg VMs showed similar structures of fibrils, which joined, branched, and ran in straight lines for long distances up to 30 μm; however, the widths of the fibrils shown in ESOL and ESIL of duck egg VM were 0.1 and 0.7−1.4 μm, respectively, and were greater than those (<0.1 and 0.5−0.8 μm) of hen egg VM. The continuous membranes of both hen and duck egg VMs were still attached to the outer layers when separated. The content of protein, the major component of VM, was higher in duck egg VM (88.6%) than in hen egg VM (81.6%). Four and six major SDS-soluble protein patterns with distinct localization were observed in hen and duck egg VMs, respectively. Overall, the different histological structures of hen and duck egg VMs were suggested to be majorly attributable to the diverse protein components.

KEYWORDS: Duck egg; hen egg; vitelline membrane; cryo-SEM

INTRODUCTION

The vitelline membrane (VM) of bird's egg is formed from secretions emitted from the follicular epithelium of the follicle and oviduct (*1*). The VM from a freshly ovulated egg consists of an inner layer (IL) that is derived from the collagenous membrane, which lies in the epithelium of the follicle. The outer layer (OL) of the VM is laid down by the oviduct secretions of mucin (*2*). The VM of a freshly laid egg is a thin sheer structure with significant strength separating the yolk from the albumen (*2*), and it also acts a semipermeable barrier permitting certain materials to pass across it (*3*−*5*). The VM of hen egg is composed of IL and OL, with a continuous membrane imbedded between the two layers (*6*−*8*). The IL, 1.0−3.5 μm thick, is fibrous and composed of a meshwork of solid cylindrical fibers. The OL, 3.0−8.5 μm thick, is also fibrous and composed of many sublayers that lay on top of one another; the sublayers are made of fibrils that swell at contact points. Between them lies a continuous membrane, 500−1000 Å thick, which remains attached to the OL when separated and shows a vacillated sheet-like structure (*6*, *7*). Hen egg VM was reported to be majorly composed of protein (*6*, *7*). There have been nine proteins identified in hen egg VM. Those in the OL are ovomucin, lysozyme, lectin, vitelline membrane outer (VMO) I, and VMO II, and those in the IL are glycoprotein (GP) I, GP-II, GP-III, and GP-IV (*7*, *9*−*11*).

In Taiwan, duck eggs are usually processed as salted eggs and pidan (thousand-year eggs), which are traditional Chinese products and very popular in Asia (*12*). Generally, salted eggs are made by brining whole eggs in saturated saline or by coating the whole eggs with a soil paste mixed with salt for about 20−35 days. During brining, the yolk gradually becomes solidified, whereas the albumen loses its viscosity and becomes watery (*13*, *14*). Salted eggs are sold in two commercial types: the first one is the frozen isolated yolk (by eliminating salted albumen), which is widely

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utilized as a filling in Chinese foods such as moon cakes and glutinous rice dumplings and needs to be baked or cooked before consumption; the second one is the whole egg, which is consumed with rice gruel for breakfast after cooking at 85 °C for 90 min. The desirable characteristics of the cooked salted egg yolk include orange color, oil exudation, and gritty texture. Some researchers have attempted to produce salted egg yolks from isolated yolks brined in saturated saline for reducing brining time, but they found that the resulting yolks became watery and failed to attain the desirable characteristics (*13*). They concluded that the permeating rate of salt modulated by VM was the major factor affecting the attributes of salted egg yolks. In our previous study (*15*), we investigated the changes of yolk states of whole duck eggs during long-term brining in saturated saline. The results showed that the yolks became granular after 4 weeks of brining at ambient temperature and gelatinous and watery after longer brining periods. The factors influencing the yolk states during brining were demonstrated to be the rate of salt and water penetration and the integrity of VM structures (*15*). Therefore, the VM plays an important role oin the successful production of salted egg yolks.

The histological structures of hen egg VM were observed by scanning electron microscopy (SEM) and transmission electron microscopy (TEM) with chemical fixations (*6*−*8*). It is well-known that the VM is so soft that it must be spread in liquids. Therefore, observation of the native structure of VM requires careful sample preparation and fixation; otherwise, VM easily changes its conformation due to mechanical stretching. To prepare the specimens for SEM observation, chemical fixation is performed over several hours or more, and this may result in some conformation changes during the dehydration process. The greatest advantage of cryo-SEM over any other technique is that it enables us to successfully examine very soft specimens, such as native egg yolks (*16*). It is believed that the native VM can be completely fixed in liquid nitrogen (−196 °C) in a very short time and dehydrated under vacuum while retaining its original structure. Therefore, the fine structure of the VM is suggested to be observed by cryo-SEM. Although the histological structures of hen egg VM were investigated (*6*−*8*), those of duck egg VM were never studied. The aim of this study was to develop a new fixation method, cryo-technology, to observe the native VM structures of the duck egg and also to compare these to those of the hen egg. To understand the differences of the VM properties between the two fowls' eggs, the chemical characteristics of the VMs were also determined.

MATERIALS AND METHODS

Eggs. One-day -old duck (*Anas plotyrhyncus*) eggs and retail hen eggs stored at ambient temperature were purchased from a local retail market. The shell eggs were immediately washed by streaming tap water (23−26 °C; pH 6.5−6.8), drained for 30 min, and then used for preparation of VM. Forty-two duck eggs (18 for the whole VM; 24 for the separated VM) and 38 hen eggs (16 for the whole VM; 22 for the separated VM) were used for cryo-SEM observation in this study.

Reagents. Sodium chloride, hydrochloric acid, and acetic acid of ACS reagent grade and glycerol of EP grade were obtained from Union Chemical Works Ltd. (Hsinchu, Taiwan). Tris(hydroxymethyl)aminomethane (Tris), sodium dodecyl sulfate (SDS), bromophenol blue, and methanol of ACS reagent grade and Coomassie Brilliant Blue R250 were purchased from Sigma-Aldrich, Inc. (St. Louis, MO).

Preparation of VM. After manually breaking of the egg shell, the albumen and yolk were poured into a Petri dish. Yolk was separated from the albumen, the chalazae were clipped off by tweezers, and the yolk was then immersed in 100 mL of 1% NaCl at 4 °C. The adhering albumen in the saline solution showed a transparent and visible layer, which was carefully peeled off by tweezers. The eliminated albumen and saline solution were sucked out through a straw. After the addition of another 100 mL of 1% NaCl, the adhering albumen was washed out by gentle streams made by a straw. A clean yolk was obtained with several changes of the saline solution. The VM was ruptured at one spot, and a straw was inserted so that the yolk contents could be sucked out. The membrane was washed in several changes in 1% NaCl to remove yolk material. As the cloudiness was invisible, the membrane was further washed three times in 10 mL of 0.05 M Tris-HCl (pH 6.5). The membrane was then immersed in 10 mL of 1% NaCl at 4 °C and then observed by cryo-SEM within 24 h.

Separation of the Inner from the Outer Layers of VM. According to the method of Kido and Doi (*7*) with some modifications, the clean yolk obtained as described above was incubated in 150 mL of 0.01 N HCl (pH 1.8) at 37 °C for 45 min. During incubation, the yolk volume increased due to the penetration of the incubation medium. After removal of the medium outside the yolk by suction, the yolk was ruptured at one spot, and a straw was inserted so that the contents could be sucked out. At this stage, the two layers were partially separated, and the complete separation was achieved by tweezers. The two layers were carefully washed by several changes of 1% NaCl. As the cloudiness was invisible, the layers were gently washed three times by 0.05 M Tris-HCl (pH 6.5), then immersed in 10 mL of 1% NaCl at 4 °C, and observed by cryo-SEM within 24 h.

Cryo-SEM Observation. The whole VM and both separated layers all presented a circular shape; therefore, the convex of the VM can be identified to be the outer layer surface (OLS), the outer surface of the outer layer (OSOL), or the exposed surface of the inner layer (ESIL), whereas the concave could be known as the inner layer surface (ILS), the exposed surface of the inner layer (ESIL), or the inner surface of the inner layer (ISIL). Each sample was suspended in 20 mL of 1% NaCl at 4 °C in a Petri dish (90 mm in diameter). A 300 mesh copper grid (diameter = 3 mm, 1GC300, Power Assist Instrument Sci. Corp., Taoyuan, Taiwan) was clipped under the sample and then lifted to allow the sample to be mounted onto the grid. The sample was loaded on the cryo-specimen holder with colloidal carbon, cryo-fixed in slush nitrogen (−210 °C) for 1 min, and then immediately transferred into the vacuum space (13.3 Pa) of the cryo-unit in the frozen state for 15 min. After coating with gold, the specimen was directly observed by the cryo-stage (−176 °C) SEM (ABT-150S, TOPCON, Japan) with a temperature controller (CryoTrans System, model E7400, BioRad, U.K.) under an accelerating voltage of 5 kV. Three specimens were obtained from each sample for cryo-SEM observation. The cryo-SEM measurements were quantified using Adobe Photoshop version 5.5 (Adobe System Inc., San Jose, CA). The surfaces of the whole VM, OL, and IL observed by cryo-SEM are diagramed in **Figure 1**.

Chemical Compositions. Each sample was washed three times with deionized water and then dried at 103 °C to constant weight according to the accepted method (*17*) for determination of chemical compositions. Individual determination was repeated 10 times, and each time about 20 mg of the dry matter of the sample was used. The chemical compositions of each sample were analyzed following the AOAC methods (42.016 for crude protein; 24.005 for crude lipid; 44.1.30 for total carbohydrates; 24.036 for ash) (*18*). Briefly, crude protein content was determined using the micro-Kjeldahl method with the nitrogen factor of 6.25 [suggested by Bellairs et al. (*6*)]; crude lipid content was determined by Soxhlet extraction process; total carbohydrates contents were identified by the phenol−sulfuric acid colorimetric method carried out spectrophotometrically at 490 nm; and ash content was measured by burning the dry matter of the samples at 600 °C.

Electrophoresis. Each sample was washed three times with deionized water and then dried at 103 °C to constant weight for SDS−polyacrylamide gel electrophoresis (SDS-PAGE) analysis. According to the method of Kelley (*19*), 2 mL of 70 mM Tris-HCl (pH 6.8) buffer solution containing 1% SDS was added to the dry matter of each sample and then stirred at 24 °C with a magnetic stir rod for 18 h to dissolve the sample. The dissolved membrane solution was centrifuged (Centrifuge himac CR21, Hitachi Ltd., Katsuda, Japan) at 20000*g* and 4 °C for 10 min. Four hundred microliters of the supernatant was mixed with 400 μL of sample buffer (62.5 mM Tris-HCl, pH 6.8, containing 2% SDS and 25% glycerol), and then 40 μL of bromophenol blue was added. The mixture was heated in a water bath at 100 °C for 5 min and then stored at −20 °C until ready for electrophoresis. Fifteen microliters of each sample was loaded in each lane. VM protein banding patterns were electrophoresed using the powered mini PAGE system (AE-6531, Atto Corp., Tokyo, Japan) on

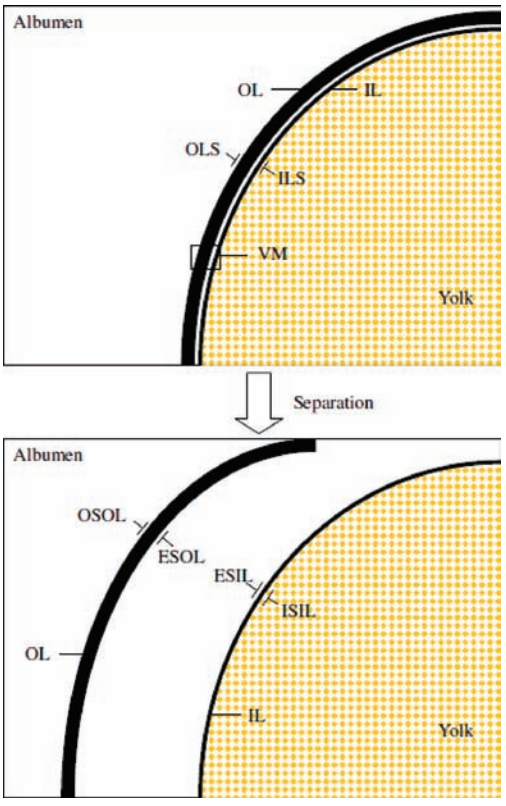

Figure 1. Diagrams of the surfaces of the whole vitelline membrane and separated layers observed by cryo-SEM. VM, vitelline membrane; OL, outer layer; OLS, outer layer surface; IL, inner layer; ILS, inner layer surface; OSOL, outer surface of the outer layer; ESOL, exposed surface of the outer layer; ESIL, exposed surface of the inner layer; ISIL, inner surface of the inner layer.

5−20% gradient gels at 20 mA for 2 h. The protein bands were stained with a solution containing 0.27% (w/v) Coomassie Brilliant Blue R250, 45% (v/v) methanol, and 45% (v/v) acetic acid and then destained in a solution containing 10% (v/v) acetic acid and 20% (v/v) methanol.

Statistical Analysis. Data of the chemical compositions are expressed as the means ± standard deviation (SD) of 20 assays from 10 independent experiments. Statistical analysis was performed by using the unpaired Student's *t* test. Differences were considered to be significant at $p < 0.05$.

RESULTS AND DISCUSSION

VM. Previous studies have reported that the transverse sections of OL of hen egg VM consisted of a variable number of sublayers, and each sublayer was composed of fibrils observed by TEM (*6*, *7*). The tangential sections showed that each sublayer was composed of fibrils which ran in straight lines for long distances up to 1 μm or more. The gaps between the fibrils were of diamond or triangular shape so that the general appearance was reminiscent of an expanded lattice. The OLS of hen egg VM observed by SEM also showed a latticework of fine fibrils (*7*, *8*). In the present study, the OLS of hen egg VM showed network structures constructed by sheets but not fibrils (**Figure 2A**). Bellairs et al. (*6*) reported that the OL showed networks of fine fibrils, which might suggest that the pieces of the samples used for TEM observation were cut into 60−100 nm slices in depth. The width of the sheets was estimated as 2−6 μm. The hollows of the networks, 5−15 μm in diameter, represented polygons. The result is consistent with previous studies (*6*−*8*). On the other hand, the OLS of duck egg VM showed network structures with round but uneven hollows which, at 1−5 μm in diameter, were smaller than those of hen egg VM (**Figure 2B**). The compact network

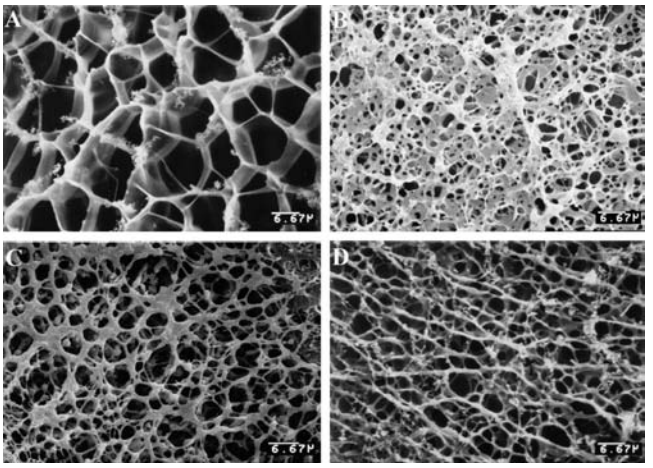

Figure 2. Micrographs of the vitelline membrane: OLS of hen egg (**A**) and duck egg (**B**); ILS of hen egg (**C**) and duck egg (**D**).

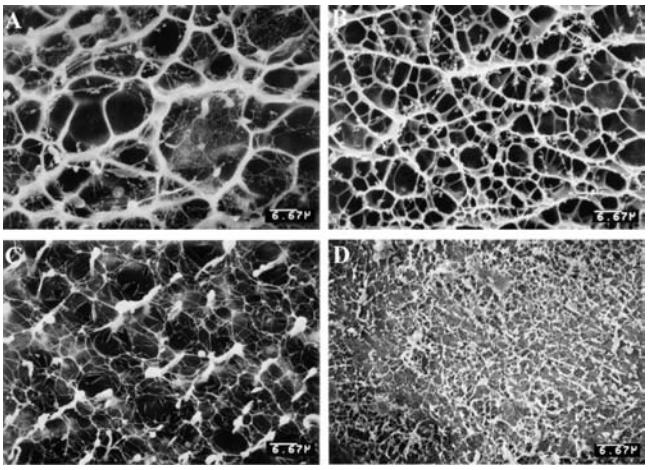

Figure 3. Micrographs of the isolated outer layer: OSOL of hen egg (**A**) and duck egg (**B**); ESOL of hen egg (**C**) and duck egg (**D**).

structures were formed by both fibrils and sheets, 0.1−5.2 μm in width. As compared to hen egg, the histological structures of OLS of duck egg VM were quite different.

Previous studies reported the IL of hen egg VM observed by TEM appeared to consist of a meshwork of solid cylindrical fibers (*6*, *7*). These fibrils ran mainly parallel to the surface of the yolk, although some of them ran vertically. The fibrils joined together and branched to form a three-dimensional network. The fibrils varied in thickness from about 0.2 to 0.6 μm in diameter. In the present study, the ILS of hen egg VM showed several layers of network structures formed by thick fibrils (**Figure 2C**). The fibrils varied in width from about 0.3 to 0.7 μm, and they also joined together and branched to form three-dimensional networks as reported previously (*6*−*8*). Most hollows of the networks were shown in a round shape and varied in diameter from 1.9 to 7.8 μm. On the other hand, the ILS of duck egg VM showed that thick fibrils (ranging from 0.5 to 1.5 μm in width) ran in straight lines for long distances and parallel to the surface of the yolk (**Figure 2D**). Network structures were formed by thick fibrils and also observed inside the ILS. The results showed that the fibril arrangement of IL of duck egg VM was quite different from that of hen egg VM.

Outer Layer of VM. Panels **A** and **C** of **Figure 3** show the microstructures of OSOL (which contacted albumen) and ESOL (which contacted IL) upon separation of hen egg VM, respectively,

and panels **B** and **D** of **Figure 3** show the OSOL and ESOL of duck egg VM. The OSOL of hen egg VM was quite similar to that of the fresh membrane (**Figures 3A** and **2A**); however, some cobweb-like thin fibrils were observed inside the network structures of OL upon separation (**Figure 3A**). Surprisingly, the OSOL of duck egg VM upon separation was quite different from that of the fresh membrane (**Figures 3B** and **2B**), but it was similar to that of fresh hen egg VM (**Figure 2A**). The OSOL of duck egg VM also showed network structures constructed by sheets. The hollows of the networks represented polygons, 1.2−7.4 μm in diameter, smaller than those of hen egg VM.

Thin fibrils (<0.1 μm in width) interlaced above a bumpy structure were observed at the ESOL of hen egg VM (**Figure 3C**). Kido and Doi (*7*) also found a vacillated sheet-like structure that represented the continuous membrane appearing to cover the ESOL. We suggested that the bumpy structure observed in the present study was identical to the continuous membrane which was reported to be still attached to the OL when separated (*6*). The ESOL of duck egg VM showed that thin (about 0.1 μm in width) and interlaced fibrils were attached to the flat substances (**Figure 3D**). The thin fibrils of the duck egg were coarser than those of the hen egg. The flat substances were supposed to be a continuous membrane that was also attached to the OL when separated.

Inner Layer of VM. Panels **A** and **C** of **Figure 4** show the microstructures of ESIL (which contacted OL) and ISIL (which contacted yolk) upon separation of hen egg VM, respectively, and panels **B** and **D** of **Figure 4** show the ESIL and ISIL of duck egg VM. The ESIL of hen egg VM appeared to consist of a network of thick fibrils (0.5−0.8 μm in width) (**Figure 4A**). Some of the fibrils joined together and branched to form a fine network, some ran in straight lines for long distances up to 27 μm, and they compactly interlaced with each other. The fibrils observed from the ESIL ran parallel to the surface of the yolk. The results obtained in the present study were similar to those reported by Bellairs et al. (*6*) and Kido and Doi (*7*); however, the widths of the fibrils observed in this study were greater than those (0.2−0.6 in diameter) in the previous studies. On the other hand, the ESIL of duck egg VM was quite similar to that of hen egg, and the thick fibrils interlaced more loosely and were wider (0.7−1.4 μm in width) as compared to those of hen egg. The fibrils also ran in straight lines for long distances up to 30 μm. Several layers of the fibrils of the duck egg could be observed from the ES side, but those of the hen egg were not easily distinguished. The smudge-like substances existing on some fibrils were believed to be the residual continuous membrane and observed in both hen and duck eggs.

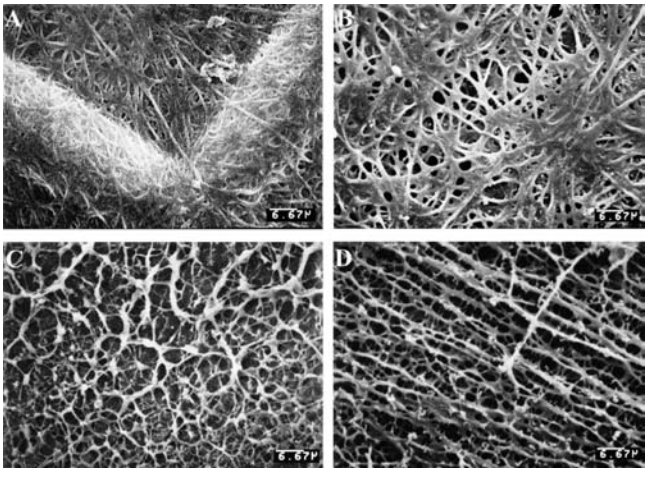

Figure 4. Micrographs of the isolated inner layer: ESIL of hen egg (**A**) and duck egg (**B**); ISIL of hen egg (**C**) and duck egg (**D**).

The ISIL of hen egg VM upon separation was similar to the fresh membrane (**Figures 4C** and **2C**). However, the layers of the network structures observed in the fresh membrane were not easily distinguished in ISIL. The ISIL of duck egg VM showed networks of thick fibrils that were quite similar to the fresh membrane (**Figures 4D** and **2D**). Some joints between the fibrils were fractured, probably due to the treatment with HCl solution. Bakst and Howarth (*20*) observed ground substances in the OS, corresponding to that of the ES, of the IL obtained from ovum at ovulation. The ground substances were not observed in the present and previous studies (*7*), and it may be suggested that this was because they were removed upon formation of the OL in the upper oviduct.

Chemical Composition. Table 1 shows the chemical compositions of the whole and separated VMs of hen and duck eggs. Average dry weights of the whole VMs of hen and duck egg were 6.0 and 10.4 mg/egg, respectively. The dry weights of OL and IL of hen egg were 3.6 and 2.1 mg/egg, and those of duck egg were 6.6 and 3.2 mg/egg, respectively. The IL and OL weight ratio of 1:1.7 of hen egg obtained in the present study was similar to that of 1:1.6 reported by Kido and Doi (*7*), whereas a ratio of 1:2.1 was observed in duck egg. In the whole VMs of both hen and duck eggs, the major component was protein, the contents of which were 81.6 and 88.6% in the dry matter, respectively (**Table 1**). In hen egg VM, the protein content of IL was 1.13 times greater than that of OL, and the carbohydrate content of OL doubled that of IL. The results were similar to those in previous studies, which reported that IL contained 1.11 and 1.83 times in total nitrogen and neutral sugar contents, respectively, greater than OL (*6*, *7*). The ash content of hen egg VM was 0.61% [0.62% reported by Trziszka and Smolinska (*17*)]. The lipid content of hen egg VM in the present study was 1.3%, and that was distinct from those reported in previous studies (*6*, *7*, *17*). The lipid content of the VM purified from the lipid fractions of the yolk which stuck to ISIL with ethanol/ether (1:3, v/v) was 5.2% (*17*), and that extracted in ether/ethanol (1:3, v/v) and subsequently with ether was 3.2% (*6*). On the other hand, the lipid content of the VM washed by 1% saline was reported to be 0.9% (*7*) and 13.26% (*14*), respectively.

Table 1. Chemical Compositions of the Dry Matter of the Vitelline Membrane of Hen and Duck Eggs[a]

	hen egg			duck egg		
chemical component	VM	OL	IL	VM	OL	IL
weight (mg/egg)	6.0 ± 0.7	3.6 ± 0.4	2.1 ± 0.3	10.4 ± 0.9*	6.6 ± 0.8**	3.2 ± 0.5***
protein (%)	81.6 ± 1.3	72.1 ± 0.9	85.6 ± 1.5	88.6 ± 0.9*	78.3 ± 0.8**	91.3 ± 1.4***
lipid (%)	1.3 ± 0.1	nd	0.24 ± 0.02	0.62 ± 0.04*	nd	nd***
carbohydrates (%)	6.5 ± 0.2	7.8 ± 0.6	3.9 ± 0.4	6.8 ± 0.3	7.6 ± 0.4	4.4 ± 0.2
ash (%)	0.61 ± 0.02	0.58 ± 0.01	nd	0.46 ± 0.04*	nd**	nd

[a] All values are means ± SE of data from 10 independent experiments ($n = 20$). nd, not determined. *, significant difference of each component of VM of duck egg with respect to that of hen egg ($p < 0.05$). **, significant difference of each component of OL of duck egg with respect to that of hen egg ($p < 0.05$). ***, significant difference of each component of IL of duck egg with respect to that of hen egg ($p < 0.05$).

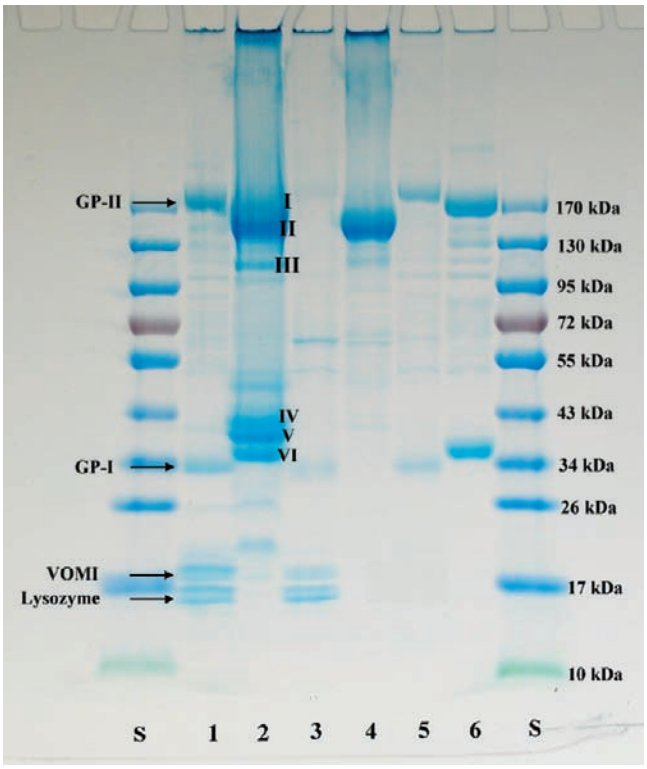

Figure 5. SDS-PAGE (5−20% gradient gel) patterns of the vitelline membrane of hen and duck eggs. Lane S represents the standard; lanes 1, 3, and 5 are the whole VM, OL, and IL of hen egg; lanes 2, 4, and 6 are the whole VM, OL, and IL of duck egg. I−VI represent COI−COVI.

We suggested that the diverse results in lipid content of VM were probably due to the eggs used from various species of hens [NX × Sussex hens were used by Trziszka and Smolinska (*17*); no information of the hens' species was provided by Bellairs et al. (*6*) or Kido and Doi (*7*)] or the content of the residual yolk materials. Furthermore, the protein content of IL of duck egg VM was also 1.13 times greater than that of OL, whereas the contents of IL and OL were both significantly higher than those of hen egg VM ($p < 0.05$). The carbohydrate contents of OL, IL, and whole VM of duck egg were almost equivalent to those of hen egg ($p > 0.05$). The lipid and ash contents of VM of the duck egg were slightly lower than those of the hen egg ($p < 0.05$). The results showed that the chemical components of the hen and duck egg VM were slightly different.

Electrophoresis. Figure 5 shows the VM protein patterns of hen and duck eggs. The macromolecular components of hen egg VM were reported to be SDS-soluble glycoproteins (GP), designated GP-I, GP-II, and GP-III (*9*−*11*), which were found to constitute the IL (*10*, *11*, *21*). The OL is mainly composed of lysozyme, VMOI, and ovomucin (*9*). In **Figure 5**, hen egg VM shows four main bands, GP-II (183 kDa) and GP-I (32 kDa) in IL and VMOI (17 kDa) and lysozyme (14 kDa) in OL, and the banding pattern of GP-III (> 1000 kDa) appeared at the top of the stacking gel probably due to its high molecular weight (*7*). Similar results were also observed in previous studies (*7*, *17*). On the other hand, duck egg VM showed six main bands, later named components I−VI (COI, COII, COIII, COIV, COV, and COVI), found to have molecular weights of about 170, 130−170, 95−130, and three between 34 and 43 kDa, respectively. The OL of duck egg VM showed two main bands, COII and COIII; meanwhile, the IL contained COI and COVI, the two major SDS-soluble components, as well as some minor patterns. There was also a banding pattern in duck egg VM at the top of the stacking gel; we suggested that one or more proteins with high molecular weight existed in the VM. The results showed that the protein components of duck egg VM were quite distinct from those of hen egg VM. Further studies on the differences of functionalities and characteristics of duck and hen egg VM proteins would allow better comparison, and changes of VM structures due to processing might be better understood.

Conclusions. The histological structures of hen and duck egg VM were successfully examined by cryo-SEM and compared. The results in this study show that each layer of the VM was constituted by network structures of fibrils varying in widths and arrangements. The OLS of duck egg VM showed networks formed by fibrils and sheets, and that of hen egg VM also showed networks formed only by sheets. The fibrils observed in the ESOL, ESIL, and ISIL of duck egg VM were thicker as compared to those of hen egg. The content of protein, the major component of the VM, was higher in duck egg VM than in hen egg VM. According to the results from electrophoresis, four and six major SDS-soluble protein patterns were observed in the VMs of hen and duck egg, respectively; the distinct localization of the macromolecular components showed various protein compositions in the two VMs. Overall, the differences in histological structures between hen and duck egg VMs were suggested to be majorly attributable to the diverse protein components, and those would result in various membrane strengths and processing qualities. The functionalities and characteristics of duck VM proteins need to be further investigated to examine their relationship to the histological structures.

ABBREVIATIONS USED

VM, vitelline membrane; SEM, scanning electron microscopy; TEM, transmission electron microscopy; cyro-SEM, cryo-scanning electron microscopy; SDS-PAGE: sodium dodecyl sulfate−polyacrylamide gel electrophoresis; OL, outer layer; OLS, outer layer surface; IL, inner layer; ILS, inner layer surface; OSOL, outer surface of the outer layer; ESOL, exposed surface of the outer layer; ESIL, exposed surface of the inner layer; ISIL, inner surface of the inner layer; GP, glycoprotein; VMOI, vitelline membrane outer I; COI−COVI, components I−VI.

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