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Changes in chemical composition, physical properties and microstructure of duck egg as influenced by salting

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ABSTRACT

Changes in chemical composition, physical properties and microstructure of duck egg, during salting for up to 14 days, were determined. Duck egg consisted of 10.87% shell, 54.73% egg white and 33.94% yolk. Salting resulted in an increase in weight proportion of egg white, but a decrease in yolk proportion. Moisture contents of both egg white and yolk decreased gradually with concomitant increases in salt and ash contents as the salting time increased. Protein and lipid contents increased slightly in both interior (viscous portion) and exterior (hardened portion) egg yolk with increasing salting time. Oil exudation was observed in yolk, particularly in exterior yolk. Triacylglycerols and phospholipid, found as the major lipids in egg yolk, underwent slight changes, but no differences in protein patterns of either egg white or egg yolk were observed during salting. Hardening ratio and hardness of egg yolk increased with increasing salting time. Adhesiveness and gumminess also increased, while springiness, cohesiveness and gumminess decreased slightly when the salting time increased. Scanning electron microscopic study revealed that yolk granule was polyhedral in shape and aligned closely when the salting proceeded. Protein spheres were distributed uniformly, together with oil droplets, in salted yolk, as visualised by transmission electron microscopy. Confocal laser scanning microscope (CLSM) micrographs indicated that the greater dehydration and release of lipids took place in egg yolk during salting.

1. Introduction

Eggs have been an important part of the human diet throughout the world. They have traditionally been used for breakfast, home meal preparation, baking and as an ingredient of many foods. Hen and duck eggs are the most commonly eaten eggs, and are highly nutritious. They supply a large amount of complete, high-quality protein (which contains all essential amino acids for humans), and provide significant amounts of several vitamins and minerals (Gutierrez, Takahashi, & Juneja, 1996). They are also one of the least expensive single-food sources of complete protein (Watkins, 1995). Salted egg is one of the most traditional and popular preserved egg products. Generally, salted egg can be made by brining eggs in saturated saline or by coating the egg with soil paste mixed with salt for about 15–30 days (Chi & Tseng, 1998; Lai, Chi, & Ko, 1999). Conventionally, salted eggs are made from duck eggs because they attain more desirable characteristics than do hen eggs (Li & Hsieh, 2004). The customer anticipates greater value in egg yolk than in egg white. The desirable characteristics of salted egg yolk include orange colour, oil exudation and gritty texture. During pickling, the yolk gradually becomes solidified and hardened. The egg white loses viscosity and becomes watery (Chi & Tseng, 1998). Chi and Tseng (1998) reported that the pickling appeared to cause moisture removal from egg yolk and the diffusion of salt into egg white and egg yolk. All changes occurring during the salting possibly determine the preferential characteristics of salted egg. However, little information regarding the composition change of egg during the salting process or microstructure of salted egg, particularly egg yolk, has been reported. Therefore, the objectives of this study were to investigate the changes in chemical composition, physical properties and microstructure of duck egg salted at different times.

2. Materials and methods

2.1. Egg samples

Fresh eggs of duck (*Anas platyrhucus*), less than 3 days after laying, having average weights of 65–75 g, were obtained from a local producer in Chaiya, Suratthani Province, Thailand. Duck eggs were salted by coating with the salting paste (mud:salt, 4:1 w/w). The thickness of coating was approximately 2–3 mm. Thereafter, the

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eggs were coated with rice hull ash. The prepared eggs were stored at room temperature for 7 and 14 days.

2.2. Chemicals

Petroleum ether, chloroform, methanol, diethyl ether, formic acid and nitric acid were purchased from Lab-Scan (Bangkok, Thailand). Glutaraldehyde, ethanol, *n*-hexane and silver nitrate were obtained from Merck (Darmstadt, Germany). Heptadecanoic acid, C17, was obtained from Sigma (St. Louis, MO, USA). Osmium tetroxide and potassium thiocyanate were purchased from Fluka (Buchs, Switzerland) and Bio-Rad (Richmond, CA, USA), respectively.

2.3. Proximate analysis and determination of salt content

Whole egg, egg yolk and egg white were analysed for moisture, ash, lipid, and protein contents (AOAC, 2000). Salt content in egg samples was measured by the method of AOAC (2000). Sample (1 g) was treated with 20 ml of 0.1 N $AgNO_3$ and 10 ml of HNO_3. The mixture was boiled gently on a hot plate until all solids except $AgCl_2$ were dissolved (usually 10 min). The mixture was cooled using running water. Five ml of 5% ferric alum indicator ($FeNH_4(SO_4)_2 \cdot 12\ H_2O$) were added. The mixture was titrated with the standardised 0.1 N KSCN until the solution became permanently light brown. The percentage of salt was then calculated as follows:

$$\text{Salt}\ (\%) = 5.8 \times [(V_1 \times N_1) - (V_2 \times N_2)]/W$$

where V_1 is the volume of $AgNO_3$ (ml); N_1 is the concentration of $AgNO_3$ (N); V_2 is the volume of KSCN (ml); N_2 is the concentration of KSCN (N); and W is the weight of sample (g).

2.4. SDS–polyacrylamide gel electrophoresis (SDS–PAGE)

Protein patterns of egg white and yolk were determined according to the method of Laemmli (1970), using 4% stacking gel and 12% separating gel. Egg samples (3 g) were homogenised with 27 ml of 5% SDS using a homogeniser (Polytron, PT 2100, Kinematica AG, Luzern, Switzerland) at a speed of 12,000 rpm for 1 min. The homogenate was heated at 85 °C for 1 h, followed by centrifugation at 7500*g* for 10 min at room temperature using a centrifuge (Sorvall, Model RC-B Plus, Newtown, CT, USA). The protein concentration of supernatant was determined by the Biuret method (Robinson & Hodgen, 1940), using bovine serum albumin (BSA) as standard. The prepared sample (20 μg protein) was loaded onto the gel. Electrophoresis was performed using a vertical gel electrophoresis unit (Mini-protein II; Bio-Rad Laboratories, Richmond, CA, USA) at the constant voltage of 200 V/plate. The gels were stained with Coomassie Brilliant Blue R-125 (0.125%) in 25% methanol and 10% acetic acid. Destaining was performed using 40% methanol and 10% acetic acid.

2.5. Extraction of lipid from egg yolk

Lipid was extracted from egg yolks (whole yolks) using the method of Bligh and Dyer (1959). Samples (25 g) were homogenised with 200 ml of the mixture of chloroform: methanol: distilled water (50:100:50, v/v/v) at 11,000 rpm, using an IKA homogeniser (Model T25, Selangor, Malaysia) for 2 min. The homogenate was treated with 50 ml of chloroform and homogenised for 1 min. Twenty-five millilitres of distilled water was added and the mixture was homogenised for 30 s at the same speed. The mixture was centrifuged at 3000*g* for 10 min and transferred into a separating funnel. The chloroform phase (bottom phase) was drained off into the Erlenmeyer flask. Sodium sulphate (anhydrous) (1–2 g) was added and the mixture was shaken thoroughly to remove the residual water. Lipid in chloroform was decanted into a rounded-bottom flask through a filter paper (Whatman No. 4). The chloroform was evaporated at 25 °C, using a rotary evaporator (Rotavapor, model R-14, Buchi, Japan) and the residual solvent was removed by flushing nitrogen. The lipid was kept in an amber vial under nitrogen at −20 °C prior to further analysis.

2.6. Determination of lipid composition and fatty acid profile of egg yolk

Egg yolk lipid composition was determined using a thin-layer chromatography/flame ionisation detection analyser (TLC-FID). Scanned quartz rods (Silica gel powder-coated chromarod S III) were dipped into 3% boric acid solution for 5 min, dried and rescanned with the TLC-FID analyser. The sample solution (1 μl) was spotted onto the rod and the separation was performed in the mixtures of benzene: chloroform: acetic acid (70:20:0.7, v/v/v) for approximately 30 min. Then the rods were dried in an oven (105 °C) for 10 min before analysing with the flame ionisation detector. The analytical condition was H_2 flow rate of 160 ml/min, air flow rate of 2000 ml/min and scanning speed of 30 s/scan. Retention time of lipid composition standards was used to identify chromatographic peaks. Peak area was quantitated and expressed as per cent of total lipid.

The fatty acid profile of egg yolk lipid was determined as fatty acid methyl esters (FAME), using a gas chromatograph HP5890 series II equipped with flame ionisation detector (FID). Silica capillary column carbowax-20 M (30 m, 0.25 mm ID) was used for separation. Helium was used as the carrier gas at a flow rate of 30 cm/s. The initial temperature of the column was set at 185 °C for 2 min, then increased at a rate of 5 °C min^{-1} to a temperature of 230 °C, and maintained at 230 °C for 24 min. The detector temperature at the injection port was maintained at 260 °C. Heptadecanoic acid (C17) was added to all samples as an internal standard before preparation of FAME (Speake, Surai, & Brotolotti, 2002). Retention times of FAME standards were used to identify chromatographic peaks. Fatty acid content was expressed as g/100 g of lipid.

2.7. Determination of oil exudation of egg yolk

Oil exudation of egg yolk was measured according to the method of Lai et al. (1999) with a slight modification. Yolk (3 g) was homogenised with 35 ml of *n*-hexane/2-propanol (3:2 v/v) at 5000 rpm for 10 min, using a homogeniser (IKA, Labortechnik, Selangor, Malaysia). The filtrate obtained through Whatman No. 1 filter paper was evaporated at 55 °C in a water bath and then dried at 105 °C to constant weight. The residue was weighed and taken as total lipid content. To determine the oil exudation, yolk (5 g) was mixed with 25 ml of distilled water and homogenate at 5000 rpm for 30 s. The homogenate was centrifuged at 9500*g* for 30 min at 25 °C and 25 ml of *n*-hexane/2-propanol (3:2 v/v) were added to the supernatant to dissolve the float. The solvent-lipid layer obtained was separated using a separating funnel. The solvent in the solvent-lipid layer was evaporated in a water bath and heated at 105 °C until a constant weight was obtained. The residue was weighed and taken as free lipid. Oil exudation was defined as the proportion of free to total lipid content.

$$\text{Oil exudation}\ (\%) = \frac{\text{Free lipid content}}{\text{Total lipid content}} \times 100 \quad (1)$$

2.8. Texture profile analysis (TPA) of egg yolk

TPA was performed as described by Bourne (1978) with a TA-XT2i texture analyser (Stable Micro Systems, Surrey, England).

Prior to analysis, salted egg yolks were rolled on a filter paper (Whatman No. 1) to remove egg white. The samples were compressed twice to 50% of their original height with a compression cylindrical aluminium probe (50 mm diameter). Textural analyses were performed at room temperature. Force–distance deformation curves was recorded at cross head speed of 5 mm/s and the recording speed was 5 mm/s. Hardness (g), adhesiveness (g s), springiness (mm), cohesiveness, gumminess (N) and chewiness (g s) were evaluated. These parameters were obtained using the Micro-Stable software (Surrey, England).

2.9. Hardening ratio of egg yolk

Hardening ratio of the salted yolk was determined, following the method of Chi and Tseng (1998). The egg yolk was rolled on a filter paper (Whatman No. 1) to remove egg white. The weight of egg yolk was measured (W_o). The egg yolk was cut with a knife and the removable interior yolk (soft or liquid) was scraped out using a teaspoon. The weight of exterior (hardened) yolk (W_{ex}) was measured. The hardening ratio of the egg yolk was calculated as follows:

$$\text{Hardening ratio} = (W_{ex}/W_o) \times 100$$

2.10. Determination of microstructures of egg shell and shell membrane

Microstructure of shell was observed by scanning electron microscope (SEM). Membrane was manually removed after cleaning. Shell membrane was dried with a series of ethanol solutions (50–100%) and sputter-coated with gold prior to analysis (Yi, Guo, Zhang, Yu, & Li, 2004).

2.11. Determination of microstructure of egg yolk using scanning electron microscopy

Microstructures of egg yolks were analysed using a scanning electron microscope (JEOL JSM-5800LV, Tokyo, Japan). Egg yolks were frozen, cut into a piece of 0.5 × 0.5 cm and fixed at room temperature in 2.5% glutaraldehyde in 0.2 M phosphate buffer (pH 7.2) for 2 h. Fixed samples were rinsed with 0.2 M phosphate buffer (pH 7.2) for 15 min, followed by fixing in 0.1% osmium solution for 2 h at room temperature. The samples were dehydrated in graded series of ethanol solutions (50%, 70%, 80%, 90% and 100%) and were then mounted on SEM stubs using a double-backed cellophane tape. The stub and samples were coated with gold and examined using a scanning electron microscope (JEOL JSM-5800LV, Tokyo, Japan).

2.12. Determination of microstructure of egg yolk using transmission electron microscopy

Microstructures of yolks were analysed using a transmission electron microscope (JEOL JEM 2010, Tokyo, Japan) at 160 kV. Egg yolk samples were fixed at room temperature in 2.5% glutaraldehyde in 0.2 M phosphate buffer (pH 7.2) for 2 h and were rinsed using 0.1 M sodium parahydroxybenzoate buffer (pH 7.4) for 1 h. The sample was then fixed in 1% osmium solution and 2% uranyl acetate for 1 h. The sample was dehydrated in graded series of ethanol solutions (70%, 80%, 90% and 100%). Ethanol was removed with two successive baths in propylene oxide. The sample was embedded in Epone resin and polymerised for 24 h at 70 °C. Thin sections were cut with a diamond knife in a LKB Ultramicrotome. The sections are 80 nm thick, and were deposited on copper grids, stained with 1% uranyl acetate, and photographed.

2.13. Determination of microstructure of egg yolk using confocal laser scanning microscopy

The microstructures of egg yolks were examined with a confocal laser scanning microscope (CLSM) (Olympus, FV300, Tokyo, Japan). Egg yolk samples were dissolved in Nile blue A solution (1:10) and manually stirred until uniformity was achieved. Fifty microlitres of sample solutions was smeared on the microscopy slide. The CLSM was operated in the fluorescence mode at the excitation wavelength of 533 nm and the emission wavelength of 630 nm using a Helium Neon Red laser (HeNe-R) for lipid analysis and at the excitation wavelength of 488 nm and the emission wavelength of 540 nm using a Helium Neon Green laser (HeNe-G) for protein analysis.

2.14. Statistical analysis

Completely randomized design was used throughout the study. The experiments were run in triplicate. Data were presented as mean values with standard deviations. One-way analysis of variance (ANOVA) was carried out and mean comparisons were run by Duncan's multiple range tests (Steel & Torrie, 1980). Statistical analyses were performed with the statistical programme (SPSS for windows, SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Weight proportions and chemical composition of fresh duck egg and salted duck egg

Changes in weight proportions of different components of duck eggs during salting for up to 14 days are shown in Table 1. Fresh duck egg consisted of 10.87% shell, 54.73% egg white, and 33.94% yolk. Generally, the proportions of egg components are dependent on the strain and age of ducks (Powrie & Nakai, 1985; Sugino, Nitoda, & Juneja, 1996). Fresh duck egg had a higher yolk proportion, than had hen egg, which had 28–29% yolk (Sugino et al., 1996). During the salting, an increase in weight of egg was observed ($p < 0.05$). This might be due to the penetration of salt into the egg. The proportion of egg white increased with increasing salting time, whereas a decrease in egg yolk proportion was observed during salting. The proximate compositions of whole egg, egg white and egg yolk during salting are shown in Table 2. Fresh duck egg contained water as the major constituent. It had protein and lipid with contents of 11.8% and 13.5%, respectively. Minerals, expressed as the ash, were also found in whole egg. Protein is the major constituent of egg white solids. The amount of lipid in egg white was negligible. Egg yolk was rich in protein and had a high content of lipids. Egg yolk had a higher content of ash than had egg white. As the salting proceeded, moisture content of egg white decreased gradually, most likely due to the loss of water from egg white to the outside caused by the osmosis process. A slight decrease in protein content was observed during salting for up to 14 days. It was

Table 1
Weight proportion of different components of fresh shell eggs and shell salted for different times

Samples	Fresh egg	Salted egg	
		Day 7	Day 14
Whole shell egg (g)	65.73 ± 2.37[a,A,B]	68.22 ± 3.04[b]	68.89 ± 1.44[b]
Egg white (%)	54.73 ± 1.68[a]	59.88 ± 1.81[b]	62.20 ± 1.74[c]
Egg yolk (%)	33.94 ± 1.65[c]	28.85 ± 2.13[b]	25.88 ± 1.59[a]
Shell and shell membrane (%)	10.87 ± 0.72[a]	11.10 ± 0.69[a]	10.87 ± 0.63[a]

[A] Means ± SD from 20 determinations.
[B] Different superscripts in the same row indicate significant differences ($p < 0.05$).

Table 2
Proximate composition and salt content of fresh egg and egg salted for different times

Samples	Composition (% wet wt. basis)[D]				
	Moisture	Protein	Lipid	Ash	Salt
Fresh egg					
Whole egg	71.77 ± 0.78[D]	11.8 ± 1.15	13.52 ± 0.14	1.17 ± 0.02	0.33 ± 0.00
Egg white	87.72 ± 0.62[z,E]	10.5 ± 0.14[z]	0.03 ± 0.012[x]	0.74 ± 0.01[x]	0.39 ± 0.07[x]
Egg yolk	43.51 ± 0.52[c,C]	16.0 ± 0.48[a,A]	37.25 ± 0.16[a,A]	1.59 ± 0.11[a,A]	0.45 ± 0.04[a,A]
Salted egg (Day 7)					
Egg white	85.19 ± 0.59[y]	10.1 ± 0.05[y]	0.03 ± 0.02[x]	3.02 ± 0.10[y]	3.96 ± 0.10[y]
Interior egg yolk	39.85 ± 0.28[b]	16.7 ± 0.35[ab]	38.39 ± 1.19[a]	1.91 ± 0.11[b]	0.54 ± 0.20[a]
Exterior egg yolk	26.57 ± 1.51[B]	19.5 ± 0.90[B]	47.59 ± 2.74[B]	2.28 ± 0.01[B]	0.67 ± 0.07B
Salted egg (Day 14)					
Egg white	83.59 ± 0.68[x]	9.55 ± 0.12[x]	0.05 ± 0.014[x]	4.04 ± 0.18[z]	6.90 ± 0.20[z]
Interior egg yolk	36.21 ± 2.44[a]	17.6 ± 0.56[b]	44.32 ± 1.40[b]	2.20 ± 0.01[c]	0.84 ± 0.15[b]
Exterior egg yolk	20.05 ± 0.29[A]	21.3 ± 0.65[C]	53.71 ± 0.39[C]	2.45 ± 0.09[B]	0.87 ± 0.10[C]

a, b and c in the same column indicate significant differences between interior salted yolks obtained from different salting times and from fresh yolk ($p < 0.05$).
A, B and C in the same column indicate significant differences between exterior salted yolks obtained from different salting times and from fresh yolk ($p < 0.05$).
[D] Means ± SD from triplicate determinations.
[E] x, y and z in the same column indicate significant differences between the egg whites obtained from different salting times ($p < 0.05$).

noted that a marked increase in salt content occurred in egg white as the salting time increased. This was in agreement with the increase in ash content. After 7 days of salting, the yolk became harder, especially at the surface, named "exterior egg yolk", while liquid yolk was found inside, termed "interior egg yolk". The reduction of moisture of egg yolk during salting was mostly associated with the hardening. High salt content in egg white might induce water migration from egg yolk. During salting, water could migrate from egg yolk to egg white, then to the environment through the egg shell, as governed by pore sizes and structure of the shell (Chi & Tseng, 1998). Protein and lipid contents slightly increased in both interior and exterior egg yolks as salting time increased, mainly owing to the dehydration of egg yolk. The dehydration resulted in the formation of exterior layer and egg yolk at the centre became more viscous, most likely associated with the lower moisture content. Ash content in salted egg yolk also increased during the extended salting, indicating the migration of salts, mainly NaCl, into the yolk. Potassium and sodium are the major minerals in albumen and the major elements in fresh yolk are calcium, potassium and phosphorus (Powrie & Nakai, 1985). Most of the minerals are in conjugated form and only a small portion is present as inorganic compounds or ions (Sugino et al., 1996). Additionally, salted eggs were produced by coating with the soil, which was the important source of minerals. NaCl content in the interior and exterior egg yolk increased after salting to a lower extent, compared with that found in egg white. The results confirmed the previous studies by Chi and Tseng (1998) and Lai et al. (1999). After yolks become solidified, the migration of NaCl could be lowered. Furthermore, high lipid content in yolk might impede the migration of NaCl into the yolk. Salt content in salted egg can be varied with salting processes. Salt content affects the acceptance of consumers. Generally, eggs salted for 7 days are recommended for pan-frying owing to the less salty taste. Eggs salted for 14 days or more are ready for boiling and commonly consumed, especially with rice gruel, for breakfast.

3.2. Oil exudation of egg yolk during salting

Oil exudation of egg yolk during salting is depicted in Fig. 1. Oil exudation increased with increasing salting time ($p < 0.05$). A greater oil exudation was observed in exterior egg yolk, compared with the interior counterpart ($p < 0.05$). Oil exudation is generally one of desirable characteristics of salted egg. Schultz, Snyder, and Forsythe (1968) pointed out that removal of water from egg yolk increased the extracted lipid. Thus, the dehydration during salting

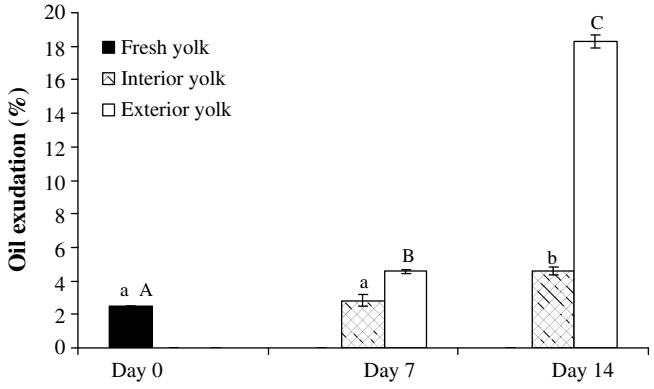

Fig. 1. Oil exudation of fresh duck egg yolk and egg yolk salted for different times. Different letters on the bar indicate significant differences ($p < 0.05$).

probably enhanced oil exudation. Free lipid might be released from low-density lipoprotein micelles, due to the structural changes of low-density lipoprotein induced by dehydration and increased salt content. The dehydration of proteins might be caused by yolk protein denaturation, associated with a loss of their emulsifying properties upon salting. Weak gel of yolk protein developed upon dehydration was attributed to hydrophobic interaction and hydrogen-bond (Paraskevopoulou, Kiosseoglou, Alevisopoulos, & Kasapis, 2000).

3.3. Changes in lipid composition and fatty acid profile of egg yolk during salting

Lipid from fresh and salted egg yolks contained triacylglycerols as the dominant constituent, followed by phospholipid. Diacylglyc-

Table 3
Lipid composition of yolk from fresh egg and egg salted for different times

Lipid composition (%(w/w) of total lipids)	Fresh egg	Salted egg	
		Day 7	Day 14
Triacylglycerols (%)	59.1 ± 1.75[b,A,B]	58.1 ± 2.62[ab]	56.1 ± 2.23[a]
1,3-Diacylglycerols (%)	0.95 ± 0.17[a]	0.83 ± 0.11[a]	0.95 ± 0.16[a]
Phospholipids (%)	39.9 ± 1.79[a]	41.0 ± 2.69[ab]	43.0 ± 2.41[b]

[A] Means ± SD from five determinations.
[B] Different superscripts in the same row indicate significant differences ($p < 0.05$).

erol was found at low concentration (0.83–0.95 g/100 g lipid). Free fatty acid was not found in egg yolks, indicating that salting had no impact on lipid lipolysis. Fatty acid compositions of lipids from fresh and salted duck eggs are shown in Table 3. The major fatty acids in fresh duck egg were oleic acid (C18:1*n*−9) and palmitic acid (16:0) which constituted 47.5% and 27.2% (w/w) of total fatty acids, respectively (Table 4). Linoleic acid (18:2*n*−6) was found at 8.08%. Arachidonic acid (C20:4*n*−6) and docosahexaenoic acid (C22:6*n*−3) were also found in egg yolk lipid. Fatty acid profiles of yolk lipid from different species of duck were similar (Speake et al., 2002). Fatty acid content of yolk lipid was influenced by

Table 4
Fatty acid profile of yolk from fresh egg and egg salted for different times

Fatty acid (% of total fatty acid)	Fresh egg	Salted egg	
		Day 7	Day 14
C14:0	0.50	0.43	0.50
C16:0	27.2	26.8	27.5
C16:1*n*−7	2.25	2.24	2.66
C18:0	6.19	5.50	5.63
C18:1*n*−9	47.5	48.0	48.4
C18:2*n*−6	8.08	8.73	6.88
C18:3*n*−3	0.33	0.31	0.30
C20:2*n*−6	0.17	0.27	0.34
C20:4*n*−6 (ARA)	2.62	2.57	2.79
C22:4*n*−6	0.18	0.15	0.18
C22:5*n*−3	0.37	0.41	0.23
C22:5*n*−6	0.35	0.22	0.31
C22:6*n*−3 (DHA)	1.66	1.95	1.61
Saturated fatty acid	33.9	32.7	33.6
Unsaturated fatty acid	63.6	64.9	63.7

Table 5
Hardening ratio and texture profile analysis (TPA) of yolk from egg salted for different times

Hardening ratio/TPA terms	Day 7	Day 14
Hardening ratio (%)	66.61 ± 3.82[a]	88.22 ± 4.50
Hardness (N)	3.45 ± 0.79	9.25 ± 3.28
Fracturability (N)	0.11 ± 0.06	3.76 ± 3.26
Adhesiveness (N s)	1.01 ± 0.71	1.68 ± 0.78
Springiness (mm)	0.58 ± 0.26	0.31 ± 0.09
Cohesiveness	0.43 ± 0.12	0.25 ± 0.05
Gumminess (N)	1.56 ± 0.76	2.27 ± 0.90
Chewiness (Nm)	1.05 ± 0.96	0.71 ± 0.31

[a] Means ± SD from 10 determinations for hardening ratio and from twenty determinations for TPA analysis.

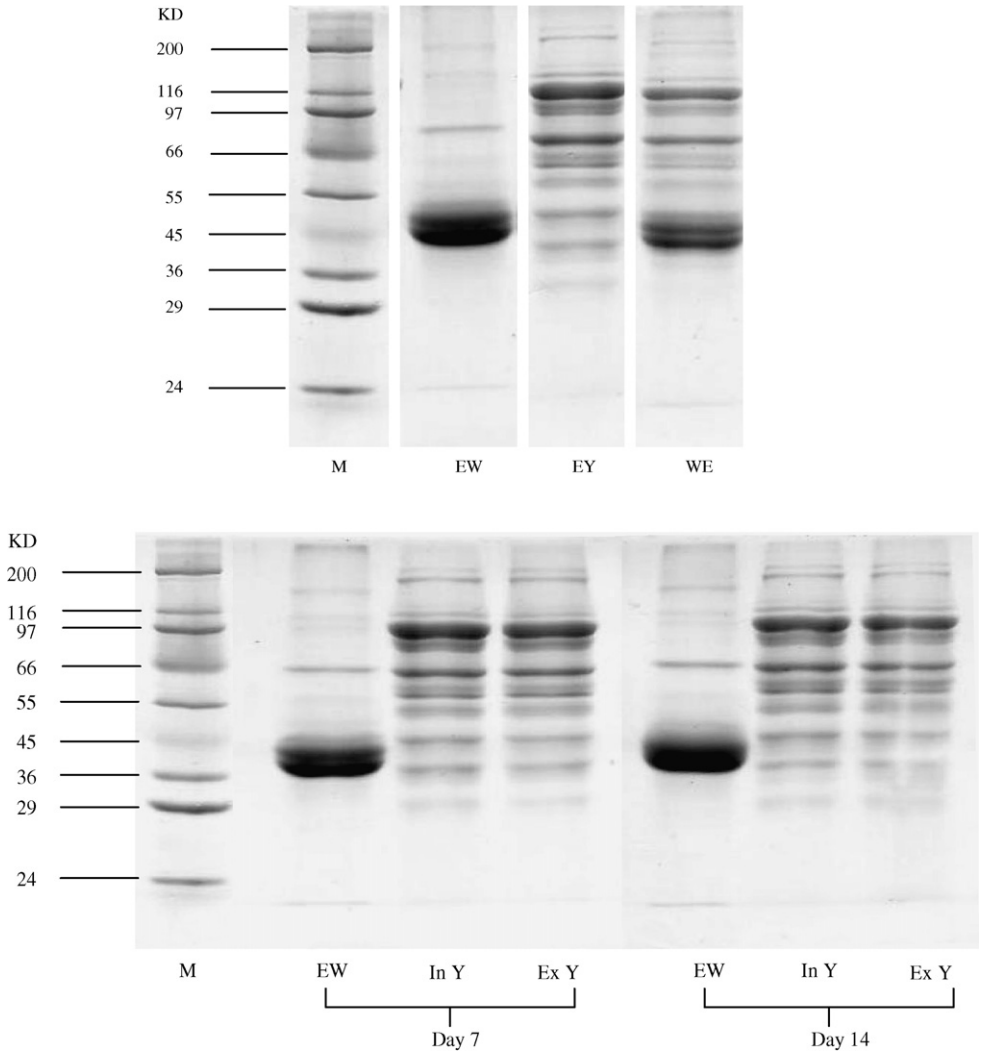

Fig. 2. SDS–PAGE patterns of different portions of fresh duck egg and duck egg salted for different times. M: molecular weight standard; EW: egg white; EY: egg yolk; WE: whole egg; InY: interior yolk and ExY: exterior yolk.

the types of fatty acid in the feed (Powrie & Nakai, 1985). Duck egg yolk lipid has unique fatty acids, including arachidonic acid and docosahexaenoic acid, which are not found in soy and other plants (Juneja & Kim, 1996). Arachidonic acid and docosahexaenoic acid are attached to phospholipids in egg yolk lipid (Juneja & Kim, 1996). ω-3 Fatty acids are now regarded as essential in the diet for brain function and visual acuity in humans (Juneja & Kim, 1996). Fatty acid profile of salted egg lipid was similar to that of fresh yolk lipid. No marked changes in fatty acid profile were observed between eggs salted for 7 and 14 days. However, stearic acid (C18:0) decreased slightly after 7 and 14 days of salting. Linoleic acid content slightly decreased after salting for 14 days. Thus, salting generally had no impact on fatty acid composition of yolk lipids.

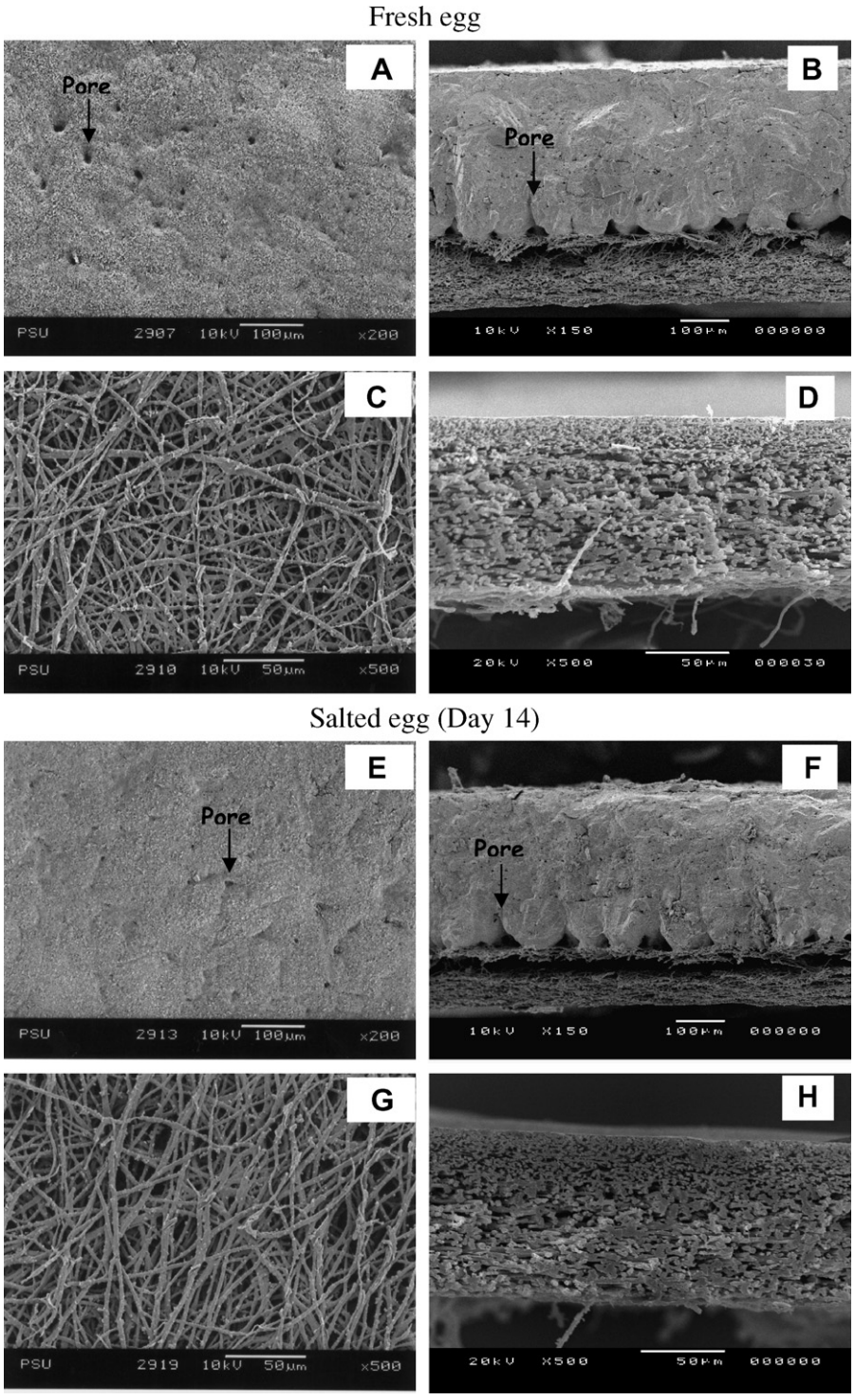

Fig. 3. Scanning electron micrograph of shell and shell membrane of fresh duck egg and salted duck egg after 14 days of salting. A, E: top surface of shell; B, F: cross section of shell; C, G: top surface of shell membrane and D, H: cross section of shell membrane.

3.4. SDS–polyacrylamide gel electrophoresis (SDS–PAGE)

Whole egg contained four major protein bands with apparent MWs of 117, 105, 85 and 47 kDa. For egg white, ovalbumin was found as the major protein with MW of 47 kDa. Ovalbumin is the most abundant protein in egg white (54%) (Li-Chan, Powrie, & Nakai, 1995; Linden & Lorient, 2000). Another protein band was found at MW of 76 kDa, most likely conalbumin (Linden & Lorient, 2000; Ternes, 2001). Electrophoretic study of yolk proteins (Fig. 2) revealed several protein bands with a MW range of 30–220 kDa. Among all proteins, that with MW of 100 kDa was dominant and most likely classified as LDL apoprotein, whose theoretical molecular weight value is estimated to be 100–180 kDa (Ternes, 2001). Proteins with MW of 46 and 56 kDa likely corresponded to HDL apoprotein (Raikos, Hansen, Campbell, & Euston, 2006). Egg yolk is a complex system of protein and lipid. All lipids of egg yolk are associated with proteins to form lipoproteins, commonly classified as low-density lipoproteins (LDL) and high-density lipoproteins (HDL) (Anton et al., 2003). For other protein bands, they might represent LDL protein of the plasma fraction of yolk. No change in protein patterns in egg white was observed between fresh egg and egg salted for 7 and 14 days. Additionally, similar protein patterns were found between yolk of fresh egg and salted egg, regardless of salting time and egg yolk portions, interior or exterior.

3.5. Hardening ratio and texture profile of egg yolk during salting

Hardening ratio of yolk was defined as the weight percent of hard exterior yolk and used as an index for the completeness of salting. Hardening ratio of egg salted for 7 days was lower than that of egg salted for 14 days (Table 5). During salting, the solidification of egg yolk was initiated near the vitelline membrane and proceeded toward the centre, the exterior formation. The interior yolk was still in liquid form, but became more viscous with further dehydration of exterior salted yolk. When yolk protein became more concentrated, interaction between protein molecules, including lipoproteins, could occur. This resulted in the formation of a gel-like network. The presence of native yolk lipids or emulsified oil droplet appears to influence the yolk gel rheological properties (Kiosseoglou, 2003). Yolk protein concentrates, having relatively high lipid content, produce gel networks at low protein concentrations, suggesting that the yolk lipid molecules are somehow involved in gel structure formation (Kiosseoglou, 2003).

Texture profiles of egg yolk salted for 7 and 14 days are shown in Table 5. The hardness of salted egg yolk increased with increasing salting time, in accordance with the increases in hardening ratio. This result suggested that the structure of salted egg yolk became more solidified. Fracturability of salted egg markedly increased from 0.11 to 3.76 N after salting for 7 and 14 days, respectively. Salted egg yolk was more dehydrated and could form a gritty texture. Grittiness is the major factor affecting consumer acceptance of salted egg product (Chi & Tseng, 1998). Adhesiveness and gumminess increased, while springiness, cohesiveness and gumminess slightly decreased when the salting time increased.

3.6. Microstructures of shell and shell membrane of fresh and salted egg

Microstructures of shell and shell membrane of fresh and salted eggs are depicted in Fig. 3. Egg shells are composed of a calcium carbonate layer, and two shell membranes (Okubo, Akachi, & Hatta, 1996). Egg shells contain funnel-shaped small holes called pore canals on the surface of the shell for gas exchange. The diameter of the pore canal ranges from 10 to 30 μm. An egg has about 7000–17,000 pore canals on the shell surface per egg (Okubo et al., 1996; Powrie & Nakai, 1985), allowing salt and water to pass through during the salting process. No differences in shell structures were observed between fresh egg (Fig. 3A and B) and salted egg (Fig. 3E and F). The egg shell membrane consisted of inner and outer membranes. The structure was entangled thread or randomly knitted net in shape. The structure of shell membrane of fresh egg (Fig. 3C and D) was similar to that of salted egg (Fig. 3G and H).

3.7. Microstructures of egg yolk of fresh and salted egg

Microstructures of yolk from fresh and salted egg obtained after 14 days of salting, visualised by SEM, are shown in Fig. 4A and B, respectively. Egg yolk and salted egg yolk had polyhedral granules with a size range of 50–100 μm. Yang and Hsu (1989) reported polyhedral granules with diameters ranging from 23 to 127 μm in salted duck egg yolk. Chi and Tseng (1998) found granules with a size range of 90–100 μm in salted egg yolk. The microstructure of salted egg yolk by SEM indicated that polyhedral granules were more closely localised than those in fresh egg yolk (Fig. 4B), mainly due to dehydration during salting. The presence of such granules probably provided the gritty texture sensation. Therefore, the stacking of granules was necessary to produce salted egg yolk with a gritty texture. The polyhedral granules were formed by yolk spheres (Mineki & Kobayashi, 1997). During salting, the dehydration was more pronounced

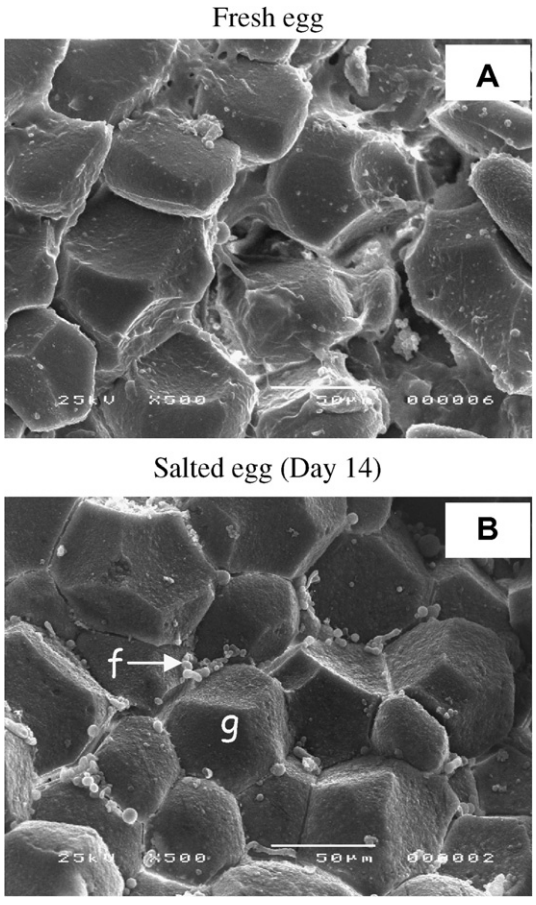

Fig. 4. Scanning electron micrograph of yolk granule from fresh duck egg (A) and after 14 days of salting (B). Magnification: 500×. f: Free fat released from the granule; g: granule.

and the granules were located closer, leading to the denser structure.

Transmission electron micrographs of fresh and salted egg yolk are shown in Fig. 5. Larger protein spheres were observed in fresh egg (Fig. 5FA), than in salted egg (Fig. 5SA). Protein granules or yolk spheres with electron density and the diameter of 0.6–4 μm (Fig. 5FB) and 0.2–2 μm (Fig. 5SB) were found in fresh egg yolk and salted egg yolk, respectively. For salted yolk, oil spheres were observed at the interface of the yolk spheres (Fig. 5SC), suggesting that oil droplets were released from the lipid–protein structure. However, no oil droplets were found at the interface of fresh yolk (Fig. 5FC). Egg yolk contains 22% of granule fraction and 72% of plasma (Sugino et al., 1996). Granules are composed of 17% phosvitin, 70% high-density lipoprotein (HDL) and 12% low-density lipoprotein, whereas plasma consists of 85% LDL and 15% livitin (McBee & Cotterill, 1979; Powrie & Nakai 1985; Sugino et al., 1996). A low-density lipoprotein (LDL) fraction (lipovitellenin) contains 90% lipids. After salting, the structure of lipoprotein was destroyed and some parts of the lipid in egg yolk became free (Lai et al., 1999).

The confocal laser scanning microscope (CLSM) micrographs of fresh egg yolk and salted egg yolk are illustrated in Fig. 6. Lipid and protein are distributed uniformly in fresh egg yolk, as shown in Fig. 6FA and FB, respectively. On the other hand, shapes of lipid and protein turned out to be irregular with a discontinuous distribution in dye solution. CLSM micrographs indicated that egg yolk is actually a source of lipids and proteins, which are easily dispersed in water, thus permitting emulsification of other substances. Most of the proteins in yolk are organised into micellar and granular structures, together with polar and non-polar lipid molecules (Kiosseoglou, 2003). All constituents of yolk (LDL, HDL, phosvitin and livitin) have a strong capacity to adsorb at the oil–water interface (Anton et al., 2003). Among yolk components, proteins (including apoprotein of lipoproteins) are the main molecules that take part in adsorption at the water interface. Furthermore, they also control the colloidal interaction between covered oil droplets. As salting proceeds, the greater dehydration, together with the release of lipids in egg yolk, might reduce the emulsion capacity of the protein portion. Irregular shapes of both lipid and protein were found in both interior (Fig. 6InA and InB) and exterior (Fig. 6ExA and ExB) yolk after salting for 14 days. Increased viscosity in interior salted egg yolk and hardening of exterior salted egg yolk also made the yolk more difficult to disperse in the dye solution.

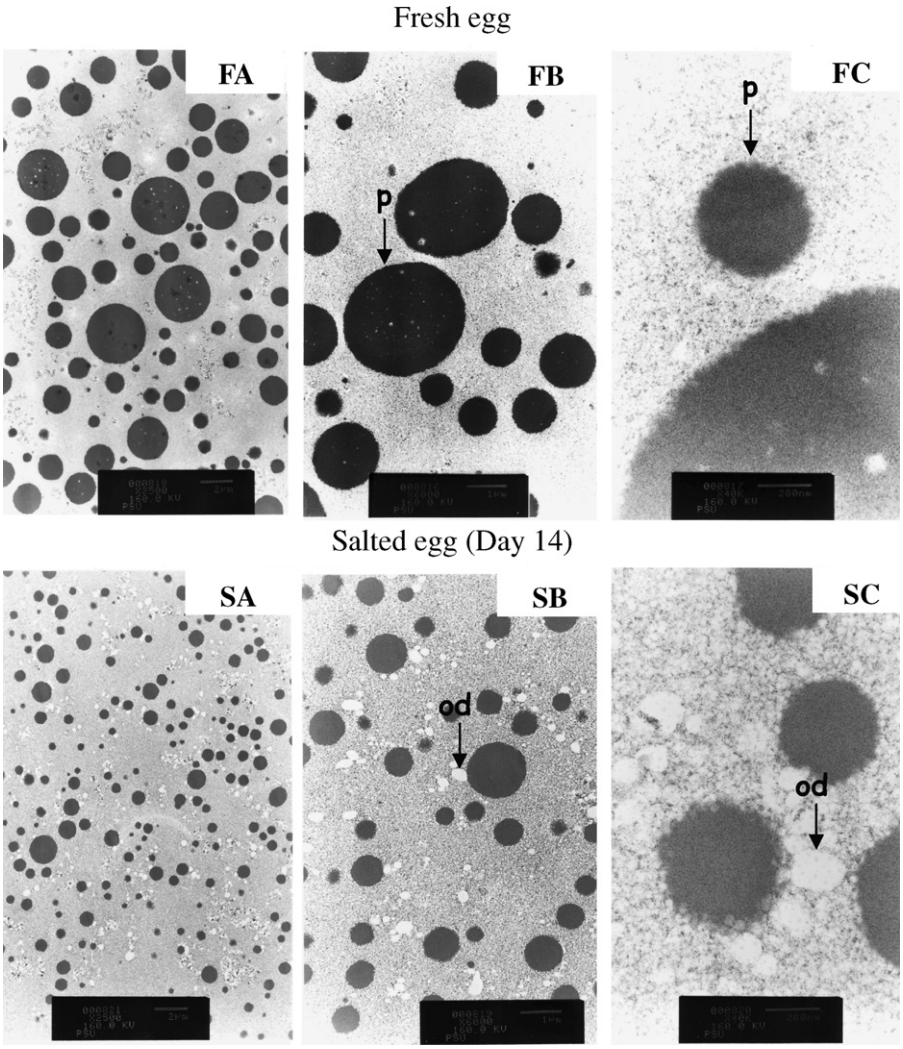

Fig. 5. Transmission electron micrograph of internal structure of yolk sphere from fresh egg yolk (F) and after 14 days of salting (S). Magnification; 2500× (A); 6000× (B); 40,000× (C). od: Oil droplet; p: protein granule.

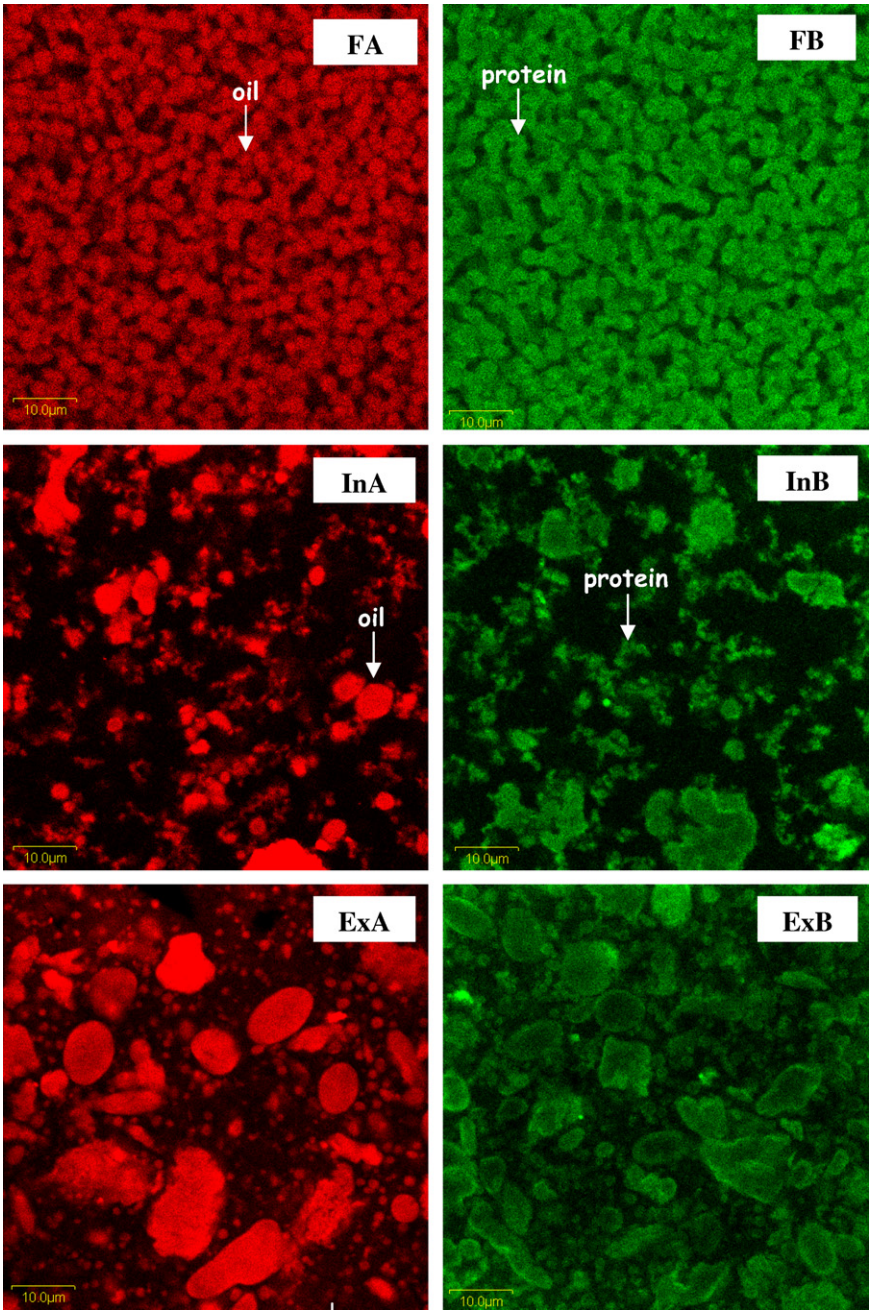

Fig. 6. Confocal laser scanning micrograph of fresh egg yolk (F), interior yolk (In) and exterior yolk (Ex) of egg after 14 days of salting. Magnification: 600× (zoom X3.5); oil distribution (A) and protein distribution (B). Scale bar = 10×μm.

4. Conclusion

Salted duck eggs were rich in protein and fat. Only slight changes in lipid composition and fatty acid profile were found in egg during salting, compared with fresh egg. Salting induced solidification of yolk, accompanied with oil exudation and the development of a gritty texture. Additionally, moisture removal and the association of egg yolk granules most likely contributed to the development of solidified yolk.

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