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Proteomics Analysis of Egg White Proteins from Different Egg Varieties

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ABSTRACT: The market of specialty eggs, such as omega-3-enriched eggs, organic eggs, and free-range eggs, is continuously growing. The nutritional composition of egg yolk can be manipulated by feed diet; however, it is not known if there is any difference in the composition of egg white proteins among different egg varieties. The purpose of the study was to compare the egg white proteins among six different egg varieties using proteomics analysis. Egg white proteins were analyzed using two-dimensional gel electrophoresis (2-DE), and 89 protein spots were subjected to LC-MS/MS. A total of 23 proteins, belonging to *Gallus gallus*, were identified from 72 detected protein spots. A quiescence-specific protein precursor in egg white was identified for the first time in this study. Significant differences in the abundant levels of 19 proteins (from 65 protein spots) were observed among six egg varieties. Four proteins, ovalbumin-related protein Y, cystatin, avidin, and albumin precursor, were not different among these six egg varieties. These findings suggest that the abundance, but not the composition, of egg white proteins varied among the egg varieties.

KEYWORDS: *specialty eggs, egg whites, 2-DE, proteome*

INTRODUCTION

Eggs contain every nutrient essential to sustaining a new life. The protein in egg white is of such high quality that it is the standard against which other proteins are judged.[1] Egg yolk contains a significant amount of vitamins and minerals, in addition to proteins and lipids.[2] To meet consumers' demands, eggs with additional attributes, such as organic, free-range, and nutrient-enriched specialty eggs, have been successfully marketed.[3−8] The volume of these specialty eggs has doubled over the past decades in the United States.[9]

It is known that the nutrient composition of egg yolk can be manipulated by the hen's feed; however, it is not known if there is any difference in the composition of egg white proteins among various egg varieties. Egg whites are widely used in the food industry as an important protein ingredient due to their excellent nutritive value and functional properties.[10] Egg white proteins or their hydrolysates also possess various bioactivities, such as antimicrobial, immunomodulating, anticancer, anti-hypertensive, and antithrombotic activities.[10,11]

There is a great potential for identifying novel egg white proteins. Novel egg white proteins, such as antibacterial ovodefensins, have been recently characterized.[12] Two-dimensional gel electrophoresis (2-DE) combined with mass spectrometry, as one high-resolution techniques for proteome analysis, has been successfully applied in egg white characterization. The first proteome-level investigation of hen egg white was performed by Desert et al.,[13] which led to the characterization of a small acidic protein, Ch21. Subsequently, Raikos et al. investigated the whole egg proteins using 2-DE.[14] Guerin-Dubiard identified a total of 16 proteins including two egg white proteins, Tenp, a protein with strong homology with a bacterial permeability-increasing protein family (BPI), and VMO-1, an outer layer vitelline membrane protein, respectively, for the first time.[15] Recently, Mann[16] detected 78 egg white proteins using 1-DE and LC-MS/MS, and 54 proteins were identified for the first time. D'Ambrosio et al.[17] reported a total of 148 proteins from egg white using 2-DE combined with a protein enrichment technology (peptide ligand libraries). Proteomics analysis of egg white proteins during storage were also reported.[18] The purpose of the study was to compare the egg white proteins among six egg varieties using proteomics analysis.

MATERIALS AND METHODS

Materials. Six egg varieties, conventional white-shell eggs (W), conventional brown-shell eggs (B), lutein-enriched eggs (L), organic eggs (O), omega-3-enriched eggs (ω), and vitamin-enriched eggs (V), were products of Lucerne Food Co. (Calgary, AB, Canada) in May 2009. The conventional white-shell eggs, lutein-enriched eggs, organic eggs, omega-3-enriched eggs, and vitamin-enriched eggs are from lines of Single Comb White Leghorns. The conventional brown-shell eggs are from lines of Rhode Island Reds.

Extraction of Egg White Proteins. Egg white proteins were extracted as described previously with slight modifications.[18] Briefly, three egg whites were manually separated from egg yolk and homogenized, and then 100 μL of egg white was finely homogenized in 1.5 mL of ice-cold acetone containing 10% (v/v) trichloroacetic acid (TCA, Sigma, St. Louis, MO) and 0.07% (v/v) dithiothreitol (DTT, Bio-Rad, Hercules, CA). After incubation at −20 °C for 1 h, the mixture was centrifuged (10000*g*, 15 min, 4 °C), and the resulting pellet was washed three times with 1 mL of ice-cold acetone containing 0.07% (v/v) DTT. The pellet was air-dried for 20 min in a Speedvac (Hetovac VR-1; Heto Laboratory Equipment A/S, Birkerod, Denmark) and then resolubilized in 150 μL of rehydration buffer (Bio-Rad) containing 1.0% (v/v) tributylphosphine (TBP, Bio-Rad) and incubated overnight at 4 °C. The sample was centrifuged (10000*g*, 15 min, 4 °C), and the supernatant was subsequently transferred into a 1.5 mL centrifuge tube and stored at −20 °C until 2-DE analysis. Protein extractions were performed twice as the technical replicates

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from each of two independent biological replicates for each variety of eggs (a total of four independent extracts were subsequently used for electrophoresis on four gels for each egg variety). Protein concentrations were determined using a modified Bradford assay with protein assay dye reagent (Bio-Rad) and bovine serum albumin (BSA, Sigma) as the standard.[19]

2-DE Analysis. Isoelectric focusing (IEF) in the linear pH gradient (the first dimension) and nonlinear molecular mass gradient separation (the second dimension) by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) were performed as described previously.[18] IPG strips (11 cm, pH 4–7, Bio-Rad) were rehydrated overnight at room temperature with a total of 100 μg of extracted egg white proteins in 125 μL of rehydration sample buffer. IEF was performed on a Protean IEF cell (Bio-Rad) with the following settings: 250 V for 15 min, linear increase to 4000 V over 3 h, focused for 20000 V h, and held at 500 V. Each focused IPG strip was equilibrated in 1 mL of equilibration buffer I (6 M urea, 2% (w/v) SDS, 0.37 M Tris-HCl, pH 8.8, 20% (v/v) glycerol, and 130 mM DTT) for 10 min twice and then incubated with 1 mL of equilibration buffer II (6 M urea, 2% SDS, 0.37 M Tris-HCl, pH 8.8, 20% glycerol, and 135 mM iodoacetamide (IAA, Bio-Rad)) for 10 min twice. The equilibrated strip was placed on top of 13% (w/v) polyacrylamide gel, and the second-dimension electrophoresis was performed in a Protean II xi Cell (Bio-Rad) with the Precision Plus Protein Standard (Bio-Rad). After electrophoresis, the gel was stained with a Colloidal Blue Staining Kit (Invitrogen, Carlsbad, CA) per the manufacturer's instructions.

2-DE images were recorded with a GS-800 calibrated densitometer (Bio-Rad), and a total of 24 gel images (from two technical replicates of two biological replicates in each kind egg white) from six egg varieties were assembled in a matchset using PDQuest software (version 7.3.1, Bio-Rad). Automated spot detection was performed with PDQuest software, and then the matched spots were verified and adjusted manually. The intensity of each matched spot was analyzed, and significantly ($P < 0.05$) altered spots were identified with the Student's *t*-test feature of the software. Moreover, the relative abundance of protein expression between each pair of eggs was compared.

Digestion. All of the matched protein spots were excised from gels and then were digested, in-gel, with trypsin based on the Agilent 1100 LC-MS Getting Started Guide. Briefly, protein spots excised from gels were washed for 5 min with HPLC grade water and dehydrated with acetonitrile (ACN) for 30 min. After removal of the liquid, samples were dried with speed vacuum equipment at room temperature for 10 min. The dry samples were subsequently destained with a mixture of 100% ACN and 0.1 M NH_4HCO_3 (with the volume ratio of 1:1) and then dehydrated again as described above. The proteins from these samples were treated with 0.1 M NH_4HCO_3 (containing 10 mM DTT) at 56 °C for 30 min and washed with 100% ACN. Then, these proteins were alkylated at room temperature with 0.1 M NH_4HCO_3 (containing 55 mM IAA) in the dark for 20 min. The samples were dehydrated again as described above. Digestion was performed by adding trypsin (0.02 μg/μL) with 40 mM NH_4HCO_3 and 10% ACN at 37 °C for 16 h. Digestion was terminated by adding formic acid (0.1%, v/v) and stirring for 40 min. After removal of the liquid, the hydrolysates (peptides) were extracted with 50% ACN and 0.1% formic acid for 30 min twice, and then samples were stored at −20 °C until MS analysis.

LC-MS/MS Analysis. The extracted peptide mixtures were analyzed on an Agilent 1100 Series LC/MSD Trap XCT System (Agilent Technologies, Palo Alto, CA) operated in the unique peptide scan auto-MS/MS mode. Twenty microliters of peptide mixture was loaded onto a concentration column (Zorbax 300SB-C18, 5 μm, 5 × 0.3 mm) by an autosampler, followed by a separation column (Zorbax 300S B-C_{18}, 5 μm, 150 × 0.3 mm). The column was eluted at a flow rate of 4 μL/min with 3% buffer B for 5 min, gradient increased to 15% buffer B over 3 min, to 45% buffer B over 42 min, and to 90% buffer B over 10 min, then decreased to 3% buffer B over 1 min, and maintained at 3% buffer B for 14 min for the next injection, in which buffer A was 0.1% FA in H_2O and buffer B was 0.1% FA in ACN (v/v). The peptide ion fragmentation was performed with an MS 300–2000 m/z scan, and the most intense ions were analyzed. All MS/MS data were shown with the ChemStation Data Analysis module in a Mascot Generic File (*.mgf) and subsequently analyzed using MS/MS ion search module of Mascot software. The NCBI nonredundant (NCBInr) database and all taxonomic categories were considered during the search: one missing cleavage allowance by trypsin, fixed modification of carbamidomethyl, ±2.0 Da of peptide tolerance, ±0.8 Da of MS/MS tolerance, 1+, 2+, and 3+ of peptide charges, and monoisotopic ions with no precursor and ESI-TRAP instrument. Individual ion scores obtained by the Mascot search based on peptide mass fingerprints were calculated as $-10 \times \log(P)$, where a random was presented as the probability (P) of the observed match. All ion scores with a threshold value >66 were considered to indicate identity or extensive homology ($P < 0.05$) as defined by the Mascot probability analysis.

RESULT AND DISCUSSION

A total of 89 protein spots were detected in the 2-DE analysis of egg white proteins (Figure 1). All protein spots were subsequently analyzed by LC-MS/MS, and 72 protein spots were successfully identified. A total of 23 proteins were characterized on the basis of the accession number in NCBI; a quiescence-specific protein precursor was identified from hen egg whites for the first time. Significant differences were observed in 19 egg white proteins among 6 egg varieties, whereas 4 proteins did not show any significant difference.

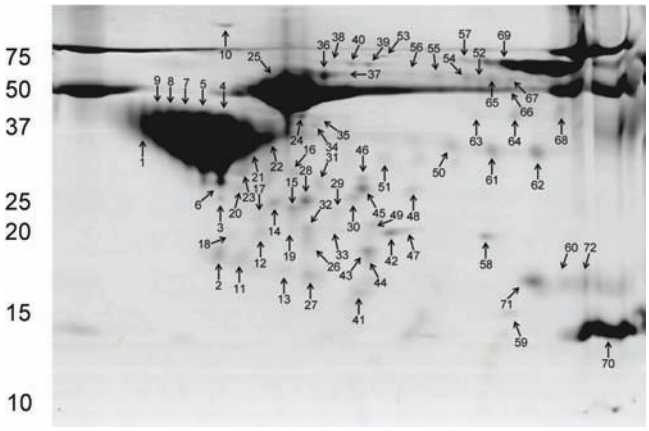

Figure 1. Representative 2-DE gel image of hen egg white proteins (white-shell egg). An amount of 100 μg of total protein was loaded on the strip (11 cm, pH 4–7) and performed on SDS-PAGE following staining with a Colloidal Blue Staining Kit.

There is no difference in nutritional value between white-shell and brown-shell eggs or between conventional eggs and organic eggs, if fed on the same diet. However, eggs enriched with nutrients such as omega-3 and vitamins may provide additional healthy benefits.[4−7] In this study, 25 spots (10 proteins) were significantly ($P < 0.05$) different in relative abundance between brown-shell eggs and white-shell eggs. Four protein spots (4 proteins) were significantly ($P < 0.05$) different between omega-3-enriched eggs and white-shell eggs, 18 protein spots (11 proteins) between vitamin-enriched eggs and white-shell eggs, 21 protein spots (14 proteins) between lutein-enriched eggs and white-shell eggs, and 17 protein spots (5 proteins) between organic eggs and white-shell eggs.

Proteins with Significant Differences among Six Egg Varieities. *Ovalbumin.* Ovalbumin, accounting for 54% (w/w) of egg white proteins, is a glycoprotein with a molecular

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mass of 45 kDa.[20] Guerin-Dubiard et al. identified 11 protein spots (from a total of 69 protein spots identified) as ovalbumin.[15] In the present study, a total of 37 protein spots were identified as ovalbumin (gi: 129293, spots 2, 3, 11−13, 18, 19, 23, 29, 31, 41, 43, 44, 46; gi: 28566340, spots 10, 14−17, 20−22, 24−28, 30, 32, 34−38, 45, 48, 49) as listed in Table 1. The abundance of ovalbumin was significantly different ($P < 0.05$) among six egg varieties. Ovalbumin spots in brown-shell eggs were 35, 65, 65, 73, and 81% higher than those of white-shell, omega-3-enriched, vitamin-enriched, lutein-enriched, and organic eggs, respectively (Figure 2). Ovalbumin spots in white-shell eggs were 5, 19, 19, and 24% higher than those of omega-3-enriched, vitamin-enriched, lutein-enriched, and organic eggs, respectively.

Ovotransferrin. Ovotransferrin (conalbumin), a member of the transferrin family, is responsible for the transfer of ferric ions from hen oviduct to the developing embryo. Ovotransferrin has three glycoforms with three pI values for aferric, monoferric, and diferric transferrin.[21] Guerin-Dubiard et al.[15] found five ovotransferrin protein spots using 2-DE but did not characterize them further. In our study, a total of nine ovotransferrin protein spots were observed, which is consistent with the result of van Eijk et al.[22] Three types of ovotransferrin, ovotransferrin BB type (gi: 71274075, spots 52 and 65−67), ovotransferrin CC type (gi: 71274077, spot 52), and ovotransferrin chain A (gi: 17942831, spot 51), were also identified in the study without using protein enrichment technology.[17] Protein spots (gi: 83754919, spots 63, 64, and 68) were identified as crystal structures of aluminum-bound ovotransferrin at 2.15 Å resolution. A colorless needle-shaped crystal of aluminum-bound ovotransferrin was first identified by Mizutani et al.[23] Our study showed that there is no difference among various egg types with the exception of spot 51 (between L/O), spot 65 (between W/V, W/L, and L/O), and spot 66 (between W/B, B/V, B/L, B/O, and ω /O).

Ovoglycoprotein. In this study, seven protein spots were identified as ovoglycoprotein (gi: 45383093, spots 4, 5, 7−9, 21, and 23). All ovoglycoprotein spots, except for spot 23, showed a much higher apparent molecular weight than the theoretical one. Guerin-Dubiard et al. explained that the higher apparent molecular weight might be caused by the interactions between ovoglycoprotein and ovomucoid or riboflavin-binding protein.[15] Besides ovoglycoprotein, another two proteins were also detected in spot 21, in which one is a riboflavin-binding protein and the other is ovalbumin, but not ovomucoid as mentioned by Guerin-Dubiard et al.[15] (Table 1). However, Li-Chan and Nakai's study suggested that the presence of 30% of sugar content in ovoglycoprotein was probably the reason for the higher apparent molecular weight.[24] Ovoglycoprotein spots from brown-shell eggs always show significantly higher ($P < 0.05$) abundance levels than those of the other four egg varieties (ω, V, L, and O).

Clusterin. Clusterin, a widely expressed secretory glycoprotein in numerous biological fluids,[25] mainly acts as an extracellular chaperone and inhibits stress-induced aggregation and precipitation, such as stabilizing lysozyme, ovotransferrin, and other proteins during embryo development.[26] In this study, three protein spots (gi: 45382467, spots 50, 61, and 62) were identified as clusterin. Significant differences were observed in clusterin protein spots 50 (between L/O), 61 (between W/V, W/L, B/L, and L/O), and 62 (between W/L); lutein-enriched eggs showed significantly lower abundance of clusterin in egg whites. The observed molecular weight (~32 kDa) of all three spots is close to the molecular weight of the fragments of clusterin but lower than the theoretical one (51.9 kDa),[15] which suggests that clusterin protein might be degraded during 2-DE analysis.

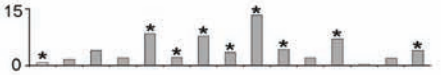
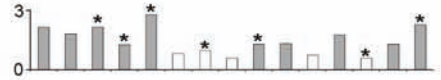
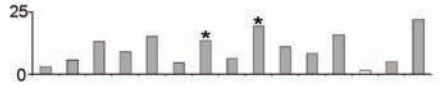
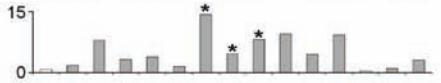
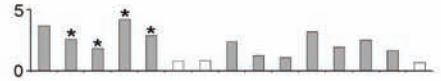
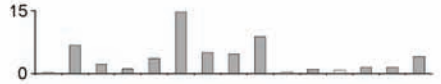
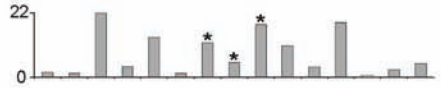
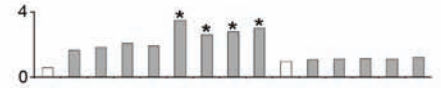
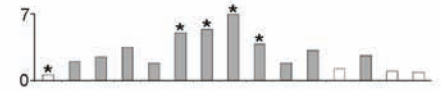
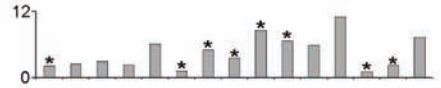
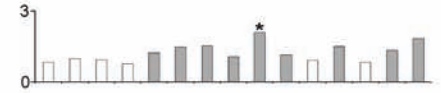
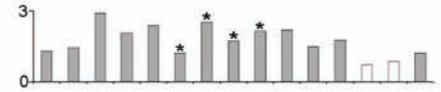
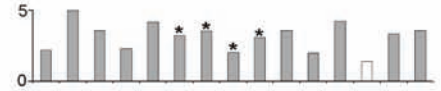
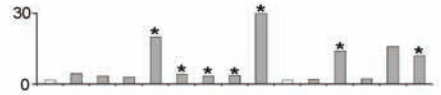
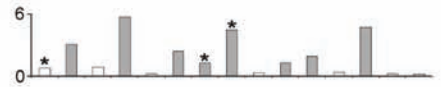
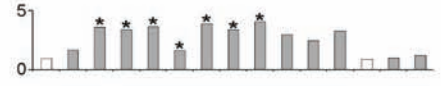
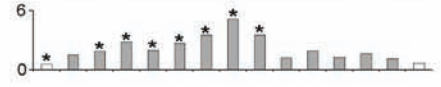
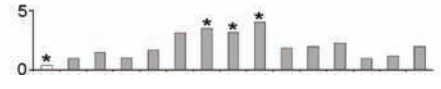
Hemopexin. Chicken homopexin was previously found in chicken serum and cerebrospinal fluid.[32,33] In this study, three protein spots were identified (gi: 16805334, spots 38−40) with the sequence coverage of 31, 29, and 49%, respectively, compared to 18% sequence coverage by Mann.[16] For the first time, homopexin was successfully separated and identified using 2-DE. The observational molecular weight of homopexin was much higher than the theoretical one, which is consistent with the previous study.[16] Significant differences were observed in spot 38 (between B/ω), spot 39 (between W/B, W/O, B/ ω, B/L, ω/O, and L/O), and spot 40 (between W/V, W/L, B/ ω, B/V, and B/L).

Ovalbumin-Related Protein Y. Members of the ovalbumin family belong to serine (or cysteine) proteinase inhibitors (serpins), a large structurally heterogeneous and functionally diverse family of proteins from various organisms, and show strong inhibition against serine or cysteine proteases, with the exception of ovalbumin.[27,28] In this study, ovalbumin-related protein Y was identified in three protein spots (gi: 71897377, spots 36, 37, and 54). The apparent molecular weights, ranging from 56.7 to 58.8 kDa, are much higher than the theoretical one (44.0 kDa) as reported elsewhere.[40] It was reported previously that one protein spot could contain multiple proteins.[15] In our study, besides ovalbumin-related protein Y, ovalbumin was also detected in spots 36 and 37, and ovalbumin-related protein X was detected in spot 54 as shown in Table 1, which probably was the reason for the higher observed molecular weight. Two protein spots (36 and 37) showed significantly higher levels in brown-shell eggs than others (ω, V, L, and O), and there was no significant difference among white-shell eggs and others (ω, V, L, and O).

Serine (or Cysteine) Proteinase Inhibitor. Serine (or cysteine) proteinase inhibitors, a large structurally heterogeneous and functionally diverse family of proteins from various organisms, show strong inhibition against serine or cysteine proteases, with the exception of ovalbumin.[27,28] In this study, serine or cysteine proteinase inhibitor was identified in three protein spots (gi: 71897377, spots 36, 37, and 54). The apparent molecular weights, ranging from 56.7 to 58.8 kDa, are much higher than the theoretical one (44.0 kDa). It was reported previously that one protein spot could contain multiple proteins.[15] In our study, besides serine (or cysteine) proteinase inhibitor, ovalbumin was also detected in spots 36 and 37, and ovalbumin-related protein Y was detected in spot 54 as shown in Table 1, which probably was the reason for the higher observed molecular weight. Two protein spots (36 and 37) showed significantly higher levels in brown-shell eggs than others (ω, V, L, and O), and there was no significant difference among white-shell eggs and others (ω, V, L, and O).

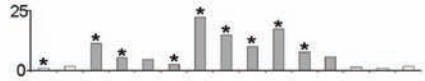
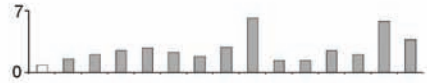
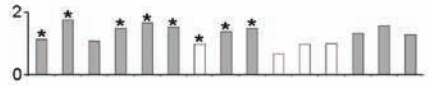
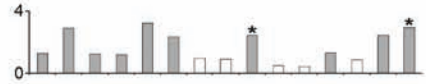
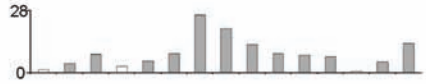
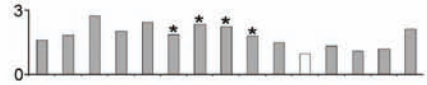
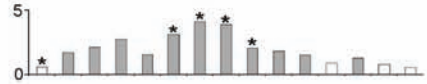
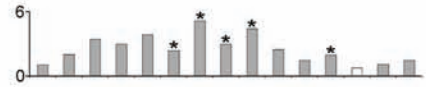
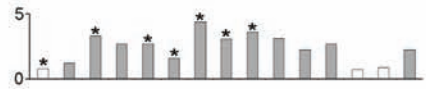
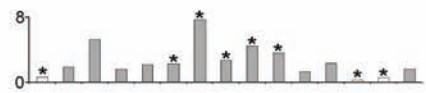
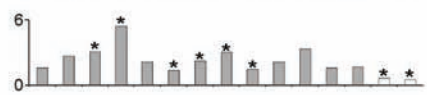
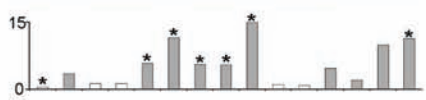
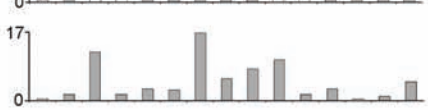
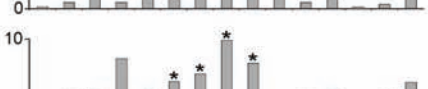
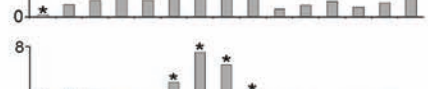
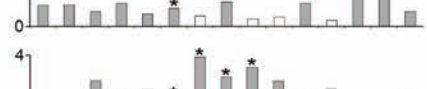
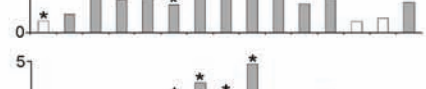
Marker Protein. Marker protein (Ch21), a developmentally regulated low molecular weight protein, was first found in chick embryo skeletal tissues.[29] It could selectively bind with high affinity fatty acids playing an important role during chick embryogenesis.[30] Two marker protein spots (gi: 211503; spot 33 and 42) were identified in this study. Protein spot 42, but not protein spot 33, was observed to be significantly up-regulated between W/L and B/L and down-regulated between B/V. Two Ch21 protein spots (gi: 82200597) have been previously detected and identified from global proteome

Table 1. Proteins Identified by 2-DE and LC-MS/MS[a]

No.[a]	gi[b]	Name	S[c]	C[d]	M[e]	Thr. Mr/pI	Obs. Mr/pI	Relative abundance (fold changes)[f]
2	129293	Ovalbumin	125	9	3	43.2/5.19	18.1/4.94	
3	129293	Ovalbumin	203	14	5	43.2/5.19	24.2/4.95	
11	129293	Ovalbumin	191	15	5	43.2/5.19	18.0/5.07	
12	129293	Ovalbumin	227	14	7	43.2/5.19	19.4/5.15	
13	129293	Ovalbumin	75	6	2	43.2/5.19	17.1/5.26	
18	129293	Ovalbumin	110	7	2	43.2/5.19	19.2/5.01	
19	129293	Ovalbumin	170	11	4	43.2/5.19	20.0/5.32	
23	129293	Ovalbumin	183	19	8	43.2/5.19	27.8/5.06	
29	129293	Ovalbumin	139	14	5	43.2/5.19	22.3/5.53	
31	129293	Ovalbumin	364	34	9	43.2/5.19	26.3/5.45	
41	129293	Ovalbumin	206	19	5	43.2/5.19	15.8/5.65	
43	129293	Ovalbumin	128	12	4	43.2/5.19	18.2/5.69	
44	129293	Ovalbumin	175	16	7	43.2/5.19	17.7/5.69	
46	129293	Ovalbumin	104	12	3	43.2/5.19	27.1/5.66	
10	28566340	Ovalbumin	288	29	7	43.2/5.19	155/4.98	
14	28566340	Ovalbumin	302	28	9	43.2/5.19	23.3/5.22	
15	28566340	Ovalbumin	286	23	12	42.9/5.19	22.4/5.31	
16	28566340	Ovalbumin	491	41	16	42.9/5.19	28.1/5.32	

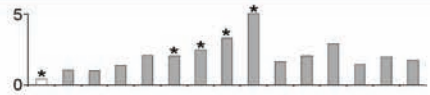
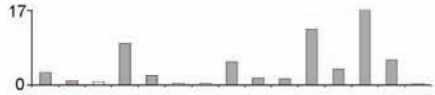
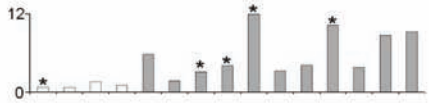
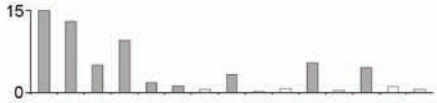
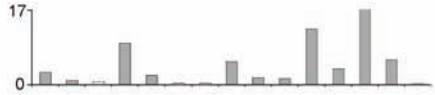
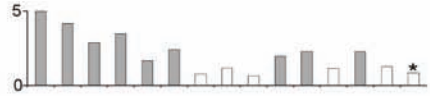
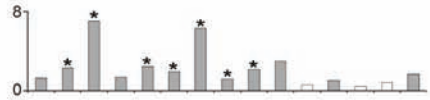
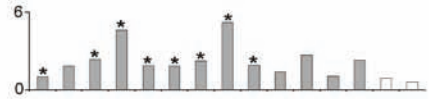
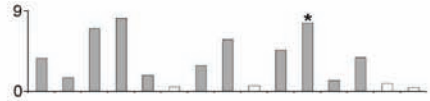
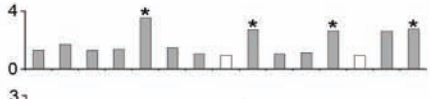
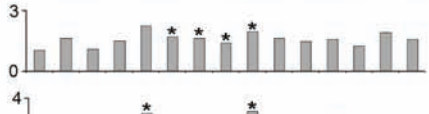
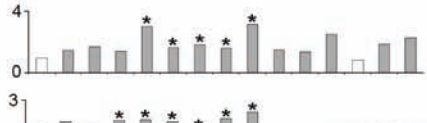
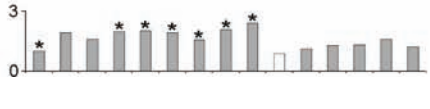
dx.doi.org/10.1021/jf2033973 | J. Agric.Food Chem. 2012, 60, 272–282

Table 1. continued

No.[a]	gi[b]	Name	S[c]	C[d]	M[e]	Thr. Mr/pI	Obs. Mr/pI	Relative abundance (fold changes)[f]
17	28566340	Ovalbumin	271	27	7	43.2/5.19	21.6/5.15	
20	28566340	Ovalbumin	228	22	5	43.2/5.19	25.8/5.05	
21	28566340	Ovalbumin	191	17	5	43.2/5.19	34.0/5.08	
22	28566340	Ovalbumin	370	32	11	43.2/5.19	34.3/5.19	
24	28566340	Ovalbumin	628	49	18	43.2/5.19	39.0/5.36	
25	28566340	Ovalbumin	660	61	46	43.2/5.19	47.2/5.29	
26	28566340	Ovalbumin	206	24	7	43.2/5.19	18.4/5.38	
27	28566340	Ovalbumin	161	14	4	43.2/5.19	16.7/5.40	
28	28566340	Ovalbumin	503	41	16	43.2/5.19	23.7/5.38	
30	28566340	Ovalbumin	240	22	9	43.2/5.19	24.3/5.62	
32	28566340	Ovalbumin	233	26	8	43.2/5.19	19.6/5.39	
34	28566340	Ovalbumin	311	29	9	43.2/5.19	36.3/5.43	
35	28566340	Ovalbumin	380	42	11	43.2/5.19	38.4/5.45	
36	28566340	Ovalbumin	426	38	14	43.2/5.19	57.4/5.47	
37	28566340	Ovalbumin	327	27	9	43.2/5.19	58.8/5.56	
38	28566340	Ovalbumin	199	23	6	43.2/5.19	67.3/5.52	
45	28566340	Ovalbumin	322	25	13	43.2/5.19	25.7/5.66	
48	28566340	Ovalbumin	161	15	5	43.2/5.19	25.5/5.91	

dx.doi.org/10.1021/jf2033973 | J. Agric.Food Chem. 2012, 60, 272−282

Table 1. continued

No.[a]	gi[b]	Name	S[c]	C[d]	M[e]	Thr. Mr/pI	Obs. Mr/pI	Relative abundance (fold changes)[f]
49	28566340	Ovalbumin	150	13	6	43.2/5.19	20.0/5.71	
52	71274075	Ovotransferrin BB type	448	26	14	79.6/6.85	51.3/6.25	
65	71274075	Ovotransferrin BB type	249	17	9	79.6/6.85	54.8/6.31	
66	71274075	Ovotransferrin BB type	411	25	13	79.6/6.85	48.9/6.39	
67	71274075	Ovotransferrin BB type	437	20	14	79.6/6.85	54.3/6.40	
52	71274077	Ovotransferrin CC type	439	26	14	79.6/7.08	51.3/6.25	
51	17942831	Chain A, Ovotransferrin, C-Terminal Lobe, Apo Form	88	20	3	39.4/6.31	30.3/5.77	
63	83754919	Chain A, crystal structure of aluminum-bound ovotransferrin at 2.15 angstrom resolution	259	21	8	77.5/6.70	39.4/6.23	
64	83754919	Chain A, crystal structure of aluminum-bound ovotransferrin at 2.15 angstrom resolution	515	31	16	77.5/6.70	39.3/6.42	
68	83754919	Chain A, crystal structure of aluminum-bound ovotransferrin at 2.15 angstrom resolution	667	41	21	77.5/6.70	40.8/6.65	
4	45383093	Ovoglycoprotein	203	29	4	22.5/5.11	33.3/4.97	
5	45383093	Ovoglycoprotein	202	12	4	22.5/5.11	34.0/4.87	
7	45383093	Ovoglycoprotein	220	18	6	22.5/5.11	34.3/4.78	
8	45383093	Ovoglycoprotein	190	18	5	22.5/5.11	35.1/4.70	

dx.doi.org/10.1021/jf2033973 | J. Agric.Food Chem. 2012, 60, 272−282

Table 1. continued

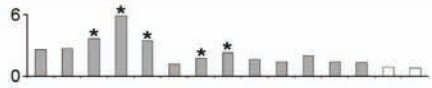
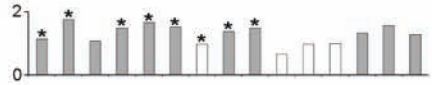
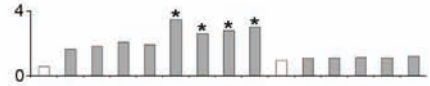
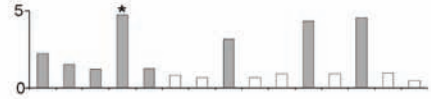
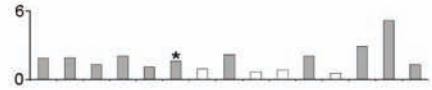
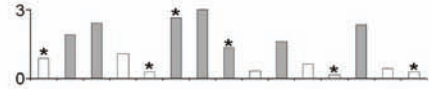
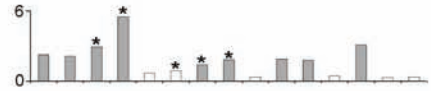
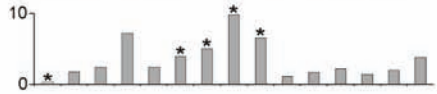
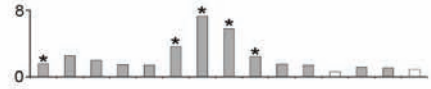
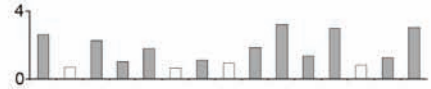
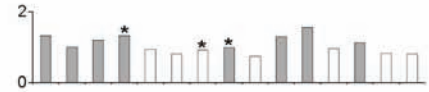
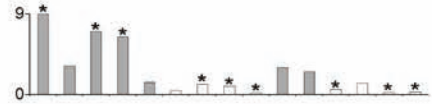
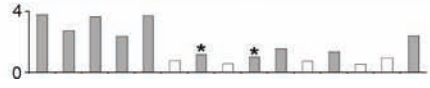
No.[a]	gi[b]	Name	S[c]	C[d]	M[e]	Thr. Mr/pI	Obs. Mr/pI	Relative abundance (fold changes)[f]
9	45383093	Ovoglycoprotein	111	8	2	22.5/5.11	35.8/4.63	
21	45383093	Ovoglycoprotein	242	18	6	22.5/5.11	34.0/5.08	
23	45383093	Ovoglycoprotein	118	10	2	22.5/5.11	27.8/5.06	
50	45382467	Clusterin	84	10	3	51.9/5.48	32.5/6.11	
61	45382467	Clusterin	171	13	6	51.9/5.48	31.8/6.31	
62	45382467	Clusterin	168	14	5	51.9/5.48	31.4/6.53	
38	16805334	Hemopexin	216	31	5	29.8/5.92	67.3/5.52	
39	16805334	Hemopexin	244	29	7	29.8/5.92	66.2/5.69	
40	16805334	Hemopexin	394	49	12	29.8/5.92	66.8/5.61	
36	71897377	ovalbumin-related protein Y	523	30	16	44.0/5.20	57.4/5.47	
37	71897377	ovalbumin-related protein Y	408	31	16	44.0/5.20	58.8/5.56	
54	71897377	ovalbumin-related protein Y	201	21	6	44.0/5.20	56.7/6.18	
33	211503	Marker protein	83	11	2	20.2/5.37	19.7/5.55	
42	211503	Marker protein	213	39	6	20.2/5.37	19.5/5.80	
57	71895337	Ovoinhibitor precursor	155	15	5	54.4/6.16	68.3/6.13	
69	71895337	Ovoinhibitor precursor	554	38	15	54.4/6.16	67.4/6.37	
6	124757	Ovomucoid	89	36	4	23.7/4.75	26.8/4.95	

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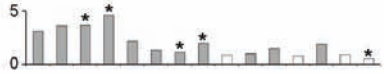
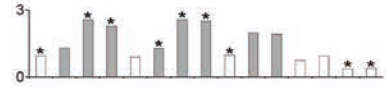
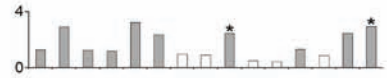
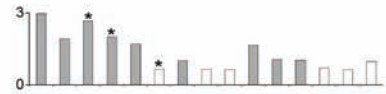
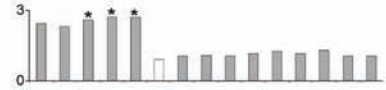
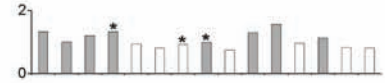
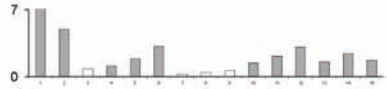
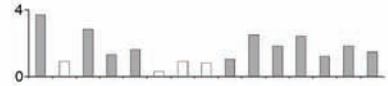
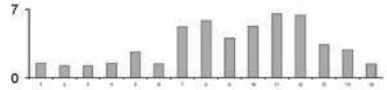
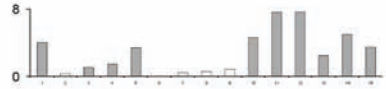
Table 1. continued

No.[a]	gi[b]	Name	S[c]	C[d]	M[e]	Thr. Mr/pI	Obs. Mr/pI	Relative abundance (fold changes)[f]
1	223464	Ovomucoid	77	28	3	21.2/4.78	36.4/4.57	
47	45383612	Prostaglandin D_2 synthase, brain	155	28	4	21.0/6.30	19.6/5.86	
58	45383612	Prostaglandin D_2 synthase, brain	281	40	8	21.0/6.30	19.2/6.28	
21	352173	Protein, riboflavin binding	215	15	5	26.1/4.95	34.0/5.08	
22	352173	Protein, riboflavin binding	216	21	4	26.1/4.95	34.3/5.19	
70	15826593	Chain A, covalent glycosyl-enzyme intermediate of hen egg white lysozyme	402	68	28	14.8/9.46	13.5/6.88	
71	45383131	Hep21 protein	75	33	3	12.6/6.55	16.5/6.52	
42	45382221	Quiescence-specific protein precursor	236	37	7	20.2/5.56	19.5/5.80	
54	118086485	Ovalbumin-related protein X	271	19	7	43.8/5.97	56.7/6.18	
55	118086485	Ovalbumin-related protein X	208	19	6	43.8/5.97	57.5/6.03	
56	118086485	Ovalbumin-related protein X	275	25	7	43.8/5.97	58.7/5.90	
59	45382805	Cystatin C precursor	68	18	3	15.6/7.60	14.6/6.39	
60	451889	Avidin	209	43	6	16.9/9.69	16.3/6.64	
72	451889	Avidin	190	36	5	16.9/9.69	16.4/6.79	
53	45383974	Albumin precursor	513	31	16	71.9/5.51	73.5/5.76	

[a]No., protein sport number corresponding in Figure 1 (ssp); gi, protein accession number in NCBI database; S, scores of Mascot and the threshold of score over 66; C, sequence coverage (%); M, matched peptide; relative abundance (fold changes), the *Y*-axis is the relative abundance (fold changes) between any two egg varieties. In the *X*-axis from left to right, the columns denote the pairwise comparison of W/B (white-shell eggs against brown-shell eggs), W/ω, W/V, W/L, W/O, B/ω, B/V, B/L, B/O, ω/V, ω /L, ω /O, V/L, V/O, and L/O, respectively. Shaded columns indicate up-regulated expression, whereas white columns indicate down-regulated expression of two egg varieties. An asterisk indicates that there is a significant difference ($P < 0.05$) between pairs of eggs by the Student's *t*-test analysis. Six egg varieties, conventional white-shell eggs (W), conventional brown-shell eggs (B), lutein-enriched eggs (L), organic eggs (O), omega-3-enriched eggs (ω), and vitamin-enriched eggs (V), were products of Lucerne Food Co. (Calgary, AB, Canada) in May 2009. The conventional white-shell eggs, lutein-enriched eggs, organic eggs, omega-3-enriched eggs, and vitamin-enriched eggs are from lines of Single Comb White Leghorns. The conventional brown-shell eggs are from lines of Rhode Island Reds.

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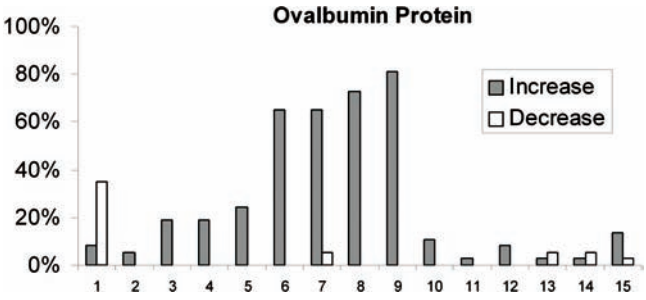

Figure 2. Comparison of 37 ovalbumin protein spots among each pair of various eggs. Column means the percentage of significantly different protein spots from a total of 37 ovalbumin protein spots in each pairs. Columns 1−15 indicate the pairs W/B, W/ω, W/V, W/L, W/O, B/ω, B/V, B/L, B/O, ω /V, ω /L, ω /O, V/L, V/O, and L/O, respectively, from left to right on the *X*-axis. Shaded columns indicate the up-regulated expression, and white columns indicate the down-regulated expression of each pair. Six egg varieties, conventional white-shell eggs (W), conventional brown-shell eggs (B), lutein-enriched eggs (L), organic eggs (O), omega-3-enriched eggs (ω), and vitamin-enriched eggs (V), were products of Lucerne Food Co. (Calgary, AB, Canada) in May 2009. The conventional white-shell eggs, lutein-enriched eggs, organic eggs, omega-3-enriched eggs, and vitamin-enriched eggs are from lines of Single Comb White Leghorns. The conventional brown-shell eggs are from lines of Rhode Island Reds.

analysis of white-shell eggs.[15] These results suggest that Ch21 protein comprises two isoforms in egg whites and only one spot (42) was significantly up- or down-regulated.

Ovoinhibitor. Ovoinhibitor, a member of protease inhibitor Kazal family, contains seven domains in a single polypeptide chain.[15] Two protein spots (gi: 71895337, spots 57 and 69) were identified as ovoinhibitor precursors in this investigation, in which protein spot 69, but not spot 57, was significantly different among O and others (B, ω, V, and L).

Ovomucoid. Ovomucoid, one of the major egg white proteins (11%), is a member of the protease inhibitor Kazal family.[24] Two protein spots (gi: 223464, spot 1; and gi: 124757, spot 6) were identified as ovomucoid. The apparent molecular weight of protein spot 1 is much higher than the theoretical one, which is consistent with previous studies that the apparent molecular weight of ovomucoid ranges between 27 and 35 kDa.[31] Significant differences in the abundance of these two ovomucoid spots were observed, and, specifically, both of these two spots of brown-shell eggs showed significantly higher abundance levels compared to the vitamin-enriched eggs.

Prostaglandin D_2 Synthase. Prostaglandin (PG) D_2 synthase has been observed in many organs that may play important roles in reproduction and in regulation of sleep and pain responses.[32] Two protein spots (gi: 45383612, spots 47 and 58) were identified as PGD_2 synthase. Significant differences were determined between white-shell and brown-shell eggs for spot 58 and between omega-3- and lutein-enriched eggs for spot 47. Moreover, both spots showed significantly ($P < 0.05$) higher abundance in organic eggs than in another two varieties (V and L) (Table 1).

Riboflavin-Binding Protein. Egg-white riboflavin-binding protein, composed of 219 amino acid residues, was first isolated and characterized by Hamazume et al.[33] Two protein spots (gi: 352173, spots 21 and 22) were identified as riboflavin-binding protein. Significant differences in the abundance of riboflavin-binding protein were observed among various egg varieties, such as between W/B, W/ω, W/L, W/O, B/ ω, B/V, B/L, and B/O for spot 21.

Lysozyme. Hen egg-white lysozyme, a well-known antimicrobial protein (3.4% of total egg white proteins), has been well studied.[24,34] Protein spot 70 (gi: 15826593) was identified as a covalent glycosyl-enzyme intermediate of hen egg white lysozyme. Significant differences were only found between W/V, W/L and B/ω.

Hep21 Protein. Hep21 protein is a member of the uPAR/CD59/LY-6/snake neurotoxin superfamily. It was first characterized with 2-DE.[35] Protein spot 71 (gi: 45383131) was identified as hep21 protein. Significant difference was observed among white-shell eggs and other three egg varieties (V, L, and O), which suggested that the abundance of hep21 protein in the egg whites of vitamin-enriched, lutein-enriched, and organic eggs are lower than those of white-shell eggs.

Quiescence-Specific Protein Precursor. A cDNA clone encoding p20K (a major gene encoding quiescence-specific protein) was first characterized by Bedard et al.[36] In this study, protein spot 42 (gi: 45382221) was identified as a quiescence-specific protein precursor (Table 1). To the best of our knowledge, this is the first time the presence of this protein in egg whites has been demonstrated even though it was previously identified in chicken embryo fibroblasts and chicken heart mesenchymal cells.[36] Significant differences in the abundance of quiescence-specific protein precursor were observed between L/W, L/B, and B/V.

Proteins without Significant Changes among Egg Varieties. Four proteins, ovalbumin-related protein Y (gi: 118086485, spotd 54−56), cystatin C (gi: 45382805, spot 59), avidin (gi: 451889, spots 53 and 72), and albumin precursor (gi: 45383974, spot 53), were found to have no significant difference among any variety of eggs (Table 1).

Ovalbumin-Related Protein X. Ovalbumin, ovalbumin-related protein X, and ovalbumin-related protein Y are three proteins encoded by three distinct gens, the X, Y, and ovalbumin genes.[38,39] Guerin-Dubiard et al.[15] and Mann[16] also found the ovalbumin-related protein X in 1-DE and 2-DE, respectively. Three protein spots identified as ovalbumin-related protein X (spots 54, 55, and 56) have been detected in all egg varieties in this study. However, there was no significant difference in spots 55 and 56, with the exception of spot 54, where the white-shell eggs showed significantly higher abundance than brown-shell eggs. This difference probably was caused by interaction with ovalbumin-related protein Y (gi: 71897377), which was also identified in spot 54 as shown in Table 1. Our results showed that there is no difference in the abundance of ovalbumin-related protein X among six egg varieties, although significant differences were observed in the abundance of ovalbumin.

Cystatin. Cystatin, a phosphoprotein, is a protein inhibitor of cysteine proteinase. Chicken cystatin, belonging to family 2 of the cystatin superfamily, was first isolated from egg white by Fossum and Whitaker.[37] Protein spot 59 (gi: 45382805) was identified as a cystatin C precursor. There is no significant difference in the abundance of cystatin C precursor among any egg varieties.

A total of 23 proteins were characterized from 89 egg white protein spots in this study, and the presence of a quiescence-specific protein precursor was identified for the first time in egg whites. Moreover, three ovotransferrin types, ovotransferrin BB type, ovotransferrin CC type, and ovotransferrin chain A, were also identified in egg white. Our results showed that 19 proteins, of a total of 23 proteins identified, were significantly

different among 6 egg varieties. Four proteins, ovalbumin-related protein Y, cystatin, avidin, and albumin precursor, were not different among any egg varieties. The results obtained in this study indicate that there is no obvious difference in the nutritional quality of egg white proteins from different egg varieties. Although our results showed that the abundance of but not the composition of egg white proteins could change depending on the egg variety, further study is needed to understand the cause of the change as a number of variables including genetics, lines, cage system, management, age of birds, feeding methods, temperature, etc., might contribute to the change of protein expression.

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