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Co-extraction of egg white proteins using ion-exchange chromatography from ovomucin-removed egg whites

Dileep A. Omana, Jiapei Wang, Jianping Wu*

Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta, Canada T6G 2P5

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ABSTRACT

Efficient isolation of egg white components is desired due to its potential uses. Existing methods mainly targeted on one specific protein; an attempt has been made in the study to co-extract all the valuable egg white components in a continuous process. Ovomucin was first isolated by our newly developed two-step method; the resultant supernatant obtained after ovomucin isolation was used as the starting material for ion-exchange chromatography. Anion-exchange chromatography of 100 mM supernatant yielded a flow-through fraction and three other fractions representing ovotransferrin, ovalbumin and flavoproteins. The flow-through fraction was further separated into ovoinhibitor, lysozyme, ovotransferrin and an unidentified fraction which represents 4% of total egg white proteins. Chromatographic separation of 500 mM supernatant resulted in fractions representing lysozyme, ovotransferrin and ovalbumin. This co-extraction protocol represents a global recovery of 71.0% proteins.

1. Introduction

Besides its excellent functional properties, hen egg white is known for its wide bioactive properties [1,2]. Ovalbumin (54%), ovotransferrin (13%), lysozyme (3.5%) and ovomucin (3.5%) represent the major egg white proteins. Ovalbumin, which is the major constituent of egg white proteins, is used in food industry because of its foaming and gelling properties. Ovalbumin contains ovokinin which can acts as antihypertensive agent [3]. Ovotransferrin is known for antimicrobial activity which is associated with its iron binding property [4]. Lysozyme is a known antimicrobial, antiviral and antalgic agent [5,6]. Besides antimicrobial activity, avidin serves as vitamin carrier [7] while flavoprotein serves as vitamin stabilizer [3]. Ovomucin, a glycoprotein in egg white, is important for its antiviral, anti-tumor and immunomodulating effects [8,9]. Therefore, isolation and purification of valuable egg white components appears to be promising due to their potential uses.

Different methods have been developed for purifying major egg white proteins such as salt precipitation, ionic strength reduction and liquid chromatography [2]. Precipitation of proteins by manipulating salt (ionic) strength is widely used despite the denaturation of the proteins caused by these processes. The major development in protein purification is liquid chromatography. Liquid chromatography purifies proteins without protein denaturation in addition to its high efficiency in separation. Lysozyme can be prepared by the conventional crystallization and precipitation method or using chromatographic methods [10–12]. Ovalbumin and ovotransferrin are prepared mainly by ion-exchange chromatography [13–16]. Ovalbumin is prepared using anion exchanger based on the principle of frontal chromatography. Isocratic elution with 0.14 M NaCl yielded 94% pure ovalbumin. Ovotransferrin was prepared by passing egg white through Q-Sepharose fast flow column [15]. Flavoprotein was prepared by salt precipitation, anion-exchange chromatography and gel filtration [17,18]. Piskarev et al. [18] prepared riboflavin as a by-product during the preparation of avidin using HPLC on a powerful cation-exchanger (TSK SP-5PW) column.

However, most of the methods reported lead to isolation of individual proteins rather than a continuous co-extraction process, which enables to separate major egg white proteins in an integrated manner. As a protein responsible for the viscous nature of egg white, ovomucin used to be isolated by dilution with three volumes of water at pH 6 [19,20]. However, ovomucin prepared by this method was contaminated with lysozyme and ovalbumin [19,20]. Recently, a new method has been developed to isolate ovomucin of high purity and yield, using a two-step precipitation method using NaCl solutions [21]. Since this protocol involved two-step treatments with 100 mM NaCl solution followed by treating the precipitate with 500 mM NaCl solution, other proteins are dissolved in these salt solutions. In an earlier work Guerin-Dubiard et al. [22] attempted co-extraction technology from mucin-free egg white and separated the major egg white proteins and two unidentified fractions. However, in this method mucin-free egg white was pre-

* Corresponding author at: Department of Agricultural, Food and Nutritional Science (AFNS), 4-10 Ag/For Building, University of Alberta, Edmonton, Alberta, Canada T6G 2P5. Tel.: +1 780 492 6885; fax: +1 780 492 4265.
E-mail address: jwu3@ualberta.ca (J. Wu).

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pared by precipitation using water while the present work involved usage of 100 mM NaCl solution for ovomucin separation. It is well established that precipitation of ovomucin using water causes huge loss of other proteins especially ovalbumin and lysozyme. Precipitation with 100 mM NaCl helps to precipitate mainly ovomucin while leaving most of the other proteins in the supernatant. Therefore, the objective of the present study was to determine if other egg white proteins could be further purified from the salt solutions after ovomucin separation.

2. Materials and methods

2.1. Materials

Fresh eggs laid within 24 h from White Leghorn were collected in the morning from the Poultry Research Centre farm of the University of Alberta (Edmonton, Canada) and used within the same day for ovomucin preparation. Hydrochloric acid was purchased from Fisher Scientific (Ottawa, ON, Canada). 2-mercaptoethanol was obtained from MP Biomedicals, LLC (Solon, OH, USA) and sodium chloride was purchased from Acros Organics (Morris Plains, NJ, USA). Citrate and sodium citrate were obtained from Sigma Chemicals (St. Louis, MO, USA).

2.2. Methods

2.2.1. Preparation of ovomucin using a two-step method

Ovomucin was prepared as per the method of Omana and Wu [21]. Briefly, ovomucin was first prepared using isoelectric precipitation of egg white in the presence of 100 mM NaCl solution. The dispersion was kept overnight at 4 °C and separated by centrifugation at 15,300 × *g* (Avanti® J-E refrigerated centrifuge, Beckman Coulter, Inc., Fullerton, CA, USA) for 10 min at 4 °C. The precipitate was further suspended in 500 mM NaCl solution while stirring for 4 h followed by overnight settling at 4 °C. After centrifugation at 15,300 × *g* for 10 min at 4 °C, the precipitate was freeze dried and stored at −20 °C. The supernatants obtained during the first step (with 100 mM NaCl solution) and the second step (with 500 mM NaCl solution) were further used for ion-exchange chromatography to separate other egg white proteins. The experiment was repeated two times.

2.2.2. Separation of proteins from 100 mM supernatant

Proteins from 100 mM supernatant were separated by two-step chromatographic methods. Initially the supernatant was allowed to pass through an anion exchange chromatographic column to separate different fractions. The unbound fractions were then passed through a cation exchange chromatographic column to separate further.

2.2.2.1. Anion-exchange chromatography. Anion-exchange chromatography of 100 mM supernatant was carried out using a High-Prep 16/10 column (Q Sepharose FF, GE Healthcare Bio-Sciences AB, Uppsala, Sweden) coupled with a Fast-Performance Liquid Chromatography (FPLC) system (AKTA explorer, GE Healthcare Bio-Sciences, Uppsala, Sweden). The elution protocol was followed as per Guerin-Dubiard et al. [22], with slight modifications in flow rates and volumes of washing used. Unlike Guerin-Dubiard et al. [22], in the present study we tried to separate ovalbumin during the first separation, since it is the most abundant protein in the egg white. The column was equilibrated with water and the pH of the sample was adjusted to 8.0 before injection. After sample injection (10 mL), flow-through fraction (4 column volume [CV]) was collected using water as the eluent, followed by isocratic elution (4 CV) of the sample using 0.14 M NaCl. Finally the bound fraction was eluted (5 CV) using gradient elution (0.14–0.5 M) of NaCl. The unbound fraction (F1) was collected and used as starting material for cation-exchange chromatography. The fractions were then collected, freeze dried and stored at −20 °C until further analysis.

2.2.2.2. Cation-exchange chromatography. Cation-exchange chromatography of the unbound fraction (F1) was carried out using a High-Prep 16/10 column (SP Sepharose FF, GE Healthcare Bio-Sciences AB, Uppsala, Sweden). The column was equilibrated with 10 mM citrate buffer, which was used as the starting buffer. Flow rate was 5 mL/min and the injection volume was 40 mL. After sample injection, the column was eluted by isocratic elution (4 CV) using 0.14 M NaCl solution followed by gradient elution (5 CV) from 0.14 M to 0.50 M NaCl solution. The fractions were then collected, freeze dried and stored at −20 °C.

2.2.3. Separation of proteins from 500 mM supernatant

Proteins from 500 mM supernatant were also separated using two-step chromatographic methods. Since salt content was higher in this supernatant, dialysis was carried out before injecting into chromatographic column. Initially the supernatant was allowed to pass through the cation-exchange chromatography column (High-Prep 16/10 column, SP Sepharose FF, GE Healthcare Bio-Sciences AB, Uppsala, Sweden). The unbound fraction was again passed through anion exchange chromatographic column (High-Prep 16/10 column, Q Sepharose FF, GE Healthcare Bio-Sciences AB, Uppsala, Sweden) for further separation.

2.2.4. Purity determination using sodium dodecyl sulphate polyacrylmide gel electrophoresis (SDS-PAGE)

SDS-PAGE was carried out [23] using 10–20% ready gels (Bio-Rad Laboratories, Inc., Hercules, CA) at a constant voltage mode in a Mini-PROTEAN® tetra cell (Bio-Rad Laboratories, Inc., Hercules, CA) attached to PowerPac™ Basic electrophoresis apparatus (Bio-Rad Laboratories Inc., 1000 Alfred Nobel Drive, Hercules, CA, USA). The loaded amount of proteins was 50 μg for all the samples. Protein markers of high range molecular weight obtained from Bio-Rad (Bio-Rad Laboratories, Inc., Hercules, CA) were loaded into a separate well for comparison of molecular weight. The gels were scanned using Alpha Innotech gel scanner (Alpha Innotech Corp., San Leandro, CA) with FluorChem SP software. Purity and molecular weight of the proteins were determined using the same software.

2.2.5. LC–MS/MS analysis

The freeze dried protein fraction (F2: obtained during anion-exchange chromatography of 100 mM supernatant) was digested with trypsin based on 'Agilent 1100 LC–MS getting started guide. The protein fraction was initially reduced with 0.1 M of NH_4HCO_3 containing 10 mM dithiothreitol at 56 °C for 30 min and washed with 100% acetonitrile (ACN). These proteins were further alkylated at room temperature with 0.1 M of NH_4HCO_3 (containing 55 mM of iodoacetamide) in the dark for 20 min. After dehydration, digestion was performed by adding trypsin (0.02 μg/μL) with 40 mM NH_4HCO_3 and 10% of ACN at 37 °C for 16 h. Termination of digestion was carried out by adding formic acid (0.1%, v/v) for 40 min. Peptides were then extracted twice with 50% of ACN and 0.1% of formic acid for 30 min and kept for MS analysis.

MS analysis of the extracted peptide mixtures was carried out on an Agilent 1100 Series LC/MSD Trap XCT System (Agilent Technologies, Palo Alto, CA, USA). Peptide sample (20 μL) was injected to a concentration column (Zorbax 300SB-C18, 5 μm, 5 mm × 0.3 mm), followed by a separation column (Zorbax 300S B-C_{18}, 5 μm, 150 mm × 0.3 mm). Activation of the columns was done at a flow rate of 4 μL/min and the HPLC gradient was set as the following linear programs: a isocratic mode of 3% buffer B for 5 min, gradient to 15% buffer B for 3 min, gradient to 45% buffer B for 42 min, gradient to 90% buffer B for 10 min, gradient to 3% buffer

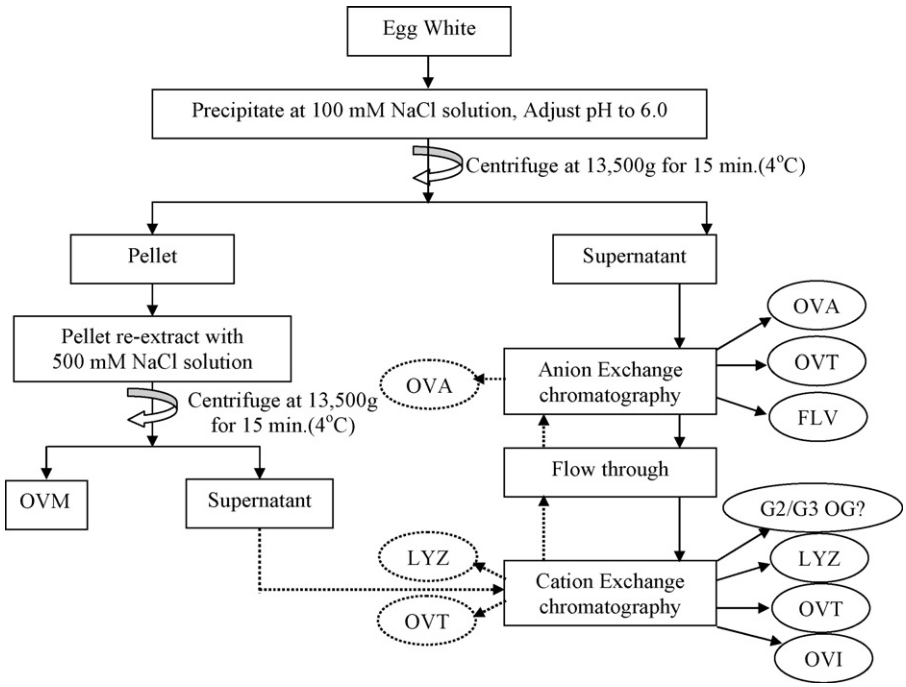

Fig. 1. Schematic diagram, showing the co-extraction protocol for egg white proteins. OVM: Ovomucin; OVA: Ovalbumin; OVT: Ovotransferrin; FLV: Flavoprotein; LYZ: Lysozyme; G2/G3 OG: G2/G3-Ovomacroglobulin; OVI: Ovoinhibitor.

B for 1 min; and finally a isocratic mode of 3% buffer B for 14 min to clean the column (buffer A = 0.1% FA in water; buffer B = 0.1% FA in ACN). The peptide ion fragmentation was performed with an MS 300–2000 *m/z* scan and the most intense ions were analyzed with a MS/MS. Every MS/MS data was 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.

2.2.6. Yield determination

Yield of the proteins was determined as ratio between protein amount in respective fraction and that of the total protein amount in 100 mL of egg white using the formula given below.

$$\text{Yield}\,(\%) = \frac{\text{Dry weight of fraction} \times \text{Protein content of the fraction}}{\text{Protein content of egg white} \times \text{Dry weight of egg white}} \times 100$$

Protein content in egg white was determined to be 10.80% (85.03% of dry weight) using a TruSpec CN carbon/nitrogen determinator (Leco Corp., St. Joseph, MI) and multiplying nitrogen content by a factor of 6.25. Total dried weight of 100 mL egg white was found to be 11.57 g. Estimated yield was also determined considering purity into account. This estimated yield is also expressed as percentage of total proteins.

2.2.7. Analysis

Ovomucin was prepared in duplicate and the supernatant was used for chromatography. For 100 mM supernatant 20 injections were carried out for each trial while for 500 mM supernatant it was 7 injections. Results are presented as mean values with standard deviations.

3. Results and discussion

Fast-performance liquid chromatography in combination with various chromatographic techniques can be effectively used to separate proteins from a mixture of proteins. Among these separation techniques, separation by ion-exchange chromatography is the most widely used method because the extracted proteins appeared less denatured and the protocol developed could be scaled up for industry purpose. Except few studies, most of the available procedures are for a specific target protein. Separation of egg white proteins by co-extraction method has earlier been attempted by Guerin-Dubiard et al. [22] using mucin-free egg white, obtained after precipitation of ovomucin. Recently Tankrathok et al. [24] could co-extract ovalbumin, ovotransferrin, ovomucoid and lysozyme using egg white treated overnight with 10 mM β-mercaptoethanol. In the present study ovomucin-free egg white was prepared based on a novel method of preparation of ovomucin [21]. Here the precipitated ovomucin was over 90% purity with a yield of 400 mg/100 mL of egg white and rest of the proteins were left in the salt solutions, which was further separated using ion-exchange chromatography. Schematic diagram representing the co-extraction protocol is given in Fig. 1.

3.1. Separation of protein fractions from 100 mM supernatant

3.1.1. Anion-exchange chromatography

The supernatant obtained after the first step of ovomucin preparation was loaded to anion exchange column. Before loading the sample, its pH was adjusted to 8.0. Egg white proteins except lysozyme and avidin will be of negative charge at this pH value. Hence the major egg white proteins viz. ovalbumin and ovotransferrin were expected to bind with anion exchange column. The positively charged avidin and lysozyme were expected to pass through the flow-through fraction (F1). Anion-exchange chromatography of 100 mM supernatant resulted in four fractions (Fig. 2). Proteins in these fractions were subjected to SDS-PAGE analysis (Fig. 3). The first fraction (F1) eluted was collected and used for further separation. The loosely bound second fraction (F2) was eluted during the isocratic elution with 0.14 M NaCl solution. The molecular weight of F2 fraction was found to be 77.3 kDa with a purity of 47.7% of ovotransferrin (Table 1). More than 50% of fraction 2 was contaminated with ovoinhibitor and lysozyme. Tankrathok et al. [24] separated lysozyme and ovalbumin using Q Sepharose Fast Flow anion-exchange chromatography as a first step of co-extraction. In some fractions like F1, F2 (Fig. 3) and F8

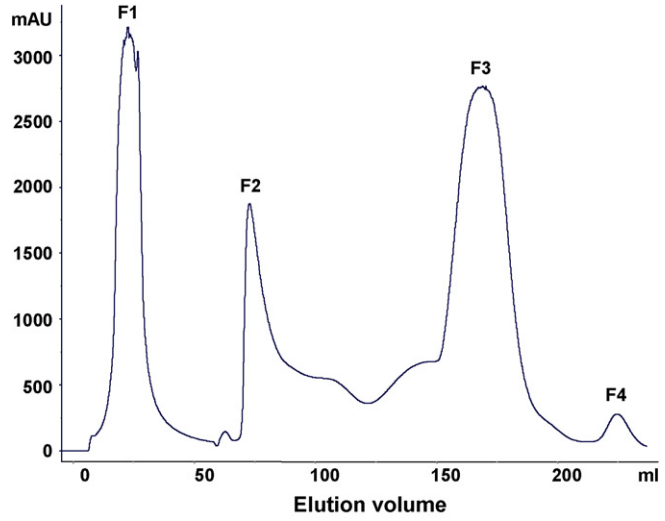

Fig. 2. Anion exchange chromatogram of supernatant obtained after treatment of egg white with 100 mM NaCl solution.

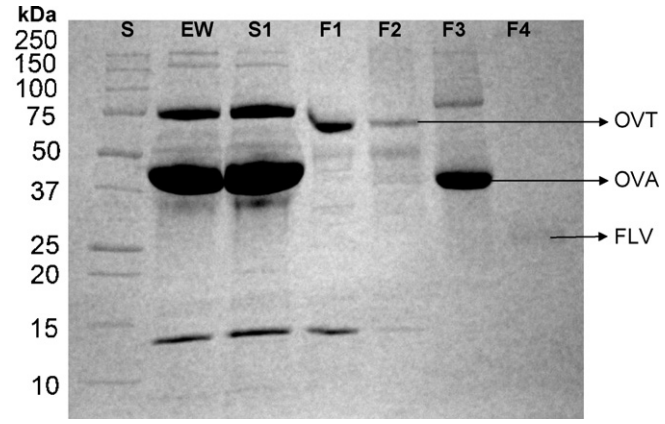

Fig. 3. SDS-PAGE pattern of fractions obtained by anion-exchange chromatography of supernatant after treatment of egg white with 100 mM NaCl solution. S: standard marker; EW: Egg white; S1: 100 mM supernatant; F1: Unbound fraction; F2–F4: Fractions collected for freeze drying.

(Fig. 5), one band appeared around 75 kDa. However, LC–MS/MS analysis of this fraction revealed that the band is corresponding to ovotransferrin (*data not shown*). The reason for the difference in relative mobility between this protein band and that of egg white sample is unknown. Further elution with a gradient from 0.14 M NaCl to 0.50 M NaCl solution yielded 2 fractions (F3 and F4). The third fraction (F3) was predominantly ovalbumin with a molecular weight and purity of 43.4 kDa and 91.2% respectively, while 8.8% was found to be ovotransferrin. Unlike the previous research wherein [14,22] ovalbumin fraction was obtained by isocratic elution, in the present study ovalbumin was eluted during

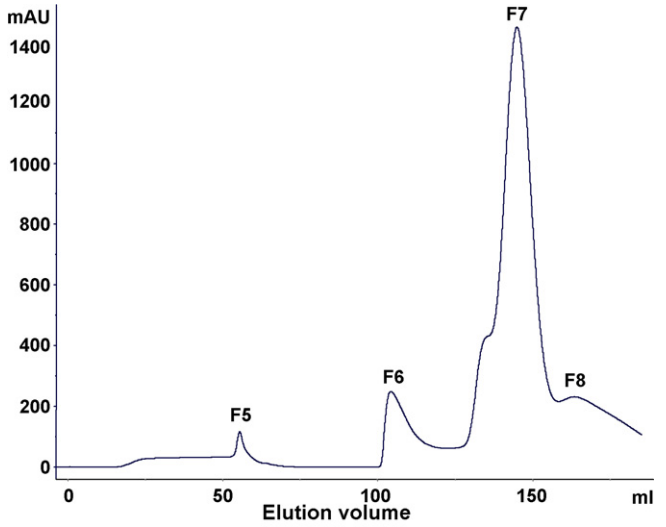

Fig. 4. Cation-exchange chromatography of unbound fraction (F1) from anion-exchange chromatography.

the gradient elution using 0.14–0.5 M NaCl solutions. The last fraction (F4) was found to be 100% pure flavoprotein with molecular weight of 29.9 kDa (Table 1). Flavoprotein was further confirmed by FPLC chromatography of standard flavoprotein using the same conditions (*data not shown*).

3.1.2. Cation-exchange chromatography

The unbound fraction (F1) was further separated into 4 different fractions (F5–F8) using cation-exchange chromatography (Fig. 4). The first fraction (F5) was eluted during the isocratic elution using 0.14 M NaCl and all other fractions (F6–F8) eluted during the gradient elution (0.14–0.50 M NaCl). The SDS-PAGE pattern of the protein fractions is given in Fig. 5. The first peak (F5) could not be identified using SDS-PAGE. However, yield calculation showed that this fractions accounts for 4% of total egg white proteins. Thus this fraction can probably be G2/G3 ovoglobulin. This has to be further confirmed. This study is still ongoing with our research group. G2- and G3-ovoglobulin accounts for 4% each of total egg white protein, with molecular weight of 49 kDa each. pI values of G2- and G3-ovoglobulin correspond to 5.5 and 5.8 respectively [25]. Ovoglobulins have 3.2–3.7% hexoses and 2.4–2.5% hexosamines [26]. The second fraction (F6) showed a band with molecular weight of 51.9 kDa and a purity of 82.5%. Molecular weight details from literature [27] suggest that this fraction could be ovoinhibitor. The third fraction was found to be with 100% pure lysozyme (Table 1). Avidin could not be separated from lysozyme using this method; probably avidin might have eluted along with lysozyme. In earlier research, avidin was separated from lysozyme fraction using direct crystallisation at pH 9.5 [10]. The last fraction (F8) also showed a completely clear single band corresponding to ovotransferrin (molecular weight 76.4). SDS-PAGE pattern showed an

Table 1
Molecular weight of the isolated fractions from 100 mM supernatant and the estimated purity of the major proteins.

Fractions	Molecular weight from software	Similar molecular weight from literature	Identified protein	Purity%
F2	77.3	77.7 [2]	OVT	47.7
F3	43.4	45.0 [2]	OVA	91.2
F4	29.9	32.0 [2]	FLV	100.0
F5	No bands	ND	G2/G3 OG?	ND
F6	51.9	49.0 [24]	OVI	82.5
F7	11.0	14.3 [2]	LYZ	100.0
F8	76.4	77.7 [2]	OVT	100.0

OVT: Ovotransferrin; OVA: Ovalbumin; FLV: Flavoprotein; OVI: Ovoinhibitor; LYZ: Lysozyme; G2/G3 OG: Ovomacroglobulin; ND: Not determined.

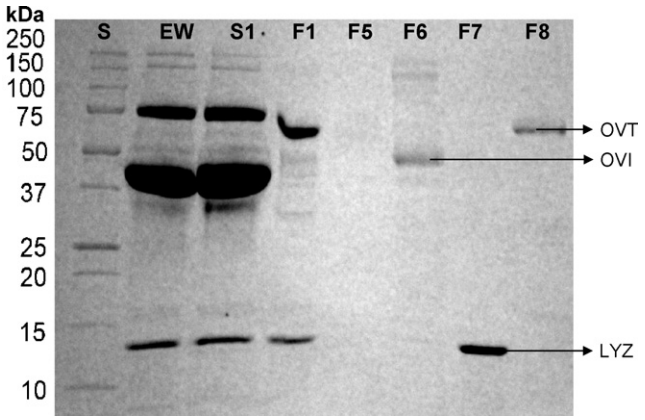

Fig. 5. SDS-PAGE pattern of fractions obtained by cation-exchange chromatography of unbound fraction (F1) from anion-exchange chromatography of 100 mM supernatant. S: Standard marker; EW: Egg white; S1: 100 mM supernatant; F1: Unbound fraction of anion-exchange chromatography of 100 mM supernatant; F5–F8: Fractions obtained by cation-exchange chromatography. Fractions were then freeze-dried. OVT: Ovotransferrin; OVI: Ovoinhibitor; LYZ: Lysozyme.

intense band at molecular weight of 77.7 kDa corresponding to ovotransferrin [22]. Unlike previous work [22] wherein lysozyme and ovotransferrin were separated by two separate steps, the present study could separate lysozyme and ovotransferrin simultaneously using cation column at pH 5.2. Ovotransferrin of 80% purity was separated during the second step of co-extraction using a CM-Toyopearl 650 M cation-exchange chromatography [24].

3.2. Separation of protein from 500 mM supernatant

During the second step of ovomucin preparation, the precipitate was suspended in 500 mM NaCl solution. After centrifugation, ovomucin was separated and the supernatant was used for further protein separation. Cation exchange chromatogram of 500 mM supernatant showed one unbound fraction (F9) and other two fractions (F10 and F11) identified as lysozyme and ovotransferrin respectively. The unbound fraction (F9) was further separated into three fractions (F12–F14) using anion-exchange chromatography (*data not shown*). SDS-PAGE profile revealed that all these fractions were ovalbumin. Purity of these fractions is given in Table 2.

In the present study ovomucoid could not be identified using SDS-PAGE. Ovomucoid has a theoretical pI of 4.1 [2]. Molecular weight of ovomucoid is 28 kDa [28]. Ovomucoid is usually difficult to visualize by SDS-PAGE with regular coomassie blue or silver staining methods; could be due to its high glycosylation rate. SDS-PAGE followed by staining with coomassie blue can stain ovomucoid, if the purified protein is loaded at a relatively high concentration [29]. In this study, since we could not stain ovomucoid, the quantity of other proteins especially ovalbumin may be overestimated. Guerin-Dubiard et al. [22] identified ovomucoid during co-extraction of egg white protein using LC–MS–MS sequencing. One sequenced peptide matched with the ovomucoid sequence (137–146). Another alternative may be to stain the gels using colloidal coomassie blue which can stain even very low quantity of proteins. Tankrathok et al. [24] precipitated ovomucoid using trichloroacetic acid from the protein fractions obtained during ion-exchange chromatography and yielded 90% purity with a recovery of 21%.

3.3. Protein yield

The main hen egg white proteins, ovalbumin, ovotransferrin, ovomucoid and lysozyme, constitute, respectively, 54, 12, 11 and 3.4% of total hen egg white proteins [30]. Out of the total 10.8 g of protein present in the egg white used as the starting material, 7.67 g (including 0.4 g of ovomucin) was recovered by this co-extraction protocol, representing a global recovery of 71.0%.

3.3.1. Fractions obtained from 100 mM supernatant

The second fraction (F2) contained ovotransferrin corresponding to 5.9% of total egg white proteins (EWP). Though the yield of F2 was higher, this fraction was contaminated with other proteins; hence the estimated yield (which is calculated based on the purity of the protein) was lower (Table 3). F3 fraction was the major fraction for ovalbumin, which yielded 40.7% of EWP. The fourth fraction (F4) yielded flavoprotein which represents 0.50% of EWP. Recovery of flavoprotein was found to be over 90% of egg white flavoprotein. Guerin-Dubiard et al. [22] could recover only 50% of flavoprotein during co-extraction.

The first fraction (F5) in cation-exchange chromatography was not fully confirmed (Table 3). Since the fraction corresponded to 3.6% of EWP, it may be G2/G3-ovoglobulin; which represents 4% of

Table 2
Molecular weight of the isolated fractions from 500 mM supernatant and the estimated purity of the major proteins.

Fractions	Molecular weight from software	Similar molecular weight from literature	Identified protein	Purity%
F10	10.8	14.3 (*1*)	LYZ	100.0
F11	78.4	77.7 (*1*)	OVT	100.0
F12	48.3	45.0 (*1*)	OVA	82.0
F13	48.5	45.0 (*1*)	OVA	100.0
F14	48.1	45.0 (*1*)	OVA	100.0

LYZ: Lysozyme; OVT: Ovotransferrin; OVA: Ovalbumin.

Table 3
Estimated yield of different protein fractions after fractionation of 100 mM supernatant.

Fractions	Freeze dried weight of fractions (g)	Protein content (%)	Protein weight of fractions (g)	Yield (%)	Purity %	Estimated yield (%)	Identified protein
F2	1.36 (0.05)	89.9 (0.78)	1.22 (0.08)	12.43 (0.73)	47.7 (1.03)	5.93 (0.51)	OVT
F3	4.48 (0.11)	98.0 (0.95)	4.39 (0.21)	44.63 (1.97)	91.2 (1.11)	40.70 (2.43)	OVA
F4	0.09 (0.01)	55.0 (0.53)	0.05 (0.01)	0.50 (0.08)	100.0 (0.00)	0.50 (0.07)	FLV
F5	0.39 (0.04)	95.3 (1.09)	0.37 (0.06)	3.78 (0.55)	ND	ND	G2/G3 OG?
F6	0.07 (0.00)	92.0 (1.15)	0.06 (0.01)	0.65 (0.01)	82.5 (0.98)	0.54 (0.02)	OVI
F7	0.48 (0.03)	51.5 (0.86)	0.27 (0.03)	2.71 (0.27)	100.0 (0.00)	2.51 (0.22)	LYZ
F8	1.16 (0.07)	42.8 (0.94)	0.52 (0.06)	5.05 (0.55)	100.0 (0.00)	5.05 (0.52)	OVT

OVT: Ovotransferrin; OVA: Ovalbumin; FLV: Flavoprotein; OVI: Ovoinhibitor; G3 OG: G3 Ovomacroglobulin; LYZ: Lysozyme; ND: Not determined. Values in the parenthesis represent standard deviation.

Table 4
Estimated yield of different protein fractions after fractionation of 500 mM supernatant.

Fractions	Freeze dried weight of fractions	Protein content (%)	Protein weight of fractions (%)	Yield (%)	Purity %	Estimated yield (%)	Identified protein
F10	0.27 (0.03)	35.3 (0.58)	0.10 (0.02)	0.97 (0.16)	100.0 (0.00)	0.97 (0.17)	LYZ
F11	0.04 (0.00)	95.0 (1.31)	0.04 (0.01)	0.39 (0.03)	100.0 (0.00)	0.39 (0.02)	OVT
F12	0.05 (0.01)	95.2 (0.99)	0.05 (0.01)	0.48 (0.13)	82.0 (1.23)	0.40 (0.12)	OVA
F13	0.04 (0.01)	93.3 (0.87)	0.04 (0.01)	0.38 (0.12)	100.0 (0.00)	0.38 (0.13)	OVA
F14	0.17 (0.02)	94.0 (0.64)	0.16 (0.03)	1.62 (0.26)	100.0 (0.00)	1.62 (0.27)	OVA

OVT: Ovotransferrin; OVA: Ovalbumin; LYZ: Lysozyme. Values in the parenthesis represent standard deviation.

total egg white protein [25]. The F6 fraction showed a protein with molecular weight of 51.9 kDa and yield of 0.54% of EWP. Ovoinhibitor, which represents only 0.5–1.5% of albumen protein, has an apparent pI of 5.1 [2]. The yield of lysozyme fraction was 2.5% of total egg white proteins, which represents over 70% of total egg white lysozyme. The final fraction represents ovotransferrin, corresponding to 5.1% of EWP at a purity of 100%.

3.3.2. Fractions obtained from 500 mM supernatant

The supernatant obtained after the second treatment with 500 mM NaCl during ovomucin preparation was further used for fractionation of proteins. The fractions (F10 and F11) obtained from cation-exchange chromatography are lysozyme and ovotransferrin representing 0.97% and 0.39% of total egg white proteins (Table 4). The three fractions (F12–F14) obtained during anion-exchange chromatography yielded 0.40%, 0.38% and 1.62% of total egg white protein respectively. All these fractions represent ovalbumin, which is the major egg white protein. Hence the study revealed that there is no need to further separate F9 fraction, since all fractions contained ovalbumin. Unlike earlier studies, this study helped to separate proteins with higher purity and yield. In earlier research, the starting material was ovomucin-free egg white prepared by precipitation in water. This method is reported to have precipitated so many other proteins along with ovomucin, especially ovalbumin and lysozyme. In the present study the raw material (500 mM supernatant) contained higher salt content and hence dialysis was required before ion-exchange chromatography; however, 100 mM supernatant was fractionated without dialysis.

4. Conclusions

Major egg white proteins were isolated from the supernatants obtained during two-step precipitation method of ovomucin preparation. The method adds to the advantage of integrating ovomucin preparation along with all the other major egg white components. Hence this can be a platform for co-extraction of major egg white proteins. The supernatant obtained after treating with 100 mM NaCl yields much of the egg white proteins as revealed by yield calculation. Most of the peaks eluted were of high purity. The yield of the major proteins obtained from the 500 mM supernatant was comparatively less, which indirectly shows that the treatment of egg white with 100 mM NaCl is more important to separate most of the major egg white proteins from ovomucin. This method we reported has a global recovery yield of 71.0%. Recovery of several proteins such as flavoproteins, lysozyme, etc. could be improved. Further study is needed to isolate and identify ovomucoid, which is one of the major egg white proteins and could not be identified in this study probably because the protein was not stained properly using coomassie blue.

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