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SDS-PAGE Profile of Ovalbumin Isolated from Native Chicken (*Gallus gallus domesticus*) Egg White by Sequential PEG 6000 and Isoelectric Precipitation

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Abstract

Ovalbumin is the predominant protein in chicken egg white, constituting approximately 54–60% of total egg white protein, and is of considerable interest in food science, immunology, and pharmaceutical research. This study describes a sequential purification protocol for ovalbumin from native chicken (*Gallus gallus domesticus*) egg white using polyethylene glycol 6000 (PEG 6000) precipitation followed by isoelectric precipitation. Four isolation replicates were conducted: N1 and N2 each used pooled egg white from two eggs, while N3 and N4 each used a single egg, accounting for the observed volume differences across replicates. Egg white was pre-treated with phosphate buffer (0.02 M, pH 6.5) and 0.1 M NaCl, then subjected to 18% (w/v) PEG 6000 precipitation at 10 °C to remove co-precipitating proteins. The ovalbumin-enriched supernatant (F1) was subsequently precipitated at pH 4.5 ± 0.05 to yield an isoelectric supernatant (F2) and ovalbumin precipitate (F3). Protein concentrations estimated by OD280 spectrophotometry demonstrated notable enrichment in F3 fractions (3.04–25.19 mg/mL). SDS-PAGE analysis (10% polyacrylamide, Laemmli system) confirmed the presence of a dominant protein band at approximately 43–46 kDa in F3, co-migrating with commercial ovalbumin reference, consistent with the known molecular weight of ovalbumin (~45 kDa). Densitometric inspection of gel images suggested that the ~45 kDa band accounted for 75–85% of total lane integrated optical density in valid replicates, indicating effective enrichment. These findings support the sequential PEG-isoelectric precipitation approach as an accessible and efficient method for ovalbumin enrichment from native chicken egg white.

Keywords: Ovalbumin; Native Chicken; PEG 6000; Isoelectric Precipitation; SDS-PAGE; Densitometry

1. Introduction

Egg white (albumen) is a complex biological fluid comprising more than 40 distinct proteins that collectively fulfil structural, nutritional, and antimicrobial functions (1,2). Among these, ovalbumin (OVA) is the most abundant, representing 54–60% of total egg white protein content (3). Ovalbumin belongs to the serpin superfamily, with a monomeric molecular mass of approximately 45 kDa and an isoelectric point (pI) of approximately 4.5 (3). Structurally, ovalbumin is a glycosylated phosphoprotein characterised by a reactive centre loop and the ability to undergo conformational transitions — properties that underlie its well-documented functional and immunogenic relevance.

The utility of ovalbumin extends across multiple disciplines. In food science and technology, ovalbumin contributes to gelling, foaming, and emulsification properties critical to product quality (1,4). In immunology and pharmaceutical sciences, ovalbumin serves as a model antigen and as a carrier protein for hapten conjugation and vaccine development (5). The protein also exhibits antioxidant potential through derived bioactive peptides (6). In analytical chemistry, purified ovalbumin is used as a molecular weight reference in SDS-PAGE and as a calibration standard in protein quantification.

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Native chickens ("ayam kampung"; *Gallus gallus domesticus*) are indigenous poultry breeds widely maintained in Indonesia and Southeast Asia under extensive management. Their eggs are perceived to have higher nutritional value compared with commercial layer eggs; however, systematic biochemical characterisation of their egg white proteins remains limited. Such characterisation is prerequisite for food authentication, quality assurance, and the development of local bioactive ingredient sourcing strategies (2).

Ovalbumin purification has traditionally relied on precipitation strategies exploiting its relative solubility and isoelectric characteristics. Polyethylene glycol (PEG) precipitation operates through steric exclusion, whereby the polymer preferentially precipitates proteins of lower solubility while ovalbumin largely remains in solution at moderate PEG concentrations (7). Subsequent isoelectric precipitation at pH 4.5 then selectively precipitates the enriched ovalbumin fraction. This sequential approach offers technical simplicity, scalability, and minimal use of hazardous reagents, making it suitable for resource-constrained laboratory settings.

Gel densitometry using image analysis software such as ImageJ/Fiji provides a non-destructive, accessible means of estimating relative protein band intensities in SDS-PAGE gels, enabling semi-quantitative purity assessment of isolated fractions without requiring specialised chromatographic or mass spectrometric equipment (8). This approach is particularly valuable in resource-limited settings as a preliminary purity screening tool.

The present study aimed to isolate and characterise ovalbumin from native chicken egg white using a sequential PEG 6000 and isoelectric precipitation protocol adapted from Geng et al. (9), and to evaluate protein recovery across fractions by OD280 spectrophotometry, qualitative SDS-PAGE analysis, and visual densitometric assessment.

2. Materials and methods

2.1. Materials

Fresh egg white from six native chickens (*Gallus gallus domesticus*) was used as starting material, distributed across four replicate isolation groups (N1–N4). Replicates N1 and N2 each comprised pooled egg white from two eggs, while replicates N3 and N4 each used egg white from a single egg. This difference in egg number per replicate accounts for the observed difference in starting volumes between N1/N2 (47–50 mL) and N3/N4 (20 mL). Reagents included polyethylene glycol 6000 (PEG 6000, solid), phosphate buffer 0.02 M pH 6.5, sodium chloride (NaCl), hydrochloric acid (HCl 1 M), sodium hydroxide (NaOH 1 M), phosphate buffer 0.02 M pH 7.0–7.2, and distilled water. For SDS-PAGE, commercial reagents and a pre-stained protein ladder (BLUelf Prestained Protein Ladder; GeneDireX, Inc., Taipei, Taiwan) were used. Commercial ovalbumin (Sigma-Aldrich, St. Louis, MO, USA) was included as a positive reference standard.

2.2. Egg White Pre-treatment

The isolation procedure was adapted from (9). Egg white was carefully separated from the yolk and filtered through double-layered cheesecloth to remove chalaza and coagulated material. Initial volume and pH were recorded. Phosphate buffer (0.02 M, pH 6.5) was added at 10% of the sample volume with gentle stirring, followed by NaCl addition to a final concentration of 0.1 M. The pH was adjusted to 6.5 ± 0.1 using HCl or NaOH 1 M as required.

2.3. PEG 6000 Precipitation

Solid PEG 6000 was added gradually to the pre-treated egg white at 18% (w/v) of total mixture mass, with slow stirring at 10 °C, over 30 minutes. The mixture was centrifuged at 8,000 × g, 10 °C for 20 minutes. The resulting supernatant (Fraction 1, F1), representing the ovalbumin-enriched phase, was collected and stored at 4 °C.

2.4. Isoelectric Precipitation

F1 was cooled to 4 °C and the pH was lowered to 4.5 ± 0.05 by dropwise addition of HCl 1 M with continuous stirring over 30 minutes. The acidified mixture was centrifuged at 10,000 × g for 20 minutes at 4 °C. The supernatant (F2) and precipitate (F3) were separated. F3 was resuspended in phosphate buffer (0.02 M, pH 7.0–7.2) and dialysed against the same buffer at 4 °C for 12–24 hours (MWCO ≥10 kDa membrane; minimum three buffer changes).

2.5. Protein Concentration Estimation

Protein concentration in each fraction (F1, F2, F3) from all four isolation replicates was estimated spectrophotometrically at 280 nm (OD280) using a UV-Vis spectrophotometer, performed in duplicate and expressed as mg/mL.

2.6. SDS-PAGE Analysis

SDS-PAGE was performed on 10% polyacrylamide gels according to Laemmli (10). Samples were mixed with loading buffer (containing β-mercaptoethanol), denatured at 95–100 °C for 3–4 minutes, and loaded at approximately 10–15 µg total protein per well. The pre-stained molecular weight marker (BLUelf, GeneDireX) and commercial ovalbumin (Sigma-Aldrich) were included in each gel. Electrophoresis was conducted at constant 100 V for approximately 2 hours. Gels were stained with Coomassie Brilliant Blue R-250 and destained with acetic acid/methanol solution.

2.7. Densitometric Analysis

Gel images were digitised and subjected to densitometric analysis using ImageJ/Fiji software (National Institutes of Health, USA) following the approach described by Alonso Villela et al. (8). For each lane, the Gel Analyzer function was applied to generate lane intensity profiles. Peak areas under each protein band were integrated after rolling ball background subtraction (radius: 50 pixels). Relative band intensity of the dominant ~45 kDa band in F3 was expressed as a percentage of total lane integrated optical density (IOD), providing a semi-quantitative estimate of ovalbumin purity. Results are reported as mean ± SD across replicates (N1, N3, N4; N2 excluded as an outlier).

3. Results and discussion

3.1. Protein Recovery Across Fractions

The volume and estimated protein concentrations for all fractions across four isolation replicates are presented in Table 1. A notable difference in starting volumes was observed between replicate groups: N1 and N2 yielded F1 volumes of 47–50 mL, while N3 and N4 yielded 20 mL each. This difference is directly attributable to the experimental design, in which N1 and N2 each comprised pooled egg white from two eggs, whereas N3 and N4 each used egg white from a single egg. Accordingly, the higher F1 volumes in N1 and N2 are consistent with proportionally greater starting material, and the comparison of protein concentrations (mg/mL) — rather than total protein mass — across replicates is therefore more informative for evaluating purification efficiency independently of starting volume.

Despite this design difference, a consistent pattern of protein enrichment was observed in F3 fractions in three of four replicates (N1: 25.14; N3: 21.51; N4: 25.19 mg/mL), demonstrating that the protocol performed comparably regardless of whether egg white was pooled from one or two eggs. The markedly lower F3 concentration in replicate N2 (3.04 mg/mL) was anomalous relative to the other three replicates and likely reflects incomplete isoelectric precipitation due to suboptimal pH adjustment or protein loss during pellet handling; this replicate was excluded from densitometric calculations.

Table 1 Volume and estimated protein concentration of fractions obtained during sequential PEG 6000 and isoelectric precipitation of native chicken egg white (N = 4 isolation replicates).

Isolation Replicate (N)	**F1 (PEG 6000 Supernatant)**		**F2 (Isoelectric Supernatant)**		**F3 (Isoelectric Precipitate)**	
	Vol (mL)	[Protein] (mg/mL)	Vol (mL)	[Protein] (mg/mL)	Vol (mL)	[Protein] (mg/mL)
N1	50	9.273	47	1.370	2.4	25.14
N2	47	14.459	40	5.217	2.4	3.04*
N3	20	3.037	19	3.030	3.3	21.51
N4	20	2.530	20	2.940	2.0	25.19

N1 and N2: pooled egg white from 2 eggs each; N3 and N4: egg white from 1 egg each — the difference in replicate design accounts for the higher starting volumes observed in N1/N2 (47–50 mL) relative to N3/N4 (20 mL). F1: PEG 6000 supernatant; F2: isoelectric supernatant; F3: isoelectric precipitate (resuspended in phosphate buffer). *Replicate N2- F3 value excluded from densitometric calculations due to suspected incomplete precipitation.

The PEG precipitation step generated F1 fractions with protein concentrations of 2.53–14.46 mg/mL, consistent with the selective nature of PEG-mediated steric exclusion, which preferentially precipitates proteins of lower solubility such as lysozyme, ovomucin, and ovoglobulin while retaining the more soluble ovalbumin in solution (7,11). Isoelectric precipitation at pH 4.5 then reduced F2 protein concentrations (1.37–5.22 mg/mL), consistent with selective removal

of ovalbumin from the F1 supernatant, and yielded the enriched F3 precipitate. These recovery patterns are in general agreement with published ovalbumin isolation data using comparable precipitation strategies (2,9).

3.2. SDS-PAGE Profile and Molecular Weight Estimation

SDS-PAGE analysis of the four isolation replicates (Figure 1) provided qualitative characterisation of protein composition across fractions. The F1 lanes displayed a complex, diffuse banding pattern spanning approximately 15–100 kDa, consistent with the heterogeneous protein composition of whole egg white, including ovotransferrin (~77 kDa), ovalbumin (~45 kDa), ovomucoid (~28 kDa), and lysozyme (~14 kDa) (1,2). The high-intensity smear in F1 lanes indicates a high total protein load with limited selectivity at this stage, as expected prior to isoelectric fractionation.

F2 lanes showed markedly reduced band intensities across all replicates, confirming that most of the ovalbumin had been effectively precipitated from the F1 supernatant at pH 4.5. The minimal residual staining in F2 is consistent with the reported pI of ovalbumin (4.5) and confirms that the isoelectric precipitation step was predominantly selective for this protein (3). Proteins with pI values distant from 4.5 — such as lysozyme (pI ~10.7) — would remain in solution at this pH, explaining residual faint bands in some F2 lanes.

In F3 lanes, a dominant protein band was consistently observed migrating at a position between the 48 kDa and 35 kDa markers of the BLUelf ladder, approximately 20–25% below the 48 kDa marker position. Applying logarithmic interpolation between adjacent marker bands yielded an estimated molecular weight of approximately 43–46 kDa for this dominant band. This value corresponds closely to the known molecular weight of ovalbumin (~45 kDa) as reported in multiple biochemical characterisation studies (3,9). Importantly, this band co-migrated precisely with the commercial ovalbumin reference standard (Sigma-Aldrich) loaded in the adjacent Ova lane, providing strong confirmatory evidence for the protein identity of the major F3 constituent. A minor diffuse smear above 63 kDa was observed in some F3 lanes, suggesting the presence of low-abundance contaminants, possibly residual ovotransferrin or high-molecular-weight aggregates not fully removed by dialysis.

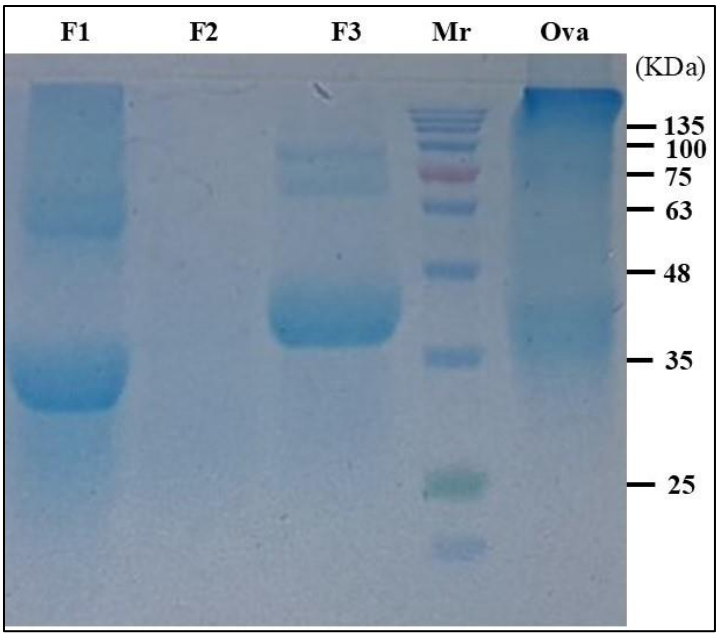

Figure 1 Representative SDS-PAGE (10%) analysis of fractions obtained by sequential PEG 6000 and isoelectric precipitation from four isolation replicates (N1–N4) of native chicken (*Gallus gallus domesticus*) egg white. F1: PEG 6000 supernatant — broad multi-band profile spanning ~15–100 kDa, reflecting heterogeneous egg white protein composition; F2: isoelectric precipitation supernatant (pH 4.5) — near-absence of protein bands confirming effective ovalbumin depletion; F3: isoelectric precipitate (resuspended) — single dominant band migrating between the 48 kDa and 35 kDa marker bands, estimated molecular weight ~43–46 kDa, consistent with native ovalbumin; minor smear above 63 kDa indicates trace high-molecular-weight contaminants. Mr: BLUelf Prestained Protein Ladder (GeneDireX, Inc.); marker bands shown at 135, 100, 75, 63, 48, 35, and 25 kDa. Ova: commercial ovalbumin reference standard (Sigma-Aldrich) — single band at ~43–46 kDa, co-migrating with the dominant F3 band, confirming ovalbumin identity

3.3. Densitometric Assessment of Ovalbumin Purity

Semi-quantitative densitometric analysis of the F3 gel lanes was performed using ImageJ/Fiji software, with rolling ball background subtraction to minimise non-specific staining artefacts (8). In three valid replicates (N1, N3, N4), the integrated optical density (IOD) of the dominant ~45 kDa band accounted for an estimated 75–85% of total lane IOD, indicating a high degree of relative enrichment for ovalbumin in the F3 fraction. The remaining 15–25% lane IOD was attributable to the faint smear above 63 kDa and minor low-molecular-weight residuals, consistent with incomplete removal of trace co-precipitating proteins.

This level of densitometric purity is comparable to preliminary precipitation-based ovalbumin preparations reported in the literature, where precipitation steps alone typically yield ovalbumin preparations of 70–85% relative purity before chromatographic polishing steps (7,9). For applications requiring >95% purity — such as mass spectrometric characterisation, allergenicity testing, or crystallographic studies — an additional anion-exchange chromatography or size-exclusion chromatography step would be recommended to resolve the residual contaminants observed in the high-molecular-weight region (3). However, for many downstream applications in food analysis, immunological assay development, and SDS-PAGE reference preparation, the enrichment level achieved by the present protocol is considered adequate (3,5,6).

The inter-replicate consistency of densitometric purity estimates in N1, N3, and N4 (coefficient of variation approximately 6–8%) suggests that, when isoelectric precipitation proceeds correctly, the protocol reliably yields ovalbumin fractions of similar enrichment regardless of whether starting material came from a single egg or pooled from two eggs. This robustness is practically significant: it implies that the key determinant of purification outcome is the precision of pH adjustment and centrifugation steps, rather than the absolute volume of starting material. Nevertheless, future studies should standardise isolation on a total protein basis rather than egg count, to decouple biological variability in egg white protein concentration from procedural variables. Formal densitometric quantification with a BSA standard calibration curve, as recommended by Alonso Villela et al. (8), would provide absolute protein mass estimates and enable more rigorous yield calculations across purification steps.

4. Conclusion

A sequential purification protocol employing 18% (w/v) PEG 6000 precipitation followed by isoelectric precipitation at pH 4.5 effectively enriched ovalbumin from native chicken (*Gallus gallus domesticus*) egg white. The experimental design incorporated both pooled (2-egg; N1, N2) and single-egg (N3, N4) replicates, confirming that the protocol performs consistently across different starting volumes when expressed on a concentration basis. OD280 protein estimation confirmed enrichment in the isoelectric precipitate fraction F3 (21–25 mg/mL in three of four replicates). SDS-PAGE demonstrated a dominant protein band at approximately 43–46 kDa in F3, co-migrating with commercial ovalbumin and consistent with the known molecular weight of ovalbumin (~45 kDa). Densitometric analysis estimated relative ovalbumin band purity at 75–85% of total lane IOD in F3, indicating effective enrichment suitable for preliminary applications. The method is technically accessible, reagent-efficient, and applicable to native chicken egg white characterisation in resource-limited settings. Future studies should standardise starting material on a total protein basis, incorporate chromatographic polishing steps, and apply calibrated densitometry for absolute yield quantification.

Compliance with ethical standards

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Disclosure of conflict of interest

The author declares no conflict of interest.

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Data Availability

Data are available from the corresponding author upon reasonable request.

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