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Time-dependent proteolytic degradation of chicken egg-white proteins by Proteinase K: qualitative and semi-quantitative evidence from 12.5% SDS-PAGE

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Abstract

Egg white is a protein-rich matrix dominated by ovalbumin, ovotransferrin, ovomucoid and lysozyme, several of which are clinically important food allergens. Controlled proteolysis can reshape the molecular-weight (MW) distribution of egg-white proteins and is widely used to generate functional hydrolysates and to attenuate allergenicity. This study qualitatively and semi-quantitatively evaluated the time-dependent digestion of diluted chicken egg white by Proteinase K (PK) using 12.5% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Diluted egg white (1 mg/mL) in Tris-HCl-$MgCl_2$ buffer was incubated at 50 °C for 30, 60 and 90 min, without PK or with PK at 200 µg/mL. Reactions were stopped by heating (95 °C, 5 min), and supernatants were resolved on 12.5% SDS-PAGE alongside a 25-245 kDa ladder. A semi-logarithmic ladder calibration (R^2 = 0.99 across the 25-100 kDa resolving range) was used to estimate apparent band masses. No-PK controls retained prominent bands at apparent ~95-120 kDa, ~70-78 kDa (ovotransferrin region) and a dominant ~45-50 kDa band (ovalbumin region). PK-treated lanes showed time-dependent loss of these intact bands, with substantial depletion by 60 min and near-complete loss of major Coomassie-detectable proteins by 90 min, whereas heating at 50 °C without PK did not reproduce this depletion. The results demonstrate efficient, enzyme-dependent proteolysis of major egg-white proteins, including the principal allergens ovalbumin and ovotransferrin, supporting PK digestion as a simple model for producing egg-white hydrolysates and a candidate strategy for allergen-epitope reduction that warrants confirmation by densitometry, peptide profiling and IgE-binding assays.

Keywords: Egg white; Proteinase K; SDS-PAGE; Ovalbumin; Food allergy; Protein hydrolysate

1. Introduction

Chicken egg white is an accessible and inexpensive protein matrix that is widely studied in food science, biotechnology and biomaterials research. It consists mainly of water and protein, the major proteins being ovalbumin (~54%), ovotransferrin (~12%), ovomucoid (~11%) and lysozyme (~3.5%), with smaller contributions from ovoglobulins, ovomucin, avidin and numerous minor proteins [1, 2]. These proteins differ markedly in abundance, glycosylation, disulphide-bond content, thermal stability and susceptibility to enzymatic hydrolysis, which makes egg white a useful substrate for studying protein denaturation and proteolysis [2].

Proteolysis is routinely exploited to modify the solubility, foaming, emulsifying and allergenic properties of egg-white proteins [3, 4] and to release bioactive peptides. Egg-white-derived peptides have been associated with antimicrobial, antihypertensive, antioxidant, immunomodulatory and gut-health-related activities, although the activity depends strongly on enzyme specificity, hydrolysis conditions and peptide sequence [5, 6].

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Egg is also one of the most frequent causes of IgE-mediated food allergy in infants and young children, and egg white is the principal source of allergens. Four proteins are recognised as the major egg-white allergens: ovomucoid (Gal d 1), ovalbumin (Gal d 2), ovotransferrin/conalbumin (Gal d 3) and lysozyme (Gal d 4) [7, 8]. Ovomucoid is regarded as the dominant and most clinically significant allergen because its extensive glycosylation and disulphide bonds confer notable resistance to heat and to digestive proteases, whereas ovalbumin is the most abundant allergen and a key diagnostic marker [7, 9]. Food-processing interventions, including thermal treatment, glycation, high-pressure processing and especially enzymatic hydrolysis, can reduce egg allergenicity by disrupting linear and/or conformational IgE-binding epitopes [7, 10]. Broad-specificity proteases are particularly effective at dismantling such epitopes, and low-IgE-binding egg-white hydrolysates can additionally modulate T-cell cytokine responses [10, 11].

Proteinase K (PK) is a broad-specificity, subtilisin-like serine protease from Parengyodontium album (formerly Tritirachium album) that preferentially cleaves peptide bonds adjacent to hydrophobic and aromatic residues and remains active over a wide range of conditions [12, 13]. It is widely used in molecular biology for protein digestion and nuclease inactivation. Moderate incubation temperatures (50-60 °C) can enhance digestion by promoting substrate unfolding and increasing protease activity, and the reaction is conveniently terminated by heat inactivation of the enzyme [12, 13].

SDS-PAGE remains a rapid, low-cost method for monitoring protein degradation. In the Laemmli discontinuous buffer system, denaturing and reducing conditions normalise the charge-to-mass ratio so that proteins separate primarily by apparent molecular mass [14]. For egg white, major Coomassie-stained bands are expected near ovotransferrin (~76-78 kDa), ovalbumin (~45 kDa), ovomucoid (~28 kDa) and lysozyme (~14.3 kDa), although assignment by SDS-PAGE alone remains provisional because glycosylation, aggregation and co-migration can affect mobility [1].

The present work systematically interprets a 12.5% SDS-PAGE gel of diluted egg white treated, or not treated, with PK at 50 °C for 30, 60 and 90 min. The primary objective was to determine whether PK treatment produces a time-dependent reduction of intact egg-white protein bands relative to no-enzyme controls. The secondary objective was to evaluate, on this basis, the potential of PK digestion both for producing egg-white hydrolysates and for reducing egg-white allergenicity.

2. Materials and methods

2.1. Materials

Fresh chicken egg white was diluted to 1 mg/mL (based on OD_{280}) in Tris-HCl-$MgCl_2$ buffer. A Proteinase K working solution was freshly prepared at 2 mg/mL in the same buffer. A pre-stained protein molecular-weight ladder (BLUelf, GeneDireX, Inc.; range 25-245 kDa) was used as the MW reference.

2.2. Experimental design and incubation

For each tube, 190 µL of diluted egg-white sample was pre-equilibrated at 50 °C for 5 min. Control tubes received 10 µL Tris-HCl-$MgCl_2$ buffer, whereas treatment tubes received 10 µL PK working solution, giving a final PK concentration of 200 µg/mL in a 200 µL reaction. Control and PK-treated samples were incubated at 50 °C for 30, 60 and 90 min. A 4 °C no-PK buffer control and an untreated egg-white sample were also included. Tubes were tightly capped to limit evaporation.

2.3. Reaction termination and clarification

Reactions were stopped by transferring tubes to a 95 °C heating block for 5 min. Samples were cooled on ice for 2 min and centrifuged at 10,000 × *g* for 5 min at 4 °C. Supernatants were transferred to fresh tubes and analysed by SDS-PAGE.

2.4. SDS-PAGE

Protein patterns were resolved on 12.5% SDS-PAGE according to the Laemmli system [14]. A 12.5% resolving gel is suitable for proteins in the approximate 10-100 kDa range, which encompasses most major egg-white proteins. Gels were stained with a Coomassie-type protein stain.

2.5. Lane assignment

Lane loading is summarised in Table 1.

Table 1 SDS-PAGE lane assignment and interpretive purpose.

Lane	Sample condition	Interpretive purpose
1	Untreated egg white	Baseline protein pattern
2	Egg white + buffer, no PK, 4 °C	Low-temperature no-enzyme control
3	Egg white + buffer, no PK, 50 °C / 30 min	Thermal/buffer control, 30 min
4	Egg white + buffer, no PK, 50 °C / 60 min	Thermal/buffer control, 60 min
5	Egg white + buffer, no PK, 50 °C / 90 min	Thermal/buffer control, 90 min
6	Egg white + buffer + PK, 50 °C / 30 min	Proteolysis, 30 min
7	Egg white + buffer + PK, 50 °C / 60 min	Proteolysis, 60 min
8	Egg white + buffer + PK, 50 °C / 90 min	Proteolysis, 90 min
9	Egg white + buffer, no PK, 50 °C / 90 min	Second 90-min no-enzyme control
Mr	Protein ladder (25-245 kDa)	Molecular-weight reference

2.6. Gel interpretation and apparent-MW estimation

Apparent molecular masses were estimated from a semi-logarithmic ladder calibration. The migration positions of the nine ladder bands (245, 180, 135, 100, 75, 63, 48, 35 and 25 kDa) were measured from the gel image, and $\log_{10}$(MW) was regressed against relative migration. Within the 25-100 kDa resolving range the calibration was linear (coefficient of determination $R^2 = 0.99$), and the apparent masses of sample bands were interpolated from this curve (full procedure in Supplementary File S1). Band identity was inferred from the expected masses of major egg-white proteins and should be regarded as provisional until confirmed by mass spectrometry, immunoblotting or targeted proteomics.

3. Results

3.1. Overall gel quality and MW range

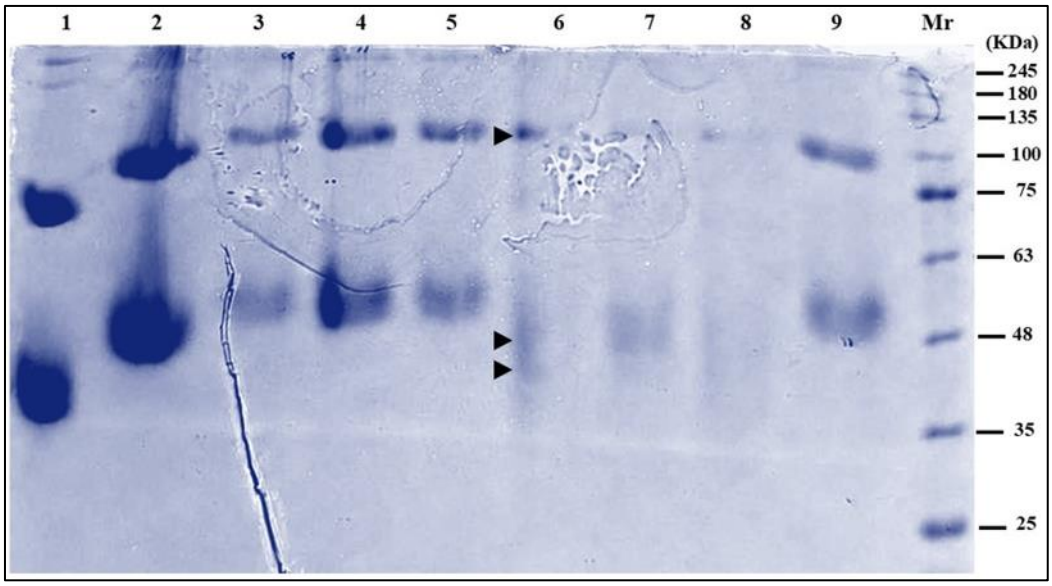

Figure 1 12.5% SDS-PAGE profile of egg-white samples. Lane 1, untreated egg white; lane 2, egg white + Tris-HCl-$MgCl_2$ buffer, no PK, 4 °C; lanes 3-5, no-PK controls at 50 °C for 30, 60 and 90 min; lanes 6-8, PK-treated samples at 50 °C for 30, 60 and 90 min; lane 9, second no-PK control at 50 °C for 90 min; Mr, molecular-weight ladder (25-245 kDa). Arrowheads indicate representative bands (~100 kDa and ~45-50 kDa regions) that are reduced in PK-treated lanes

The 12.5% gel resolved the ladder bands from approximately 25 to 245 kDa, with the best linear resolution between 25 and 100 kDa. Egg-white lanes showed Coomassie-stained protein mainly between ~35 and ~120 kDa, plus weaker low-MW staining near the gel front. Some background staining, lane-to-lane intensity variation and localised gel distortion were present, so the data are most appropriately interpreted as qualitative and semi-quantitative evidence rather than full densitometry (Figure 1).

3.2. Pattern in untreated and no-PK control lanes

The untreated and no-PK control lanes retained distinct bands after incubation. Lane 1 showed bands in the apparent ~70-78 kDa and ~40-50 kDa regions, consistent with ovotransferrin and ovalbumin, respectively. The 4 °C control (lane 2) showed strong staining, particularly in the ~45-50 kDa region, indicating good preservation of the major egg-white proteins. The 50 °C no-PK controls (lanes 3-5, 9) retained detectable protein, demonstrating that heating at 50 °C alone did not eliminate intact Coomassie-detectable egg-white proteins. Lanes 4, 5 and 9 showed clear staining in the upper/mid region, whereas lane 3 was less intense; this variation probably reflects pipetting/loading differences, partial precipitation before supernatant transfer, or local staining heterogeneity. Importantly, the repeated 90-min no-PK control (lane 9) retained its major bands, supporting an enzyme-dependent rather than purely thermal mechanism for band loss in PK-treated lanes.

3.3. Time-dependent effect of Proteinase K

PK treatment substantially altered the egg-white profile. By 30 min (lane 6), the prominent ~45-50 kDa band and the upper bands were already reduced relative to controls, with faint residual mid-MW staining indicating partial digestion. By 60 min (lane 7), the major intact bands were further depleted, with diffuse low-intensity staining consistent with the accumulation of smaller fragments below the well-resolved range of the gel. By 90 min (lane 8), few high- or mid-MW bands remained, indicating extensive digestion of intact egg-white proteins. Qualitative scoring is summarised in Table 2.

Table 2 Qualitative scoring of band changes across treatments

Condition	~95-120 kDa region	~45-50 kDa (ovalbumin)	Low-Mr fragments	Interpretation
Untreated / no-PK controls	Present; variable	Present; prominent	Weak-moderate	Major proteins retained
PK, 30 min	Reduced	Reduced; faint residual	Weak/faint	Partial digestion
PK, 60 min	Strongly reduced	Strongly reduced	Diffuse weak	Substantial digestion
PK, 90 min	Nearly absent	Nearly absent	Weak low-Mr	Extensive digestion

3.4. Apparent molecular-mass estimation and protein assignment

Using the semi-logarithmic ladder calibration (R^2 = 0.99, 25-100 kDa), the apparent masses of the main bands and their tentative assignments are given in Table 3. The dominant PK-sensitive band migrated at an apparent ~45-50 kDa, consistent with ovalbumin (true mass ~45 kDa), the most abundant egg-white protein and a major allergen. A band at apparent ~70-78 kDa (clearest in lane 1) is consistent with ovotransferrin. A persistent upper band migrated at an apparent ~95-120 kDa; because this exceeds the expected mass of ovotransferrin, it most likely represents high-MW egg-white components (e.g. ovomacroglobulin/ovomucin-derived or aggregated/incompletely dissociated material) and/or anomalous migration associated with the localised gel distortion, and its assignment is therefore deliberately conservative. Faint staining near ~28 kDa is compatible with ovomucoid, while lysozyme (~14.3 kDa) migrates at or below the 25 kDa marker, near the dye front, and was not reliably resolved on this 12.5% gel. All assignments remain provisional pending mass spectrometry or immunoblotting.

Table 3 Apparent molecular masses of major bands estimated from the ladder calibration, with tentative assignments.

Band region	Apparent mass	Tentative assignment (allergen)
Upper band	~95-120 kDa	High-MW egg-white protein / aggregated or co-migrating material
Upper-mid band	~70-78 kDa	Ovotransferrin (Gal d 3)
Dominant mid band	~45-50 kDa	Ovalbumin (Gal d 2) - PK-sensitive
Lower band	~28 kDa	Ovomucoid (Gal d 1)
Near dye front	~14 kDa	Lysozyme (Gal d 4) - not resolved

4. Discussion

The SDS-PAGE pattern supports a clear conclusion: PK treatment at 50 °C produced time-dependent degradation of major chicken egg-white proteins. No-PK controls retained visible bands after incubation at 50 °C, whereas PK-treated lanes progressively lost the major high- and mid-MW bands between 30 and 90 min. This behaviour is consistent with the broad specificity of PK and the tendency of moderate heating to expose additional cleavage sites in folded or partially folded substrates [12, 13].

The most informative observation is the loss of the apparent ovalbumin band (~45-50 kDa). Ovalbumin is the most abundant egg-white protein, so its marked reduction indicates that the enzyme-to-substrate ratio used here was sufficient to digest a large fraction of the soluble protein within the incubation period. The parallel reduction of the ~70-78 kDa (ovotransferrin) region indicates digestion of upper-range proteins as well. The near-disappearance of major bands by 90 min shows that 200 μg/mL PK at 50 °C is a strong digestion condition for a 1 mg/mL egg-white substrate.

The appearance of only faint low-MW material in PK-treated lanes should not be read as an absence of peptides. Small peptides stain poorly with Coomassie and frequently migrate with, or ahead of, the dye front in a 12.5% gel. The loss of intact bands therefore most likely reflects conversion of large proteins into peptides that are not efficiently retained or visualised in this system; tricine-SDS-PAGE, LC-MS/MS or free-amino-group (OPA/TNBS) assays would be better suited to quantify the hydrolysis products.

Heating at 50 °C alone did not reproduce the protein depletion seen with PK. Egg-white proteins differ in thermal stability: ovotransferrin is comparatively heat-sensitive, whereas ovalbumin and ovomucoid are relatively stable [7, 9]. The retention of major bands in the 50 °C no-PK controls, especially the 90-min lane, confirms that the dominant effect was enzymatic proteolysis rather than thermal denaturation or precipitation, although the lane-to-lane variation indicates that sample handling and loading should be tightened in future replicate experiments.

4.1. Relevance to egg-white allergenicity

The four major egg-white allergens - ovomucoid (Gal d 1), ovalbumin (Gal d 2), ovotransferrin (Gal d 3) and lysozyme (Gal d 4) - elicit IgE-mediated reactions through linear and conformational epitopes [7, 8]. A central principle of allergen mitigation is that extensive proteolysis fragments these epitopes, lowering IgE-binding capacity; broad-specificity proteases are especially effective because they cleave at many internal sites, and the resulting low-IgE-binding hydrolysates can additionally rebalance Th1/Th2 cytokine responses [10, 11]. In the present gel, PK abolished the intact ovalbumin and ovotransferrin bands within 60-90 min, which is precisely the kind of backbone fragmentation expected to disrupt the conformational and many of the linear epitopes carried by these allergens. This provides a reasonable mechanistic basis for proposing that PK treatment of egg white could reduce its allergenicity.

Two important caveats temper this prospect. First, ovomucoid (Gal d 1), the dominant clinical allergen, is heavily glycosylated and disulphide-stabilised, which makes it comparatively resistant to proteases and heat [7, 9]; on a non-reducing or partially denaturing basis it may persist longer than ovalbumin, so demonstrating ovomucoid epitope loss specifically (rather than overall band loss) is essential. The mild thermal unfolding at 50 °C used here, combined with the reducing conditions of SDS-PAGE sample preparation, is expected to assist PK access to such resistant substrates. Second, PK is not a food-grade enzyme and would require validated removal or inactivation before any food application; the present work is therefore best framed as a biochemical model of broad-spectrum proteolysis and an allergen-reduction screening tool rather than a ready food process. Even so, the findings are directly useful for method development, allergen-reduction screening, protein-stability assessment and teaching demonstrations of enzyme-dependent hydrolysis.

4.2. Limitations

Several limitations should be acknowledged. The evidence derives from a single gel image, precluding statistical analysis; band intensities were estimated rather than measured by densitometry; protein identity was inferred from apparent mass only; the design lacks a no-substrate PK-only lane, which would clarify whether faint ~25-35 kDa staining in treated lanes is residual PK (~28.9 kDa) or substrate-derived fragments; and peptide formation and degree of hydrolysis were not quantified.

4.3. Future prospects

To strengthen and extend this work we recommend: (i) at least three independent biological replicates with densitometric quantification of the ovalbumin and ovotransferrin bands; (ii) inclusion of a PK-only lane and post-incubation protein normalisation; (iii) degree-of-hydrolysis (OPA/TNBS) measurement and peptide-size profiling by tricine-SDS-PAGE or LC-MS/MS; (iv) targeted assessment of allergenicity, in particular IgE-binding ELISA and immunoblotting with sera from egg-allergic individuals, focusing on ovomucoid and ovalbumin epitopes, ideally before and after simulated gastrointestinal digestion; and (v) comparison of PK with food-grade proteases (e.g. alcalase, papain, pepsin) to benchmark allergen-reduction efficiency against process feasibility. Such data would convert the present qualitative observation into quantitative evidence for PK-mediated allergen-epitope reduction in egg white.

5. Conclusion

Proteinase K treatment of diluted chicken egg white at 50 °C caused progressive, enzyme-dependent degradation of the major Coomassie-detectable proteins on 12.5% SDS-PAGE. Relative to no-PK controls, PK-treated samples showed a clear decrease in the apparent ovotransferrin- and ovalbumin-region bands by 30 min, substantial depletion by 60 min and near-complete loss of major intact bands by 90 min, whereas heating alone did not reproduce this effect. Because the bands abolished by PK include the principal egg-white allergens ovalbumin and ovotransferrin, PK digestion is a promising model both for producing egg-white hydrolysates and for screening allergen-epitope reduction, provided the findings are confirmed by replicate densitometry, peptide-level analysis and IgE-binding assays.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest.

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