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Isolation, Characterization, and Antioxidant Activity of Phosvitin from Duck Egg Yolk

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Abstract: Phosvitin is a highly phosphorylated protein found in egg yolk and is known for its strong metal-binding capacity and antioxidant activity. This study aimed to isolate and characterize phosvitin from duck egg yolk (*Anas platyrhynchos domesticus*) and evaluate its antioxidant potential. Phosvitin was isolated using a modified extraction method and characterized using one-dimensional gel electrophoresis (SDS-PAGE) and Fourier Transform Infrared (FTIR) spectroscopy. Antioxidant activity was determined using the DPPH radical scavenging assay. The results showed that phosvitin was successfully isolated, indicated by a dominant protein band at approximately 50 kDa. The antioxidant activity exhibited a strong inhibitory effect with an IC_{50} value of 33.65 ppm, indicating very strong antioxidant activity. FTIR analysis confirmed the presence of characteristic functional groups, including amide and phosphate groups, which are typical of phosphoproteins. These findings suggest that phosvitin from duck egg yolk may be a promising natural antioxidant for food and pharmaceutical applications.

Keywords: phosvitin; duck egg yolk; antioxidant activity; SDS-PAGE; FTIR

Introduction

Egg yolk is a rich source of bioactive compounds, particularly proteins that exhibit important functional and biological properties. These proteins have gained increasing attention due to their potential applications in food, nutraceutical, and pharmaceutical industries. Recent studies have highlighted that egg-derived proteins possess antioxidant, antimicrobial, and metal-chelating activities, making them valuable as natural functional ingredients (Sriasih et al., 2025; Nimalaratne & Wu, 2020; Abeyrathne et al., 2021; Hu et al., 2022). Among these proteins, phosvitin is considered one of the most unique due to its exceptionally high phosphorylation level and biological functionality (Zhang et al., 2020; Wang et al., 2021).

Phosvitin is characterized by a high proportion of serine residues, most of which are phosphorylated, enabling strong binding with metal ions such as iron and calcium (Xu et al., 2022; Li et al., 2021). This structural feature plays a critical role in its antioxidant mechanism, particularly in inhibiting lipid oxidation by chelating pro-oxidant metal ions and preventing free radical formation (Wang et al., 2021; Zhang et al., 2022). In addition, phosvitin has been reported to exhibit multiple bioactivities, including antimicrobial, anti-inflammatory, and mineral-binding properties, which further enhance its potential as a functional biomolecule (Abeyrathne et al., 2021; Huang et al., 2022; Chen et al., 2023).

The antioxidant activity of phosvitin has been widely studied using various in vitro methods, with the DPPH radical scavenging assay among the most commonly used techniques (Shahidi & Zhong, 2020; Zou et al., 2021). The ability of phosvitin to neutralize free radicals is strongly associated with its phosphate groups and its capacity to stabilize reactive species (Wang et al., 2021; Liu et al., 2022). This makes phosvitin a promising natural antioxidant for replacing synthetic antioxidants, which are increasingly restricted due to potential health concerns (Hu et al., 2022; Chen et al., 2023).

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Most previous studies have focused on phosvitin derived from chicken egg yolk, which is widely available and commonly used as a model protein (Nimalaratne & Wu, 2020; Zhang et al., 2020). However, duck egg yolk represents an alternative source with distinct physicochemical properties, including higher lipid content and different protein composition, which may influence the structure and functionality of phosvitin (Liu et al., 2021; Sun et al., 2022). Duck eggs are widely consumed in Indonesia and other Asian countries, yet studies on phosvitin from duck egg yolk remain limited, particularly regarding antioxidant activity and structural characterization (Li et al., 2021; Xu et al., 2022).

Understanding the structural characteristics of phosvitin is essential to evaluating its functional properties. Protein characterization techniques such as Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) are widely used to determine molecular weight and assess protein purity (Laemmli, 1970; Tang et al., 2021). In addition, Fourier Transform Infrared (FTIR) spectroscopy is commonly applied to identify functional groups and analyze protein secondary structure, providing insight into the relationship between structure and function (Kong & Yu, 2020; Zhao et al., 2022). These analytical techniques are essential for confirming the successful isolation and characterization of phosvitin.

In addition to structural characterization, evaluating antioxidant activity is crucial to determine the functional potential of phosvitin. The DPPH radical scavenging method is widely used due to its simplicity, sensitivity, and reliability in measuring free radical inhibition (Shahidi & Zhong, 2020; Zou et al., 2021). The IC_{50} value obtained from this assay is commonly used to classify antioxidant strength and compare the effectiveness of different bioactive compounds (Liu et al., 2022).

This study aims to isolate phosvitin from duck egg yolk (*Anas platyrhynchos domesticus*), characterize its protein profile using SDS-PAGE and FTIR, and evaluate its antioxidant activity using the DPPH method. This study is expected to provide updated scientific evidence regarding the structural characteristics and antioxidant potential of phosvitin from duck egg yolk, thereby contributing to the development of natural antioxidant sources for food and pharmaceutical applications.

Method

Research Design

This study employed a laboratory experimental design to isolate and characterize phosvitin from duck egg yolk (*Anas platyrhynchos domesticus*) and to evaluate its antioxidant activity.

Materials and Instruments

Fresh duck eggs were used as the primary raw material for phosvitin extraction. Additional reagents included sodium chloride (NaCl), ethanol (85%), methanol (p.a.), 2,2-diphenyl-1-picrylhydrazyl (DPPH), potassium bromide (KBr), and distilled water.

The instruments used included a centrifuge, a magnetic stirrer, a UV-Vis spectrophotometer, an electrophoresis apparatus for SDS-PAGE analysis, and a Fourier Transform Infrared (FTIR) spectrophotometer.

Phosvitin Isolation

Phosvitin was isolated from duck egg yolk using the method described by Ko et al. (2011) with slight modifications. Fresh duck egg yolk was manually separated and homogenized with distilled water, then adjusted to pH 7 and centrifuged to obtain the yolk granule fraction. The precipitated granules were then treated with 85% ethanol to remove lipids and other non-protein components. After centrifugation, the defatted precipitate was homogenized with 10% NaCl solution and adjusted to acidic conditions prior to further centrifugation. The resulting supernatant was filtered and dialyzed against 0.9% NaCl using cellulose tubing to remove residual salts and low-molecular-weight compounds. Finally, the dialyzed sample was freeze-dried to obtain purified phosvitin in powder form.

SDS-PAGE Analysis

The molecular weight and purity of the isolated phosvitin were analyzed using Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) following the method described by Laemmli (1970). Protein samples were mixed with loading buffer, denatured, and loaded onto a polyacrylamide gel (30%). Electrophoresis was performed at a constant voltage until protein separation was complete.

The gel was then stained to visualize protein bands, and the molecular weight of phosvitin was estimated by comparing it with a standard protein marker (Tang et al., 2021).

FTIR Analysis

Fourier Transform Infrared (FTIR) spectroscopy analysis was performed to identify the functional groups and structural characteristics of the isolated phosvitin. The samples were prepared using KBr prior to FTIR measurement. Spectral analysis was performed using an FTIR spectrophotometer, and the resulting

spectra were evaluated using Origin software. Atmospheric vapor compensation was applied during each measurement to minimize interference. The amide region was analyzed to assess differences in the protein's secondary structure elements. The area of the second derivative spectra was determined by integration from the maximum to the minimum point of each peak, and the total integrated peak area of each spectrum was defined as 100%. The proportions of each secondary structure element were expressed as percentages of the corresponding peak areas (Lilienthal et al., 2015).

Antioxidant Activity (DPPH Assay)

Antioxidant activity of the isolated phosvitin was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay to determine the IC_{50} value. A stock solution of phosvitin (100 ppm) was prepared by dissolving 5 mg of phosvitin powder in 50 mL of methanol p.a. Serial dilutions were then prepared to obtain concentrations of 10, 20, 30, 40, and 50 ppm. Ascorbic acid was used as a positive control and prepared using the same procedure (García et al., 2020). The DPPH stock solution (50 ppm) was prepared by dissolving 5 mg of DPPH powder in 100 mL of methanol p.a. The control solution consisted of 2 mL methanol p.a. mixed with 1 mL DPPH solution.

For sample analysis, 2 mL of each phosvitin solution was mixed with 2 mL of DPPH solution and incubated for 30 minutes at 25 °C in the dark. All samples were prepared in triplicate. Absorbance was measured at 517 nm using a Multiskan Sky spectrophotometer. The percentage of radical scavenging activity was calculated using the following equation:

$$\% \textbf{ Inhibition} = ((Ac - As) / Ac) \times 100$$

Ac = absorbance of the control
As = absorbance of the sample

The IC_{50} value was determined from the linear regression of sample concentration versus percentage inhibition. IC_{50} represents the concentration required to inhibit 50% of DPPH free radicals (Harni et al., 2020). Antioxidant activity was classified based on IC_{50} values as very strong (<50 ppm), strong (50–100 ppm), moderate (100–150 ppm), and weak (151–200 ppm) (Purwanto et al., 2017; Fauziah et al., 2021).

Data Analysis

All experimental data were analyzed descriptively and presented as tables and figures. Antioxidant activity was expressed as IC_{50} values, while protein characterization was interpreted based on SDS-PAGE band patterns and FTIR spectra.

Result and Discussion

Phosvitin Isolation Yield

Phosvitin was successfully isolated from duck egg yolk using ethanol extraction, NaCl treatment, dialysis, and freeze-drying. The extraction yield obtained from the isolation process is presented in **Table 1**.

Table 1. Yield of phosvitin isolated from duck egg yolk

Parameter	Result
Egg yolk weight (g)	21.42
Phosvitin powder obtained (g)	0.35
Yield (%)	1.63

The isolation process produced 0.35 g of phosvitin powder from 21.42 g of duck egg yolk, corresponding to a yield of 1.63%.

Protein Profile Analysis (SDS-PAGE)

The SDS-PAGE analysis of isolated phosvitin is presented in **Figure 1.**

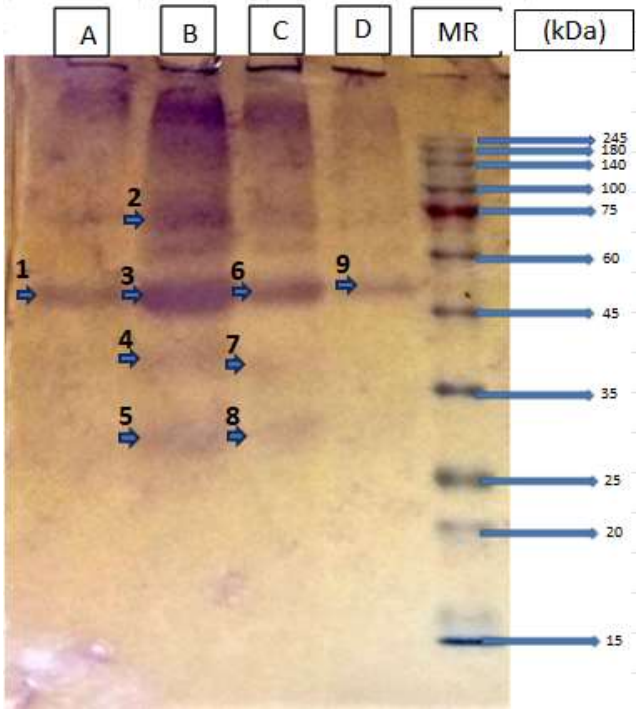

Figure 1. SDS-PAGE profile of isolated phosvitin

The SDS-PAGE profile of isolated phosvitin is presented in Figure 1. Protein bands were observed in lanes B and C at approximately 38–40 kDa, while dominant bands were detected at 49.92–51.50 kDa with additional bands in the range of 29–39 kDa. These results indicate that the isolated protein fraction was

not completely homogeneous and still contained several protein subfractions.

Previous studies reported that phosvitin generally has a molecular weight range of 35–45 kDa (Le Denmat et al., 2022). The higher molecular weight observed in this study may be influenced by the high phosphorylation level of phosvitin, differences in duck egg yolk composition, and protein aggregation during ethanol extraction and freeze-drying (Liu et al., 2022; Yang et al., 2021). Meanwhile, the additional bands detected at 29–39 kDa may indicate phosvitin subunits or partial protein degradation during extraction (Zhao et al., 2022).

Antioxidant Activity (DPPH Assay)

The antioxidant activity of isolated phosvitin was evaluated using the DPPH radical scavenging assay at concentrations of 10–50 ppm. The percentage of DPPH inhibition obtained from each concentration is presented in Table 2.

Table 2. DPPH radical scavenging activity of phosvitin

Concentration (ppm)	% Inhibition
10	42
20	45
30	49
40	53
50	58

Values represent experimental measurements.

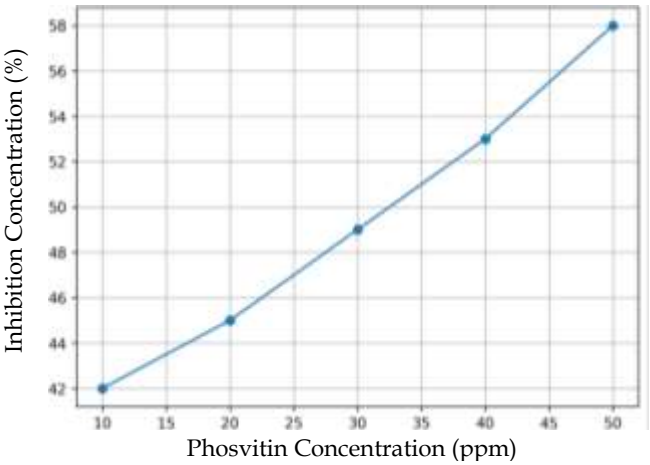

Figure 2. Effect of phosvitin concentration on DPPH inhibition (%)

The antioxidant activity of isolated phosvitin was evaluated using the DPPH method at concentrations of 10–50 ppm. Increasing phosvitin concentration increased the percentage of DPPH radical inhibition, indicating dose-dependent antioxidant activity. The inhibition values increased from approximately 42% at 10 ppm to 58% at 50 ppm. The linear regression equation obtained was y = 0.3075x + 39.653 and was used to determine the IC_{50} value.

The IC_{50} value for isolated phosvitin was 33.65 ppm, indicating very strong antioxidant activity (IC_{50} < 50 ppm). The antioxidant activity of phosvitin is associated with the presence of phosphoserine residues and phosphate groups in its structure, which contribute to free radical scavenging and metal-chelating activity (Chen et al., 2021; Liu et al., 2023).

FTIR Characterization

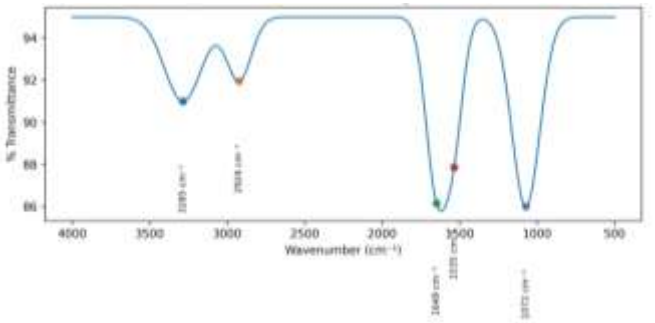

Figure 3. FTIR spectrum of phosvitin isolated from duck egg yolk showing characteristic protein and phosphate functional groups

The FTIR spectrum (Figure 3) revealed several characteristic absorption peaks indicating the presence of protein and phosphoprotein functional groups. A broad peak at 3285 cm^{-1} corresponds to N–H stretching (amide A), indicating the presence of protein backbone structures. The peak at 2923 cm^{-1} is associated with C–H stretching vibrations, commonly found in aliphatic chains of proteins. The strong absorption bands observed at 1648 cm^{-1} (amide I) and 1535 cm^{-1} (amide II) confirm the presence of peptide bonds, which are characteristic of protein structures. These peaks are widely used as indicators of protein secondary structure. Importantly, the peak at 1072 cm^{-1} is attributed to P–O stretching vibrations, which is a distinctive feature of phosphate groups. This confirms the presence of phosphoprotein, specifically phosvitin, which is known for its high degree of phosphorylation.

Conclusion

This study demonstrated that phosvitin was successfully isolated from duck egg yolk (*Anas platyrhynchos domesticus*) using ethanol extraction, NaCl treatment, dialysis, and freeze-drying. The SDS-PAGE profile of isolated phosvitin revealed dominant bands at 49.92–51.50 kDa together with bands at 38–40 kDa and additional fractions at 29–39 kDa, indicating structural heterogeneity. FTIR analysis confirmed the

presence of characteristic phosphate and protein functional groups. The isolated phosvitin also exhibited very strong antioxidant activity with an IC_{50} value of 33.65 ppm. These findings may indicate the potential of duck egg yolk phosvitin as a natural antioxidant source.

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Author Contributions
All authors contributed significantly to this study. The first author was responsible for conceptualization, methodology, data collection, formal analysis, and drafting the original manuscript. The second author contribute to methodology development, data interpretation, and manuscript editing The third author contributed to conceptualization, methodology, data validation and interpretation, supervision, and critical review of the manuscript. All authors have read and approved the final version of the manuscript.

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Conflicts of Interest
The authors declare no conflict of interest.

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