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Stability of house dust mite raw materials: Impact of storage conditions and lyophilization

Teerapong Wangapai,[1] Nitaya Indrawattana,[2,3,4] Prapagorn Vongjareonsanti,[1] Pichet Ruenchit,[1,2,3] Dichapong Kanjanawasee,[1,2,3] Thapani Srisai,[2,3] Anchalee Tungtrongchitr,[1,2,3] Nawannaporn Saelim[2,3]

Abstract

Background: *Dermatophagoides pteronyssinus* and *D. farinae* are major indoor allergens. Raw materials derived from these house dust mites (HDMs) are used to produce allergen extracts. The stability of these materials is influenced by storage form and conditions, which can affect protein integrity.

Objective: To compare storage formats and conditions for maintaining physicochemical stability of HDM raw materials.

Methods: Pure mite bodies (PMBs), prepared in fresh frozen and lyophilized forms, were stored at -80°C, -20°C, and 25°C. Over a 12-month period, samples were assessed for total protein and major allergens (Der p 1 and Der f 1) using ELISA. Protein integrity was analyzed by SDS-PAGE. Accelerated stability testing at 25°C for up to 14 days was also performed.

Results: Storage at −80°C best preserved total protein and Group 1 allergen content over 12 months. At −20°C, moderate declines were observed, with *D. farinae* showing greater reductions than *D. pteronyssinus*. Lyophilized samples were generally more stable than fresh-frozen ones at −20°C. Accelerated testing at 25°C caused marked losses over 14 days.

Conclusion: Ultra-low temperature storage (−80°C) remains optimal for preserving physicochemical stability of HDM raw materials. Lyophilization with −20°C storage is a promising, context-dependent option; however, in the absence of residual-moisture and immunological data, these findings should be interpreted as preliminary and limited to raw-material handling.

Key words: House dust mite, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, allergen stability, lyophilization, storage conditions, diagnostic reagents, vaccine production, immunotherapy

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Affiliations:
[1] Siriraj Dust Mite Center for Services and Research, Department of Parasitology,
[2] Biodesign Innovation Center, Department of Parasitology,
[3] Siriraj Center of Research and Excellence in Allergy and Immunology,
[4] Biomedical Research Incubator Division, Research Department, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

Corresponding author:
Nawannaporn Saelim
Biodesign Innovation Center, Department of Parasitology, Faculty of Medicine Siriraj Hospital, Mahidol University, 2 Wanglang Road, Bangkok Noi, Bangkok, 10700, Thailand
E-mail: nawannaporn.sae@mahidol.ac.th

Introduction

Allergic diseases have become a growing global health concern, significantly impacting healthcare systems. Among indoor allergens, house dust mites (HDMs) are the most common and potent contributors to allergic sensitization. Studies indicate that 60–80% of allergic patients exhibit sensitivity to HDMs, particularly *Dermatophagoides pteronyssinus* and *D. farinae*, which produce allergens that trigger immune responses in genetically predisposed individuals.[1,2] HDM allergenic extracts play a critical role

in both diagnostic and therapeutic applications, particularly in allergen-specific immunotherapy (AIT). These extracts, derived from natural sources, are subject to batch-to-batch variability due to differences in allergenic composition, which can impact standardization, diagnostic accuracy, and treatment efficacy.[3,4] Ensuring the stability of HDM raw materials is therefore essential to maintaining their potency and clinical value.

The protein and allergen composition of extracts is directly determined by the integrity of the raw material, especially for standardized diagnostic and therapeutic preparations. Degraded raw material can lead to lower extract yield, reduced allergen content, and greater batch-to-batch variability. Although some manufacturers use whole mite cultures (including feces and culture media), purified mite bodies provide greater consistency and reduced background contamination and are increasingly used in research-grade and clinical-grade preparations. One of the key factors influencing the stability of HDM raw materials is storage condition, particularly temperature and formulation (e.g., fresh frozen versus lyophilized forms). Previous studies have shown that inappropriate storage can result in protein degradation, reduced immunogenicity, and loss of allergen potency.[5,6] However, comparative data evaluating different storage methods specifically for HDM raw materials intended for vaccine production remain limited.

This study was designed to determine the optimal storage format for HDM raw materials used in vaccine manufacturing. It evaluates the stability of *D. pteronyssinus* and *D. farinae* raw materials in both fresh frozen and lyophilized forms under various temperature conditions over time. Both long-term and accelerated stability studies were performed to assess protein integrity and allergen retention. The findings aim to provide evidence-based recommendations for selecting appropriate storage methods, thereby supporting improved standardization, transportability, and long-term preservation of HDM raw materials for clinical and commercial use in allergy vaccine development.

Materials and Methods

Storage and stability testing of house dust mite samples

Pure mite bodies (PMB), the raw material for house dust mite (HDM) vaccine production, of *Dermatophagoides pteronyssinus* (Dp) and *D. farinae* (Df), were obtained from the Siriraj Dust Mite Center for Services and Research, Department of Parasitology, Faculty of Medicine Siriraj Hospital, Thailand. The PMBs were divided into two forms; fresh frozen mites and lyophilized mites for storage and stability studies. Fresh frozen samples were aliquoted into 2.0 mL sterile polypropylene cryovials with screw caps and O-ring seals to ensure low-temperature compatibility and sealing integrity. Lyophilized samples were stored in 10 mL borosilicate glass vials with Polytetrafluoroethylene (PTFE)-lined screw caps, sealed immediately after freeze-drying and kept at −20°C. Long-term stability analyses of raw materials were performed at predetermined intervals of 0, 3, 9, and 12 months using samples from three production batches under each storage condition. Accelerated stability testing was performed at 25°C and 70 ± 5% relative humidity for up to 14 days to simulate stress conditions and evaluate degradation trends. These accelerated stability conditions were designed to simulate temperature-induced degradation over short durations, allowing the assessment of protein and allergen stability under suboptimal storage, without relying on predictive kinetic models.

Following storage, raw materials were subjected to protein extraction, and the extracted proteins were analyzed to evaluate their quality and stability under both long-term and accelerated conditions.

Stability assessments included physical evaluations, protein quantification, and immunological analysis of allergen content. Physical changes were monitored through visual inspection, while protein concentration was quantified using the Bradford assay. Allergen content, specifically Der p 1 and Der f 1, was measured using a sandwich enzyme-linked immunosorbent assay (ELISA). Additionally, protein integrity and stability were assessed through SDS-PAGE analysis of HDM extracts, providing a comprehensive evaluation of protein profiles over time.

Protein extraction, quantification, and profile analysis

Each 500 mg aliquot of PBM from individual storage conditions was homogenized in 4 mL of phosphate-buffered saline (PBS, pH 7.0) using a sonicator (OmniRuptor 4000 Ultrasonic Homogenizer, Omni International, Georgia, USA) set at 30% amplitude with a 50% pulse-off cycle for 15 minutes on ice. The homogenate was then centrifuged at 12,000 ×g for 20 minutes at 4°C, and the supernatant was collected for protein concentration and profile analysis.

Protein concentrations in the HDM extracts were measured using the Bradford assay with Bio-Rad protein assay dye reagent concentrate (Bio-Rad, CA, USA). Ten microliters of each sample were pipetted into the wells of a 96-well microplate, followed by the addition of freshly prepared dye reagent. After mixing, the plate was incubated at 25°C for 5 minutes, and absorbance was measured at 595 nm (A595) using an ELISA reader (BioTek Instruments, Inc., Vermont, USA). Protein concentrations were calculated based on a standard curve generated from bovine serum albumin (BSA) and adjusted according to the dilution factor.

The protein profile of HDM extracts was analyzed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). A 4% stacking gel and a 12% separating gel were prepared. Protein samples were mixed with 6× SDS-reducing buffer and heated at 95°C for 5 minutes. Denatured proteins and PageRuler® pre-stained protein ladders (Thermo Scientific, MA, USA) were loaded into gel wells. Electrophoresis was performed in Tris-Glycine running buffer at a constant current of 20 mA per gel for 60 minutes. After electrophoresis, the gels were stained with Coomassie Brilliant Blue G-250 (Affymetrix, California, USA) to visualize protein bands.

Quantification of Major HDM Allergens (Group 1)

The major allergens group 1 (Der p 1 and Der f 1) were quantified using a commercial sandwich ELISA kit (INDOOR Biotechnologies, USA) from the collected supernatant which mentioned above. For Der p 1, the MAb5H8-allergen sample-biotinylated MAb4C1 format was used, while Der f 1 utilized the MAb6A8-allergen sample-biotinylated MAb4C1 format. Both assays were calibrated using a universal allergen standard (UAS). Streptavidin-HRP (Southern Biotech; 1:1,000) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) substrate (KPL, USA) were used for color development. The allergen concentrations were determined using the UAS (Universal Allergen Standard) standard curve, which provides a reference for quantifying allergen levels based on a series of known concentrations.

Statistical Analysis

Statistical analyses were performed using SPSS software (Version 25.0; IBM Corp., Armonk, NY, USA). Data are presented as mean ± standard error of the mean (SEM). Linear regression analysis was conducted to evaluate the association between storage time and protein concentration, following the recommendations of the ICH Q1E guideline for stability studies. Protein level changes were analyzed separately for each storage condition, and the regression slope (m) was used to quantify the rate of change over time, while the coefficient of determination (R^2) expressed the proportion of variance explained by time. Statistical significance of the relationship was assessed using two-tailed *p*-values, with $p < 0.05$ considered significant. For trend analysis of long-term stability (12 months), linear regression (y = m x + c) was applied to batch-averaged data across all time points (three batches × four time points; n = 12 per condition). Exact two-tailed p-values for each slope were obtained from the regression t-statistic (df = 10) and are reported in **Supplementary Table S2**, with scatter plots and regression lines for each batch illustrated in **Figure S1** to show lot-to-lot variability.

Results

Physical Property Assessment

The physical integrity of PMBs was evaluated under different storage conditions to determine their suitability for protein extraction, a critical factor in vaccine production. Ensuring that storage conditions do not compromise the physical properties of raw materials is essential for maintaining the consistency and quality of allergenic extracts.

For long-term stability assessment, fresh frozen and lyophilized PMBs were stored under controlled conditions at -80°C and -20°C for up to 12 months. Throughout the storage period, no significant changes in physical appearance were observed in either species. The fresh frozen PMBs retained their original morphology without visible discoloration or aggregation. Similarly, lyophilized PMBs remained as dark brown solid particles with no detectable odor or structural degradation. These findings suggest that fresh at both -80°C and -20°C storage effectively preserve the physical integrity of PMB.

For short-term accelerated stability testing, fresh frozen and lyophilized PMBs were incubated at 25°C with 70 ± 5% relative humidity for up to 14 days. No visible changes were noted in either *D. pteronyssinus* or *D. farinae* across all time points. However, protein degradation trends observed in subsequent biochemical analyses indicated that extended exposure to suboptimal storage conditions might impact allergen stability despite preserved physical appearance.

These findings confirm that low-temperature storage (-80°C or -20°C) effectively maintains the structural integrity of PMBs for extended periods. Furthermore, lyophilization enhances transportability and storage stability without compromising the physical properties of PMB. However, prolonged storage at ambient temperatures (25°C) should be avoided to prevent potential biochemical degradation.

Long-term stability of protein and allergen retention in Dermatophagoides pteronyssinus and D. farinae under different storage conditions

The stability of total protein and Group 1 allergens (Der p 1 and Der f 1) was assessed over 12 months under three storage conditions (fresh-frozen −80°C, fresh-frozen −20°C, and lyophilized −20°C). Storage at −80°C preserved the highest levels of total protein in both species. At −20°C, declines were more apparent in *D. farinae* than in *D. pteronyssinus*, while lyophilization at −20°C generally improved retention versus fresh-frozen at −20°C. Consistent with the regression-based trend analysis (**Supplementary Table S2**), Der f 1 did not show a statistically significant time-dependent decline under any of the tested conditions ($p = 0.183$ at −80°C; 0.113 at −20°C; 0.082 for lyophilized −20°C; df = 10). In contrast, Der p 1 demonstrated significant negative slopes under frozen storage ($p = 0.0083$ at −80°C; 1.93×10^{-5} at −20°C), but not when lyophilized at −20°C ($p = 0.361$), indicating species- and condition-specific stability patterns.

To more rigorously evaluate protein and allergen degradation over time, linear regression analysis was incorporated into the main results (previously presented only in **Supplementary Table S2**). Trend analysis revealed substantial batch-to-batch variability across all storage conditions for both *D. pteronyssinus* and *D. farinae* (**Figure 2**). For *D. pteronyssinus*, Der p 1 demonstrated statistically significant negative slopes under frozen storage (−80°C: $p = 0.0083$; −20°C: $p = 1.93 \times 10^{-5}$), whereas lyophilized samples stored at −20°C did not show a significant time-dependent decline ($p = 0.361$). For *D. farinae*, total protein exhibited significant negative trends across all long-term storage conditions (−80°C: $p = 0.015$; −20°C: $p = 1.61 \times 10^{-4}$; lyophilized at −20°C: $p = 0.024$). In contrast, Der f 1 did not demonstrate statistically significant slopes under any condition ($p = 0.183$–0.082), indicating that the absence of detectable trends may be due to high inter-batch variability rather than true stability of the allergen.

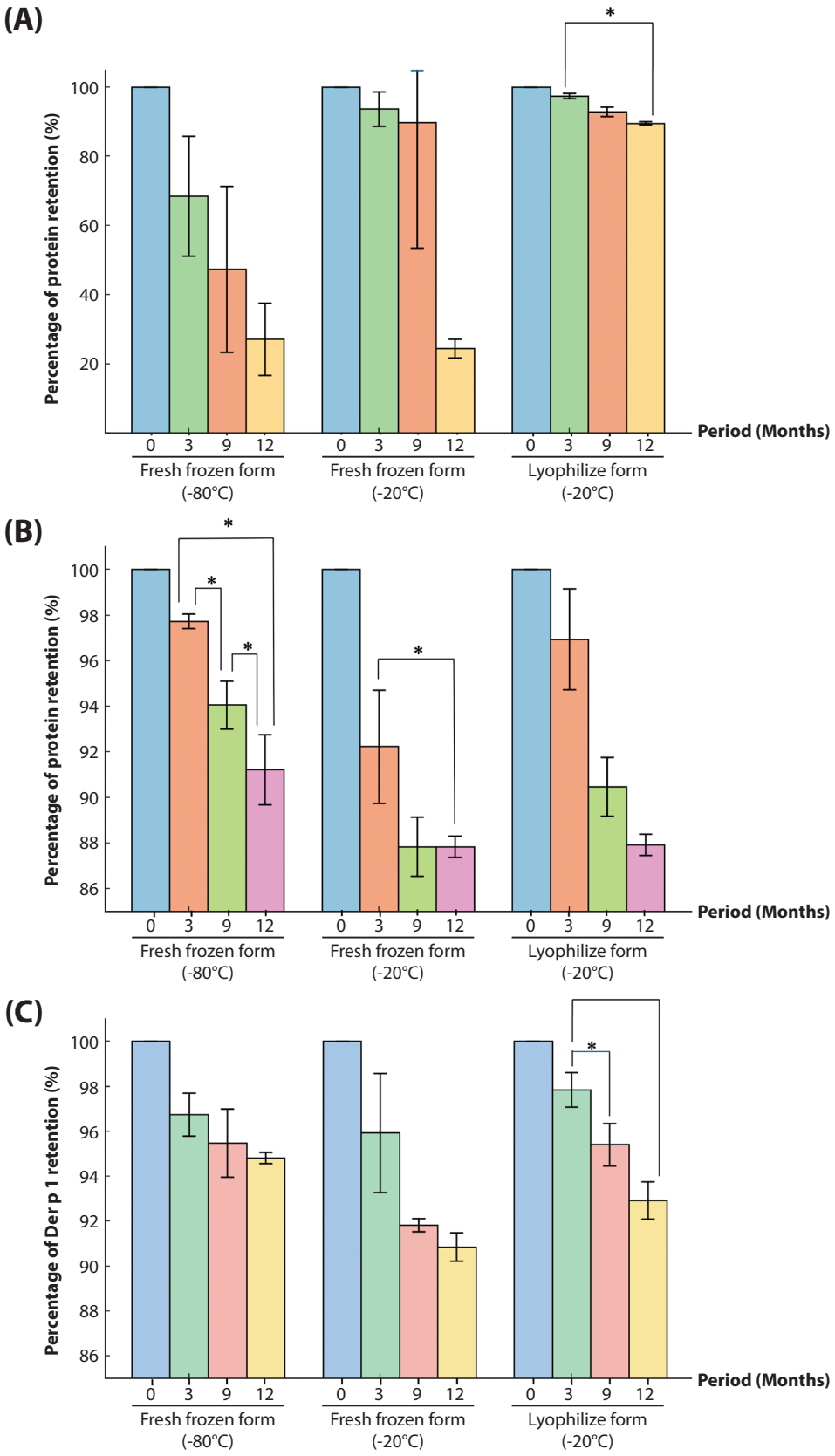
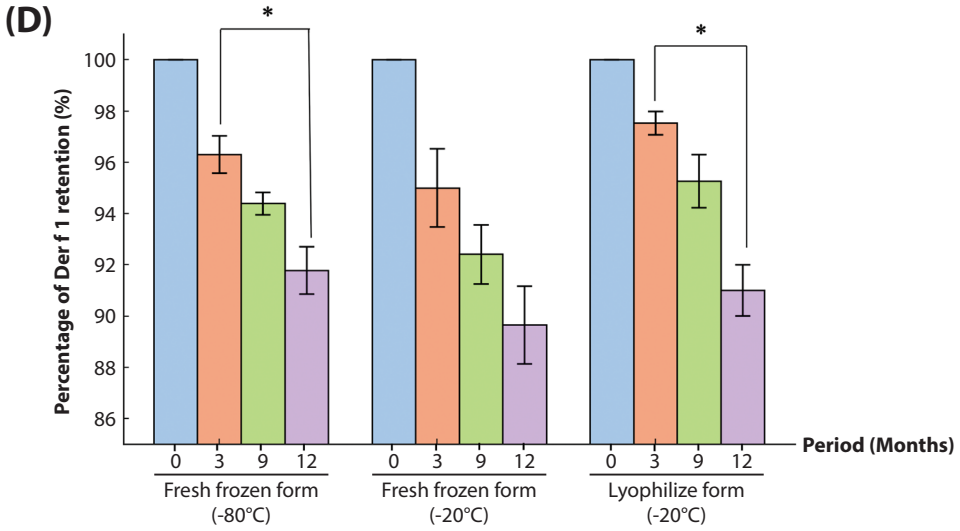


Figure 1. Protein and allergen concentrations of *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* samples stored under three different conditions—Fresh Frozen Mite at -80°C, Fresh Frozen Mite at -20°C, and Lyophilized Mite at -20°C—over 0, 3, 9, and 12 months for long-term stability. (A) Percentage of protein retention in *D. pteronyssinus*, (B) Percentage of protein retention in *D. farinae*, (C) Percentage of allergen Der p 1 retention in *D. pteronyssinus*, and (D) Percentage of allergen Der f 1 retention in *D. farinae*. Bars represent the mean percentage relative to the initial concentration at Month 0, with error bars indicating the standard deviation. Statistically significant differences between storage conditions are denoted by asterisks (*$p < 0.05$).



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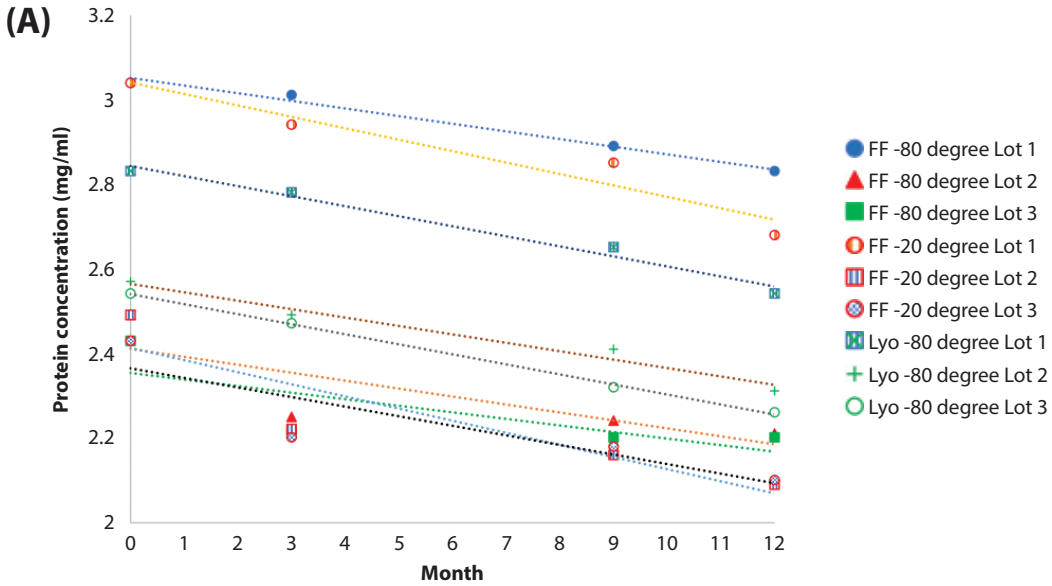


Figure 2. Scatter plots showing the stability of total protein and major allergens (Der p 1 and Der f 1) in *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* stored under different conditions over a 12-month period. Samples were stored as Fresh Frozen Mites at −80°C, Fresh Frozen Mites at −20°C, and Lyophilized Mites at −20°C. Data are presented separately for each batch (Lot 1–3) with corresponding linear regression lines. (A) Total protein concentration in *D. pteronyssinus*. (B) Total protein concentration in *D. farinae*. (C) Der p 1 allergen concentration in *D. pteronyssinus*. (D) Der f 1 allergen concentration in *D. farinae*. Each data point represents the measured concentration for the respective batch, and dotted lines indicate regression trends over time. Statistical evaluation was performed using linear regression; exact regression statistics and *p*-values are reported in **Supplementary Table S2**.

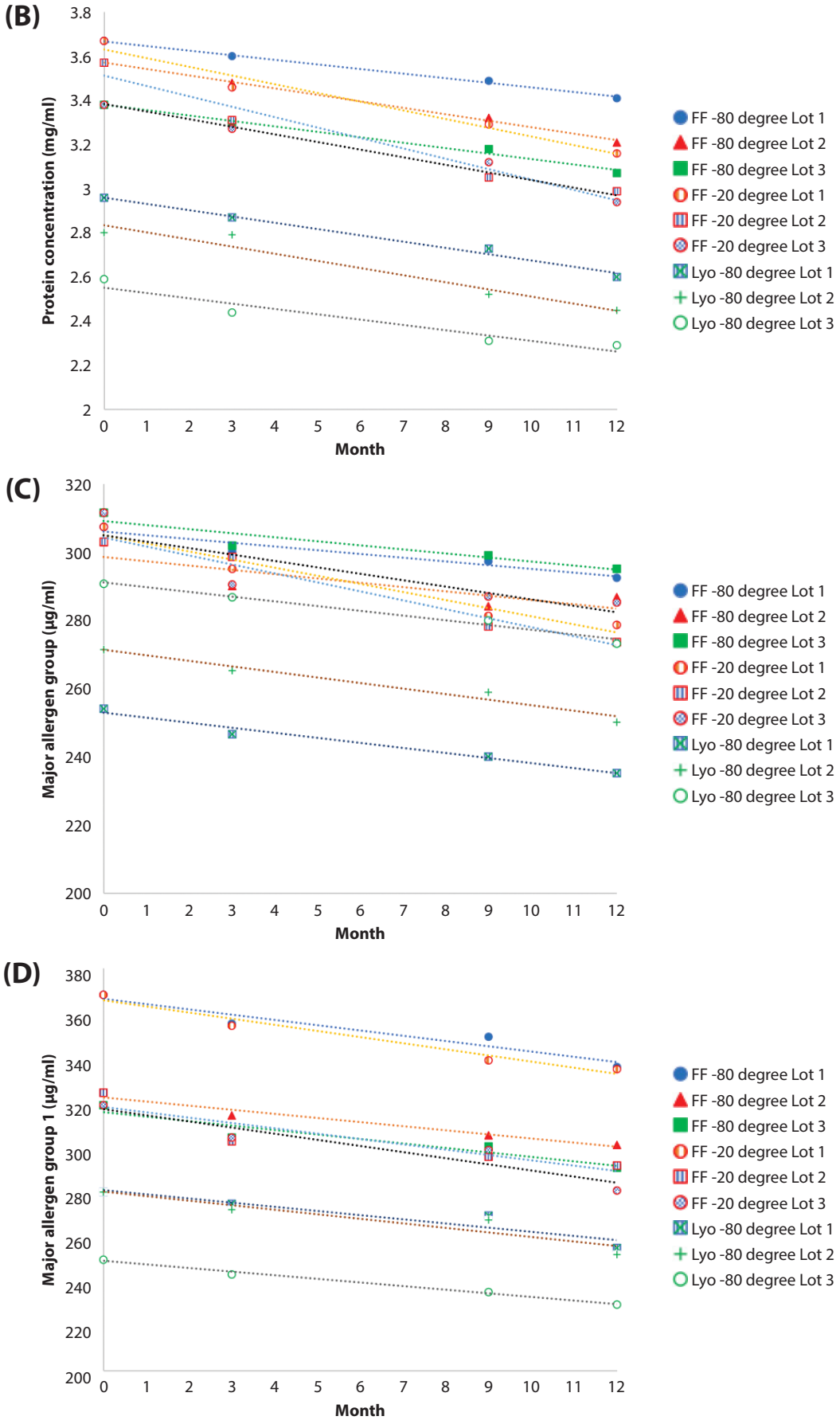


Figure 2. (Continued)

Accelerated Stability of Protein and Allergen Retention Under Suboptimal Conditions

To evaluate the effects of short-term exposure to suboptimal storage conditions, fresh frozen and lyophilized PMBs previously stored at -20°C were incubated at 25°C for up to 14 days. Protein content and allergen retention were subsequently analyzed (**Figure 3A–3D**). Protein stability declined rapidly under these conditions, with fresh frozen *D. farinae* exhibiting significantly greater degradation compared to *D. pteronyssinus* ($p < 0.05$). By day 14, fresh frozen *D. pteronyssinus* retained approximately 40% of its initial protein content, whereas fresh frozen *D. farinae* retained only 20% (**Figure 3A and 3B**). In contrast, lyophilization improved protein stability in both species, with lyophilized *D. pteronyssinus* retaining 60% and lyophilized *D. farinae* retaining 40% of their initial protein content by day 14. A similar trend analysis was performed for the accelerated stability study (**Figure 4**). Although the duration was shorter (14 days), batch-wise regression analysis again demonstrated considerable variability for both *D. pteronyssinus* and *D. farinae*. Significant negative slopes were observed for lyophilized *D. farinae* protein and fresh-frozen Der f 1 ($p = 0.022$ and 0.045, respectively), whereas other analytes did not reach statistical significance (**Supplementary Table S1**). These results support the interpretation that short-term degradation under suboptimal conditions is detectable but may be obscured by inter-batch variability, consistent with observations from the long-term dataset.

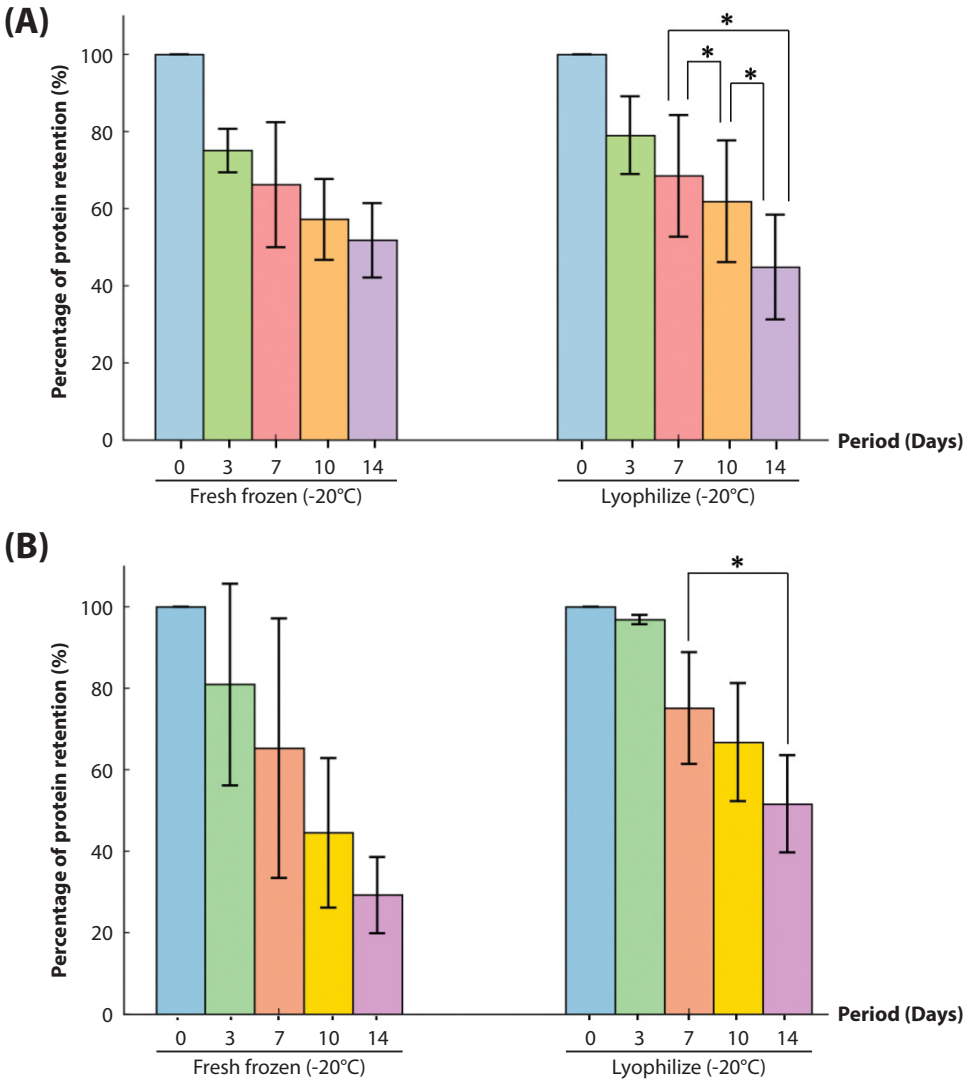
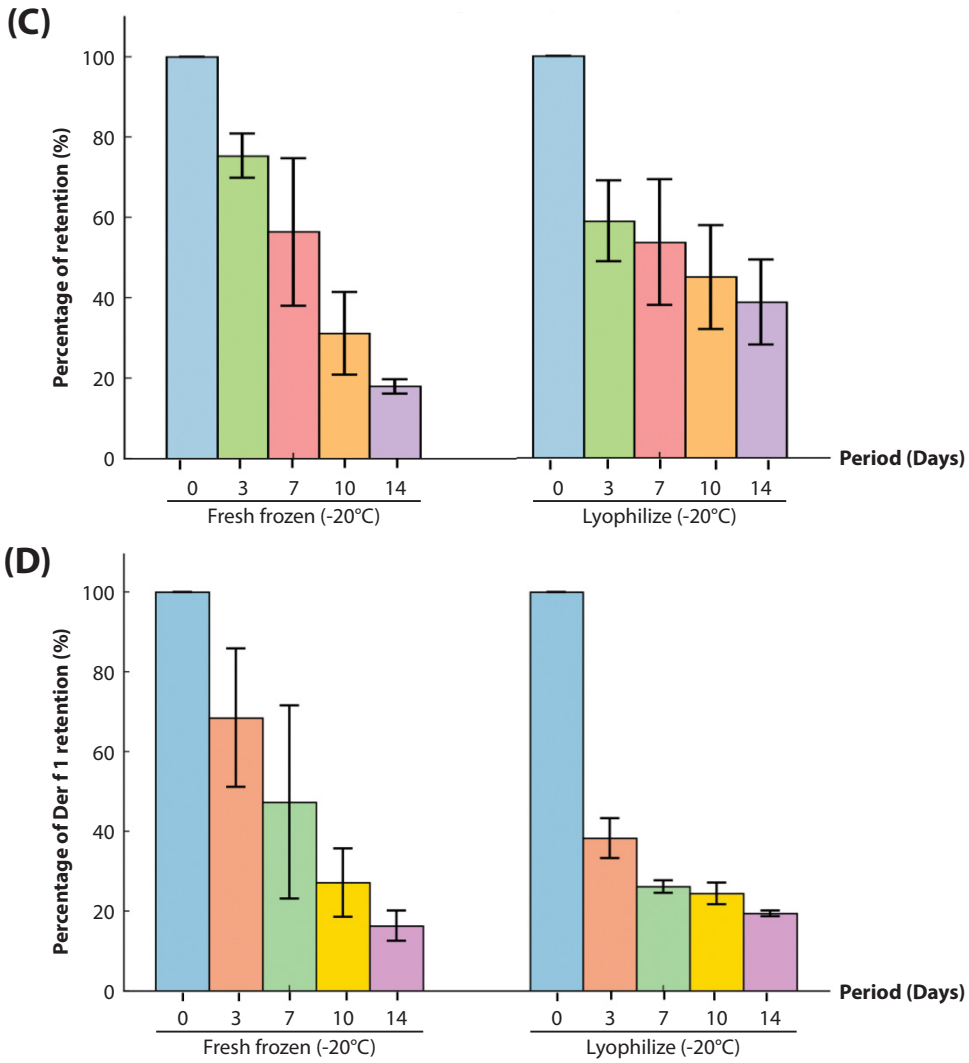


Figure 3. Protein and allergen concentrations of *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* samples stored under two different conditions—Fresh Frozen and Lyophilized Mite—over 0, 3, 7, 10, and 14 days for accelerated stability testing. (A) Percentage of protein retention in *D. pteronyssinus*, (B) Percentage of protein retention in *D. farinae*, (C) Percentage of allergen Der p 1 retention in *D. pteronyssinus*, and (D) Percentage of allergen Der f 1 retention in *D. farinae*. Bars represent the mean percentage relative to the initial concentration at Day 0, with error bars indicating the standard deviation. Statistically significant differences are denoted by asterisks (*$p < 0.05$).



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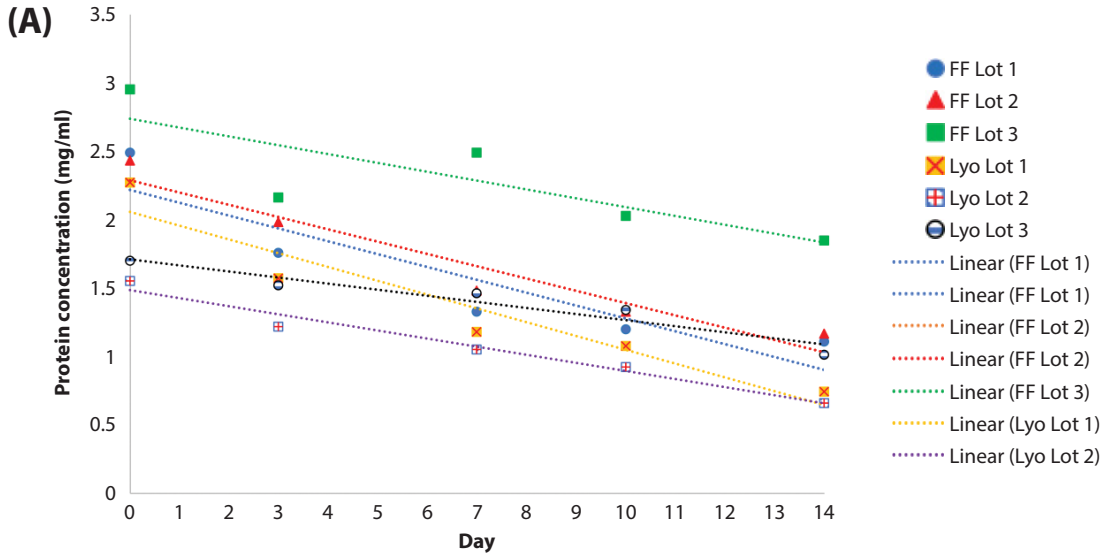

Figure 4. Scatter plots showing protein and allergen concentrations of *Dermatophagoides pteronyssinus* and *D. farinae* samples stored under two different conditions—Fresh Frozen and Lyophilized Mites—over 0, 3, 7, 10, and 14 days for accelerated stability testing. Data are presented separately for each batch (Lot 1–3) with corresponding linear regression lines. (A) Total protein concentration in *D. pteronyssinus*. (B) Total protein concentration in *D. farinae*. (C) Der p 1 allergen concentration in *D. pteronyssinus*. (D) Der f 1 allergen concentration in *D. farinae*. Each point represents the measured concentration for the respective batch, and dotted lines indicate regression trends over time. Statistical evaluation was performed using linear regression; exact *p*-values are reported in **Supplementary Table S2**.

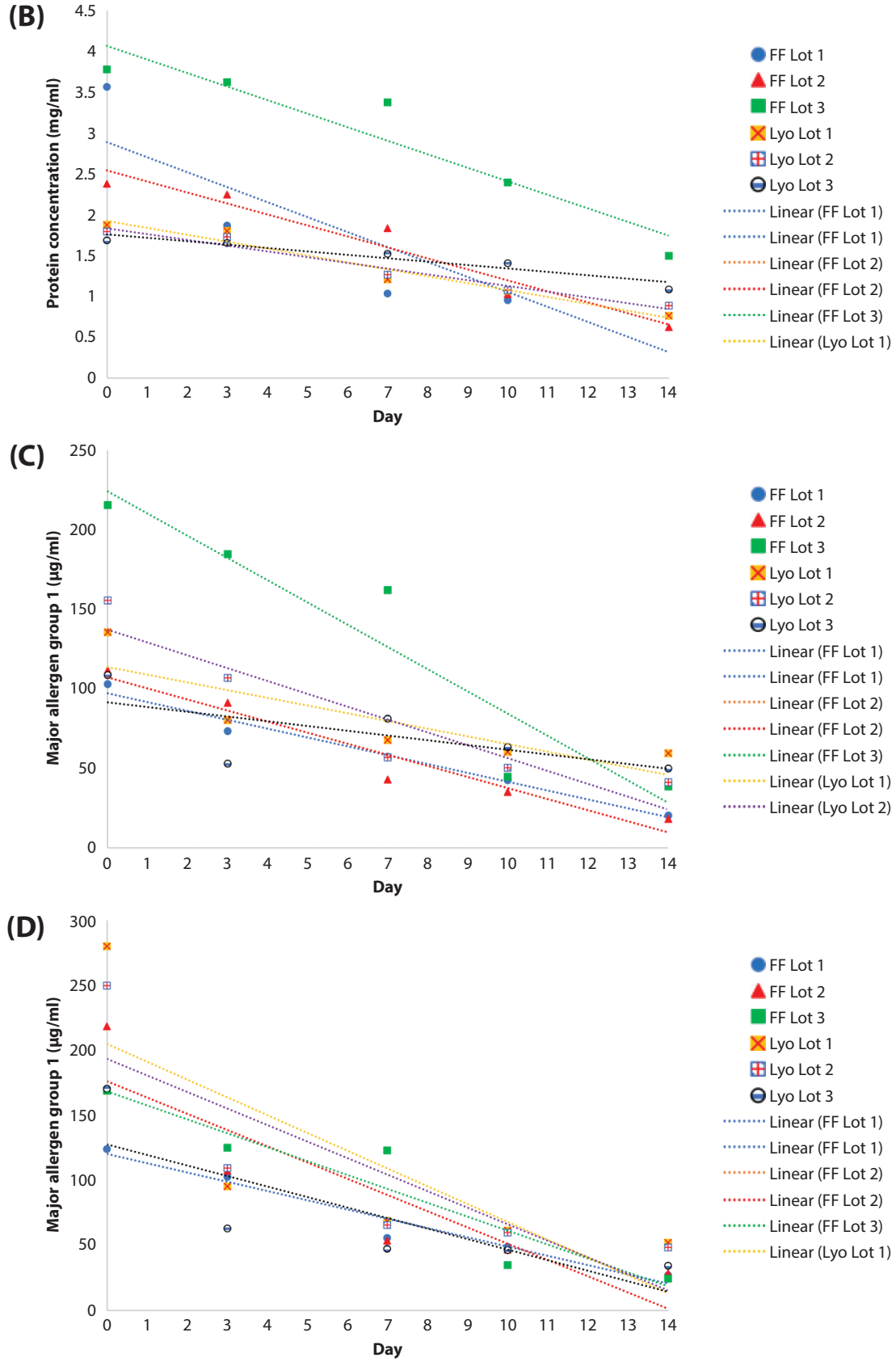

Figure 4. (Continued)

Similarly, allergen retention followed a comparable trend, with significant reductions observed in both species over 14 days (**Figure 3C and 3D**). By day 14, fresh frozen *D. pteronyssinus* retained only 30% of Der p 1, while fresh frozen *D. farinae* retained 20% of Der f 1, demonstrating greater allergen degradation in *D. farinae* ($p < 0.05$). Lyophilization partially mitigated this loss, with lyophilized *D. pteronyssinus* and *D. farinae* retaining 50% and 40%, respectively, of their initial allergen content by the end of the study. Despite these benefits, allergen degradation remained evident in all conditions, reinforcing the importance of strict temperature control to preserve extract quality.

Protein Profiles by SDS-PAGE

SDS-PAGE analysis provided a detailed view of protein stability under different storage conditions. Protein extract from fresh frozen PMBs exhibited well-defined protein bands at 12 months when stored at -80°C or -20°C. In contrast, lyophilized samples stored at 25°C showed extensive fragmentation, with smearing indicative of protein degradation. These changes were more pronounced in *D. farinae* compared to *D. pteronyssinus*, consistent with the higher allergen reduction observed in *D. farinae* samples under similar conditions (**Figure 5**). Quantitative analysis of band intensity showed statistically significant degradation in room temperature samples compared to those stored at lower temperatures ($p < 0.01$). Under accelerated conditions, SDS-PAGE analysis also demonstrated progressive band deterioration in both species, particularly in lyophilized samples stored at 25°C, consistent with rapid protein degradation observed in biochemical assays (**Figure 6**).

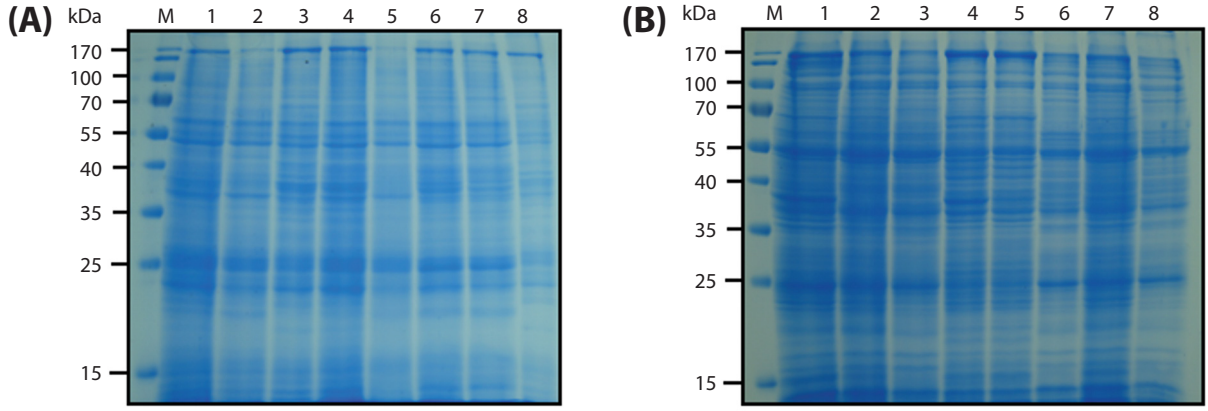

Figure 5. Protein profiles of *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* stored under different long-term conditions. (A) *D. pteronyssinus* protein pattern and (B) *D. farinae* protein pattern analyzed by SDS-PAGE. Lane M: Prestained SDS-PAGE Standards Broad Range with molecular weights (kDa) indicated on the left. Lane 2: Lyophilized mite at Month 0. Lane 3: Lyophilized mite at Month 3, stored at -20°C. Lane 4: Lyophilized mite at Month 12, stored at -20°C. Lane 5: Fresh Frozen mite at Month 0. Lane 6: Fresh Frozen mite at Month 3, stored at -20°C. Lane 7: Fresh Frozen mite at Month 3, stored at -80°C. Lane 8: Fresh Frozen mite at Month 12, stored at -20°C. Lane 9: Fresh Frozen mite at Month 12, stored at -80°C. The protein banding patterns illustrate the effects of different storage conditions on protein stability over time.

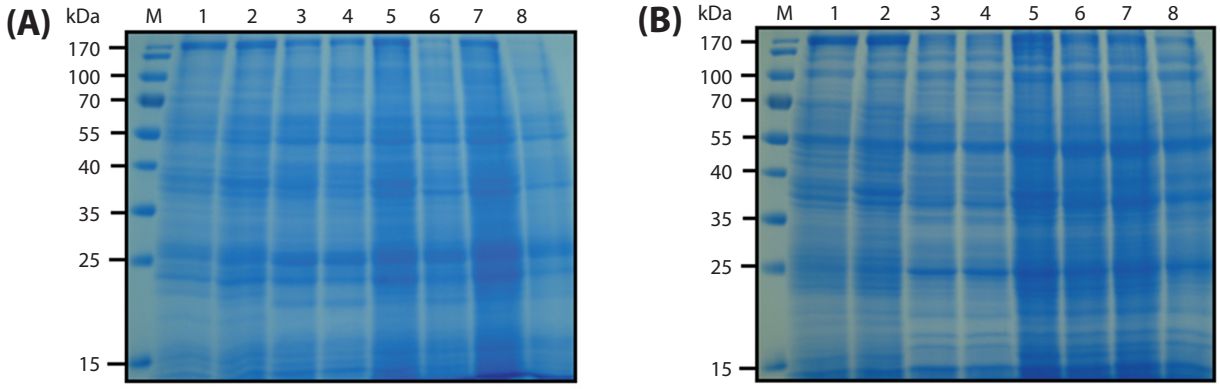

Figure 6. Protein profiles of *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* stored under accelerated conditions. SDS-PAGE analysis was performed to assess protein stability under accelerated storage conditions. Lane M: Prestained SDS-PAGE Standards Broad Range with molecular weights (kDa) indicated on the left. Lanes 1-4: Fresh Frozen mites stored for 0, 3, 7, and 10 days, respectively. Lanes 5-8: Lyophilized mites stored for 0, 3, 7, and 10 days, respectively. The protein banding patterns illustrate the effects of accelerated storage conditions on protein integrity in *D. pteronyssinus* and *D. farinae* over time.

Discussion

The stability of raw house dust mite (HDM) materials is a critical determinant of allergen extract quality. Degradation of mite proteins prior to extraction can reduce yield, alter allergenic composition, and increase batch-to-batch variability. Although whole cultures are often used in industrial settings, purified mite bodies offer a more defined allergen source with reduced microbial and environmental contaminants. Ensuring the stability of this raw material is therefore essential for producing high-quality, reproducible allergen extracts.[7] This study systematically assessed the long-term and short-term stability of pure mite bodies (PMBs) under different storage conditions. The results revealed species-specific differences in protein and allergen degradation, underscoring the importance of optimizing storage protocols for different HDM species.

Low-temperature storage at -80°C was the most effective condition for preserving PMBs, including total protein and major allergen content (Der p 1 and Der f 1) in both *D. pteronyssinus* and *D. farinae*. These findings are consistent with previous reports highlighting the importance of deep-freeze storage in preventing protein denaturation and maintaining allergenic stability.[4,8] Similar observations have been reported in other biological materials, such as pollen and food proteins, where storage at -80°C effectively maintained structural integrity and immunogenicity. In contrast, fresh frozen PMBs stored at -20°C exhibited gradual degradation, with *D. farinae* showing a more pronounced decline in both protein and allergen retention than *D. pteronyssinus*, particularly after 9 months. This supports earlier studies suggesting that -20°C may be suboptimal for long-term preservation due to potential temperature fluctuations and repeated freeze-thaw cycles leading to protein denaturation.[4,8]

Lyophilized PMB stored at -20°C partially mitigated protein and allergen loss, particularly for *D. farinae*, which demonstrated better stability in lyophilized form compared to fresh frozen storage at the same temperature. This observation is consistent with previous studies reporting that lyophilization enhances protein stability by reducing water content, thereby minimizing hydrolytic degradation.[3,9] However, although lyophilization improved stability relative to fresh frozen storage at -20°C, it did not completely prevent degradation of protein and allergen content over a 12-month period. Prior research has shown that lyophilized proteins remain vulnerable to oxidation and conformational changes, which may compromise their long-term stability and immunogenicity.[7,10] Therefore, while lyophilization offers advantages in terms of transportability and storage flexibility, it cannot fully replace ultra-low temperature storage, especially for allergens prone to degradation.

Allergen retention followed a similar trend to protein stability, with Fresh frozen -80°C storage preserving over 90% of Der p 1 and Der f 1 across both HDM species. However, Fresh frozen storage at -20°C resulted in significant allergen loss, particularly in *D. farinae*, which retained only 68% of Der f 1 by 12 months, compared to 80% retention of Der p 1 in *D. pteronyssinus*. These findings highlight species-specific differences in allergen stability, possibly due to intrinsic variations in protein structure, post-translational modifications, or proteolytic activity within PMBs.[11,12] Similar trends have been observed in studies on pollen and animal-derived allergens, where certain proteins exhibited higher susceptibility to degradation under suboptimal storage conditions.[13,14] The observed discrepancy between total protein and Der p 1 retention may be due to selective degradation of non-allergenic proteins or differential stability of Der p 1, which has been shown to resist proteolysis and temperature-induced denaturation. Additionally, ELISA detects Der p 1 based on specific epitopes and may overestimate allergen preservation if degradation spares antibody-binding regions.[11]

Regression-based trend analysis (**Figures 2 and 4**) provided a clearer interpretation of the stability data by accounting for batch-to-batch variability, which was substantial across all conditions. This variability explains why mean retention values alone may be misleading. For *D. pteronyssinus*, Der p 1 showed significant time-dependent declines under frozen storage, whereas the non-significant slopes observed for Der f 1 in *D. farinae* (p = 0.183–0.082) reflect variability rather than true stability of the allergen. Trend analysis of the accelerated-stability data similarly showed that only certain analytes exhibited significant short-term degradation, while others did not reach significance due to inter-batch fluctuation rather than preservation of protein integrity. These findings underscore that degradation is occurring but may be obscured without regression-based evaluation. Based on this integrated analysis, interpretations suggesting species-specific differences must be moderated. Apparent differences between *D. pteronyssinus* and *D. farinae* are largely confounded by variability between production batches, and the current dataset does not support definitive biological species differences. Future studies with more batches and broader allergen panels will be needed to clarify this point.

Accelerated stability studies further confirmed that *D. farinae* was more susceptible to degradation than *D. pteronyssinus*. Fresh frozen *D. farinae* showed a more rapid decline in both protein and allergen content, retaining only 20% of total protein and Der f 1 by day 14. In contrast, fresh frozen *D. pteronyssinus* retained approximately 40% of total protein and 30% of Der p 1 over the same period. Lyophilization partially improved stability, but allergen retention remained suboptimal, with lyophilized *D. farinae* and *D. pteronyssinus* retaining 40% and 50% of their initial allergen content, respectively. These results emphasize the need for stringent temperature control during allergen storage and transport, as short-term exposure to suboptimal temperatures significantly compromises extract stability. This aligns with prior findings in food allergen research, where storage at -20°C for prolonged periods was associated with significant protein denaturation and loss of antigenic epitopes.[15,16]

From a practical standpoint, this study highlights the importance of selecting appropriate storage formats for HDM raw materials intended for diagnostic reagents and allergy vaccine production. Storage at -80°C remains the most effective method for preserving protein integrity and allergenic potency, representing the gold standard. However, in resource-limited settings where ultra-low temperature storage is not feasible, lyophilization combined with -20°C storage offers a practical and relatively stable alternative. These findings are particularly relevant for vaccine manufacturers, diagnostic kit developers, and immunotherapy providers, as they underscore the critical role of storage conditions in ensuring the quality and consistency of allergenic extracts. Future investigations should consider incorporating novel stabilizing agents—such as trehalose, polyethylene glycol (PEG), or recombinant protein engineering—to enhance the thermal stability of allergenic proteins under variable storage conditions.[17,18] This study primarily focused on the physicochemical stability of house dust mite (HDM) raw materials using Group 1 allergens (Der p 1 and Der f 1) as representative markers. While these proteins are relatively thermostable and widely accepted in allergen standardization, inclusion of additional major allergens such as Der p 2 and Der p 23 in future studies would provide a more comprehensive allergenic profile. Functional immunoassays (e.g., IgE-binding or inhibition ELISA) were beyond the current scope, as the emphasis was on physicochemical preservation prior to extract formulation; however, such assays will be incorporated in the next phase to validate immunological integrity. In lyophilized samples, residual moisture was not directly quantified, but the process parameters were carefully controlled to ensure batch consistency. Future investigations will include moisture quantification (e.g., Karl Fischer titration or water-activity measurement) as a quality attribute for lyophilization. Overall, this study establishes a foundation for optimizing HDM raw-material storage and processing, and forthcoming work will extend these findings through multiplex allergen analysis and immunological validation to confirm clinical relevance.

Conclusion

This study provides preliminary physicochemical evidence for the stability of house dust mite raw materials under different storage conditions. Ultra-low temperature storage (−80°C) remains the most effective strategy for preserving total protein and Group 1 allergen content. Lyophilization at −20°C showed promise as a preservation approach, but, in the absence of residual-moisture data and immunological validation, these findings should be regarded as indicative rather than conclusive. The results support optimization of raw-material preservation as a foundation for future allergen extract development, pending broader allergen profiling and functional confirmation.

Conflict of interest declaration

The authors declare no conflicts of interest.

Ethical considerations

This study and the use of animal samples were approved by the Siriraj Animal Care and Use Committee, Faculty of Medicine Siriraj Hospital, Mahidol University (SiACUC no. 017/2567). This protocol covered invertebrate animals (HDMs) only; no vertebrate animal procedures were conducted.

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Author contributions

- T.W., N.I., A.T., and N.S. conceived and designed the study and obtained ethical approval.
- P.R., P.N., and T.W. prepared the house dust mite samples and assessed their physical properties.
- N.I., T.S., and N.S. conducted the PMB stability storage experiments, protein extraction, and protein analysis.
- T.W., N.I., and N.S. performed the quantification of major HDM allergens.
- N.I., D.K., A.T., T.W., and N.S. analyzed the data.
- N.I., A.T., T.W., and N.S. drafted and revised the manuscript.
- All authors read and approved the final version of the manuscript.

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Supplementary Table

Table S1. Regression analysis result of house dust mite protein over time under accelerated conditions.

Mite (mg/ml)	Slope	R Square	*P*-value
Fresh frozen *D. pteronyssinus*	-0.083	0.547	0.153
Lyophilized *D. pteronyssinus*	-0.068	0.717	0.070
Fresh frozen *D. farinae*	-0.161	0.560	0.146
Lyophilized *D. farinae*	-0.066	0.864	0.022
Fresh frozen Der p 1	-8.837	0.539	0.158
Lyophilized Der p 1	-5.294	0.642	0.103
Fresh frozen Der f 1	-10.091	0.786	0.045
Lyophilized Der f 1	-11.488	0.598	0.125

Table S2. Regression analysis result of house dust mite protein over 1 year under different storage conditions.

Mite (mg/ml)	Slope	R Square	*P*-value
Fresh frozen *D. pteronyssinus* at -80°C	-0.018	0.064	0.428
Fresh frozen *D. pteronyssinus* at -20°C	-0.026	0.139	0.233
Lyophilized *D. pteronyssinus* at -20°C	-0.022	0.388	0.030
Fresh frozen Der p 1 at -80°C	-1.190	0.518	0.008
Fresh frozen Der p 1 at -20°C	-2.293	0.851	1.93e-5
Lyophilized Der p 1 at -20°C	-1.498	0.084	0.361
Fresh frozen D. farinae at -80°C	-0.025	0.462	0.015
Fresh frozen D. farinae at -20°C	-0.040	0.774	1.61e-4
Lyophilized D. farinae at -20°C	-0.028	0.413	0.024
Fresh frozen Der f 1 at -80°C	-2.062	0.170	0.183
Fresh frozen Der f 1 at -20°C	-2.618	0.232	0.113
Lyophilized Der f 1 at -20°C	-1.824	0.272	0.082
Lyophilized Der p 2 at -20°C	-0.022	0.388	< 0.05
Lyophilized Der f 2 at -20°C	-0.028	0.413	< 0.05

Linear regression was performed on the batch-averaged values at each time point (n = 12 observations per condition; 3 batches × 4 time points). Slopes (rate of change over time) and coefficients of determination (R^2) were obtained from simple linear regression ($y = mx + c$). Exact two-tailed *p*-values for the slope were derived from the regression t-statistic with df = 10. For completeness, batch-wise scatter plots and regression lines are provided in **Figure S1**.