Synergistic NADES–Ionic Liquid Hybrid Solvent Systems Coupled with Microwave-Assisted Extraction for Efficient Plant Protein Recovery from Moringa oleifera Seeds

Program Riset Internasional ITB Tahun 2026

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Abstract

This study evaluated NADES–IL hybrids with microwave-assisted extraction (MAE) for protein recovery from *Moringa oleifera* seeds. Four NADES and 12 NADES–IL formulations were compared with conventional alkaline extraction. MAE was performed at 300 W and 50 °C for 2 min.

Protein yield and functional properties were assessed. The highest yield was obtained with ChOH:Gly:[BMIM]Br (17.45%), versus 2.061% for conventional extraction. Hybrid formulations achieved the highest water- and oil-holding capacities and solubility. However, conventional extraction produced the highest foaming capacity and stability. Emulsifying properties varied with solvent composition. Overall, NADES–IL composition significantly influenced protein recovery and functionality, demonstrating the potential of hybrid solvents for developing moringa protein ingredients with tailored properties.

Keyword: Moringa oleifera seed protein, NADES–IL, microwave-assisted extraction.

Introduction

The growing demand for sustainable protein sources has increased interest in plant-derived alternatives. *Moringa oleifera* seeds represent a promising protein source, but efficient recovery remains challenging due to their complex matrix and limitations of conventional extraction methods.

Natural deep eutectic solvents (NADES) offer tunable extraction properties, while ionic liquids (ILs) may complement NADES by modifying solvent characteristics. However, high NADES viscosity and concerns regarding IL application highlight the need for optimized hybrid formulations. Combining these solvents with microwave-assisted extraction (MAE) may further enhance protein recovery through rapid heating.

Nevertheless, NADES–IL hybrid systems coupled with MAE for moringa seed protein extraction remain largely unexplored. This study investigates different NADES–IL formulations for protein recovery from *Moringa oleifera* seeds, comparing their performance with conventional alkaline extraction. Protein yield, physicochemical characteristics, and techno-functional properties of the recovered isolates are evaluated.

Research Method

Moringa seeds were ground and defatted using hexane. Conventional extraction was performed at pH 12 using NaOH (seed-to-water ratio of 1:10), followed by stirring for 1 h, centrifugation, and isoelectric precipitation at pH 4.5. The recovered protein was adjusted to pH 7 and freeze-dried.

Four NADES formulations were prepared using choline chloride (ChCl) or choline hydroxide (ChOH) as hydrogen bond acceptors (HBA), and glycine or glucose as hydrogen bond donors (HBD), at a 1:1 molar ratio with 30 wt% water. Three ILs ([BMIM]Br, [BMIM]BF_4, and pyrrolidinium propionate) were added at 10% (NADES = 9:1), yielding 12 hybrid formulations. Thus, 16 solvent systems (4 NADES and 12 NADES–IL) were evaluated.



Picture 1. MAE



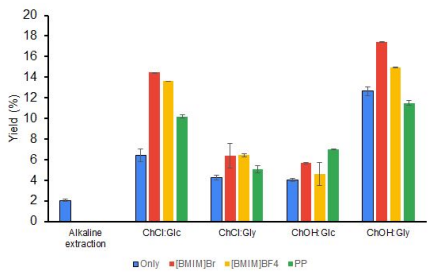
Picture 2. Moringa seeds protein isolate



Picture 3. Freeze drying process

For NADES-based extraction, defatted seeds (1:10, w/v) were stirred with each solvent for 30 min, followed by MAE (300 W, 50 °C, 2 min). Protein isolates were recovered through centrifugation, precipitation (pH 4.5), cold-water washing, pH adjustment to 7, and freeze-drying.

Protein yield, content, solubility, WHC, OHC, foaming, emulsifying properties, and zeta potential were evaluated. Selected isolates underwent FTIR, SDS-PAGE, thermal stability, and amino acid composition analyses.



Picture 4. Protein yield

Discussion & Result

One-way ANOVA showed significant differences ($p < 0.05$) among conventional extraction and the four NADES-based treatments for all evaluated parameters. Two-way ANOVA of the 12 NADES–IL formulations also indicated significant effects of NADES type, IL type, and their interaction ($p < 0.05$) across the evaluated protein properties.

The highest protein yield was obtained with ChOH:Gly:[BMIM]Br (17.45 ± 0.04%), compared with 2.061 ± 0.1% for conventional extraction. ChOH:Glu:[BMIM]BF_4 produced the highest WHC (6.58 ± 0.88 g/g), while ChOH:Glu:[BMIM]Br showed the highest OHC (9.12 ± 0.06 g/g). In contrast, conventional extraction gave the highest FC (83.76 ± 0.74%) and FS (90.30 ± 0.52%), followed by ChOH:Gly:[BMIM]Br for FC and ChOH:Gly for FS. ChOH without IL showed the highest EAI (76.89 ± 4.68%), whereas ChOH:Gly achieved the highest ESI (85.02 ± 0.13%). The highest solubility (92.87 ± 4.96%) was observed for ChOH:Gly:[BMIM]Br. ChOH exhibited the most negative zeta potential (−32.937 ± 2.6 mV).

These results indicate that solvent composition affected protein recovery and functionality differently, with no single formulation being optimal across all properties.

Conclusion

NADES–IL hybrid solvents coupled with MAE substantially improved moringa seed protein recovery compared with conventional alkaline extraction, increasing the highest yield from 2.061 ± 0.1% to 17.45 ± 0.04%. Statistical analysis confirmed that solvent composition significantly affected protein yield and the evaluated physicochemical and techno-functional properties. However, the best-performing formulation differed by property. ChOH:Gly:[BMIM]Br gave the highest yield and solubility, while ChOH hybrids provided the highest water- and oil-holding capacities. Conventional extraction retained the highest foaming capacity and stability, whereas the highest emulsifying activity and stability were obtained using ChOH and ChOH:Gly, respectively. These findings demonstrate that NADES–IL composition can be tailored to obtain specific functional properties rather than a universally superior isolate. The hybrid solvent–MAE approach is a promising alternative for moringa protein recovery, although further optimization and assessment of solvent removal, residual ILs, and protein structure are needed before food applications.