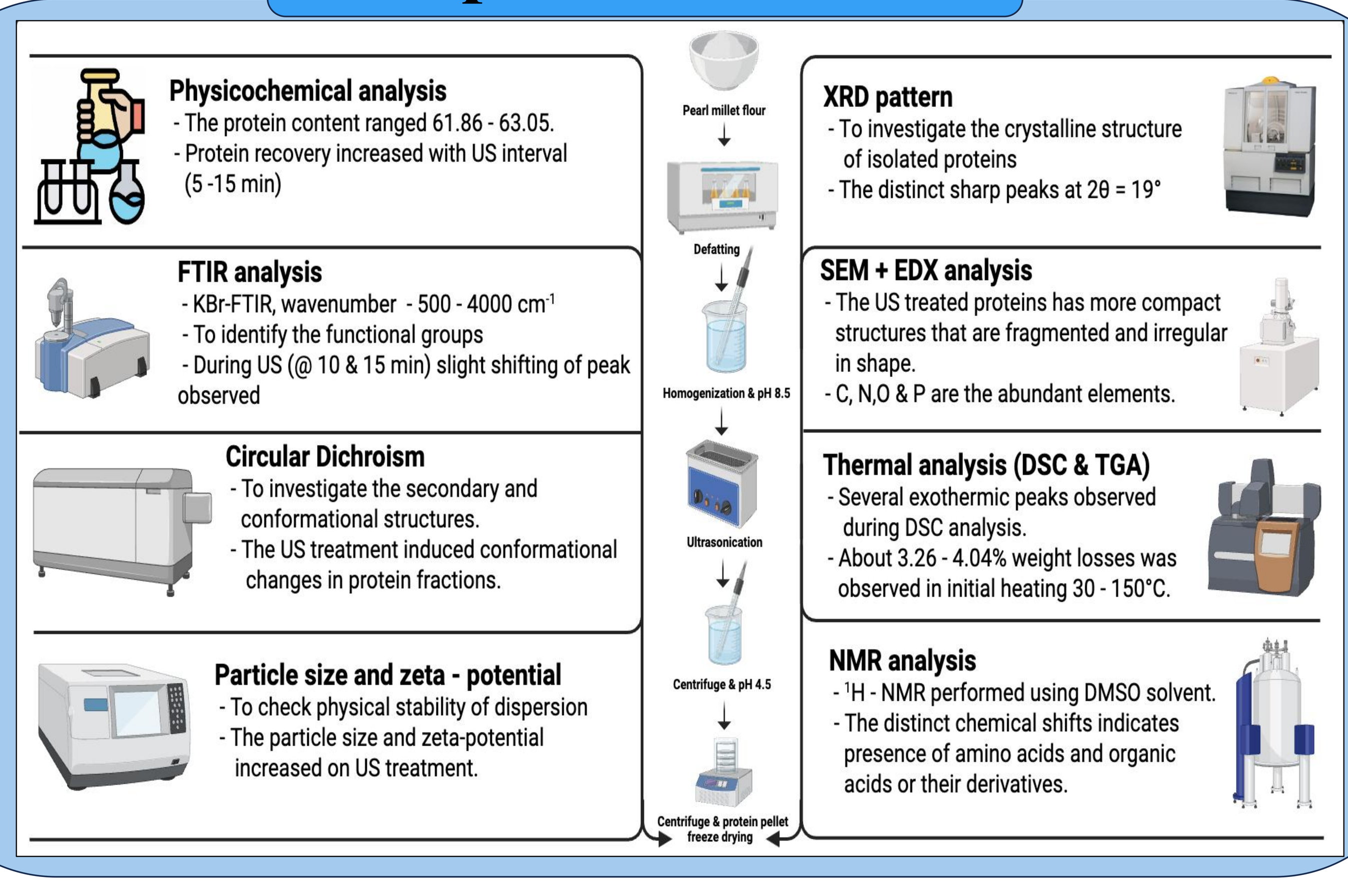
Unlocking the functional and therapeutic potential of ultrasound-assisted millet protein extract using emerging analytical techniques like IR spectrometry, NMR, FTIR, Circular dichroism, XRD, and microscopic imaging.

Sunil Meena, Upendra Singh
Department of Dairy Sciences and Food Technology, Institute of Agricultural Sciences, BHU, Varanasi, U.P, India.
Corresponding author: Dr. Sunil Meena; Email ID : sunilmeena@bhu.ac.in

Introduction

Millets are gaining global recognition as nutritious, climate-resilient crops that can address food security and dietary needs. Among them, pearl millet (*Pennisetum glaucum*) is the most extensively grown, accounting for 75% of global millet cultivation. Pearl millet is particularly valued for its high macronutrient content, including proteins and carbohydrates, and is a rich source of dietary fiber, soluble and insoluble, as well as resistant starch. Harnessing the functional and therapeutic potential of pearl millet proteins (PMPs) requires a deeper understanding of their structural and physicochemical properties. In this study, we employed advanced analytical techniques, such as Raman spectrometry, NMR, FTIR, circular dichroism, and microscopic imaging, to investigate the impact of ultrasound (US) treatment on the structural, functional, and metabolic characteristics of PMPs.

Graphical Abstract



Methodology

Proteins from pearl millet were extracted via acid-base precipitation and subjected to ultrasound treatment for 5, 10, and 15 minutes at 45 kHz. The modified proteins were then characterized using particle size and zeta potential measurements, FTIR spectroscopy, XRD, CD spectroscopy, SEM, DSC, TGA, and ^{1}H NMR. These evaluations were aimed at analyzing the impact of US on the structural, functional, and thermal behavior of the protein isolates to determine their suitability for future food processing and agricultural value chain applications.

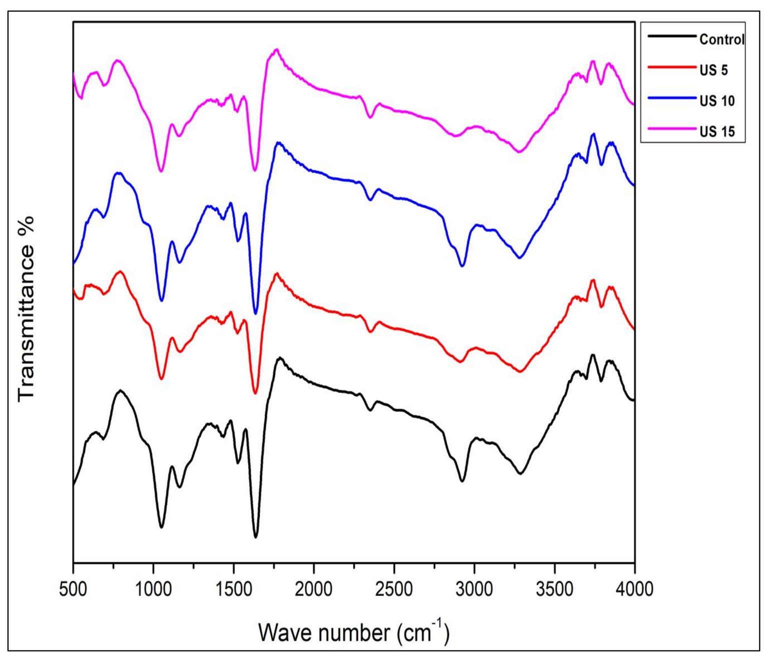


Figure 2: FTIR spectra of the control (untreated) and ultrasound-assisted PMPs (US5, US10, and US15).

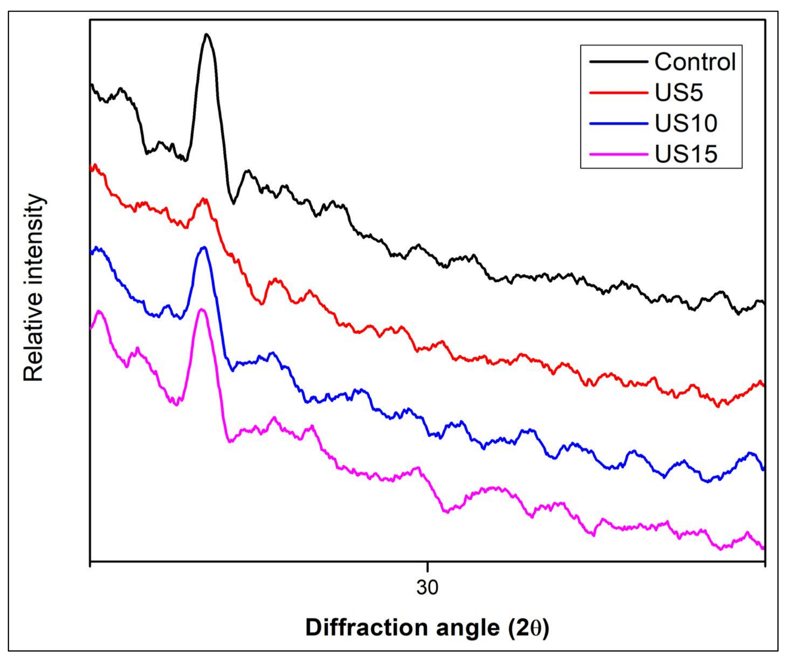


Figure 3: X-ray diffraction (XRD) pattern of control (untreated) and ultrasound-assisted PMPs (US5, US10 and US15)

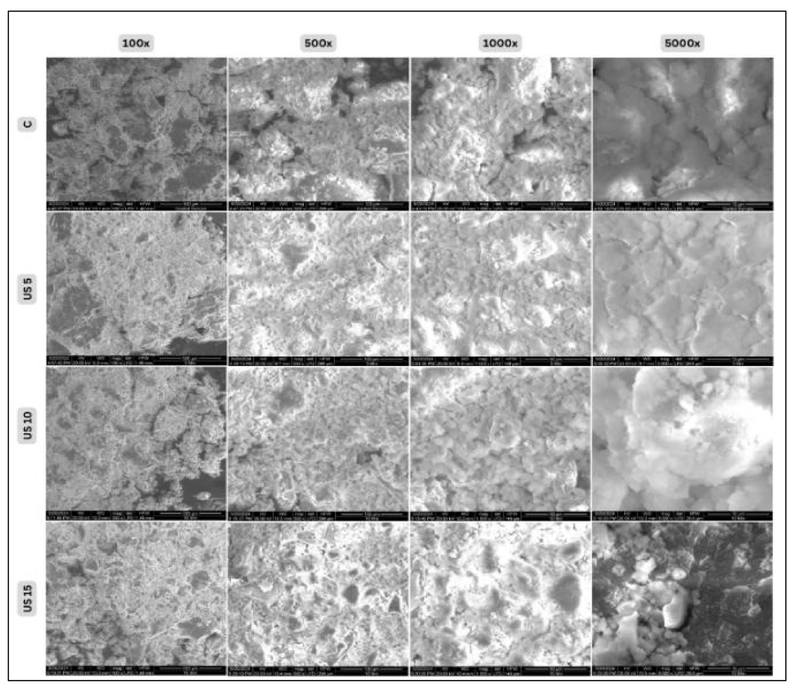


Figure 4: The scanning electron microscopy analysis of control (untreated) and ultrasound-assisted PMPs

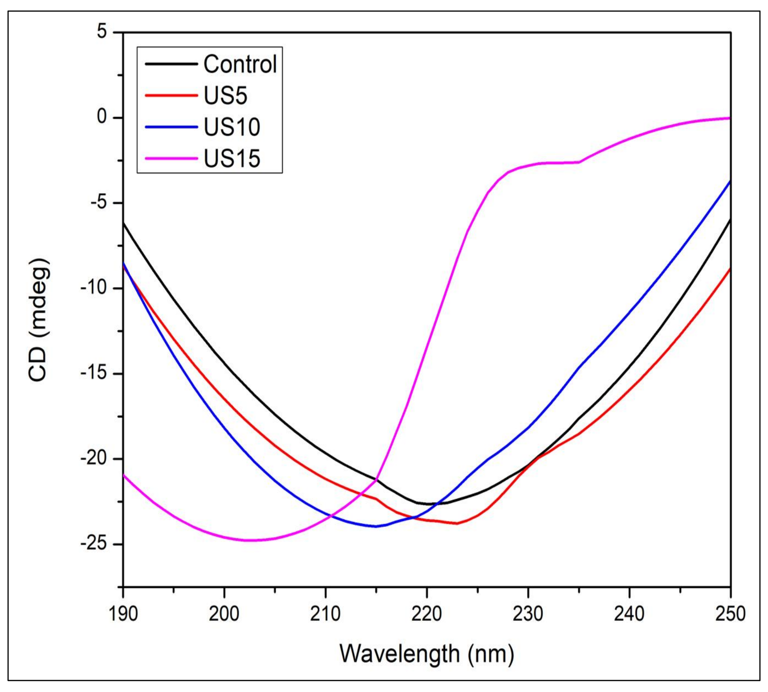


Figure 5: Secondary structure fraction and ratio as a function of the temperature of control (untreated) and ultrasound-assisted PMPs (US5, US10 and US15).

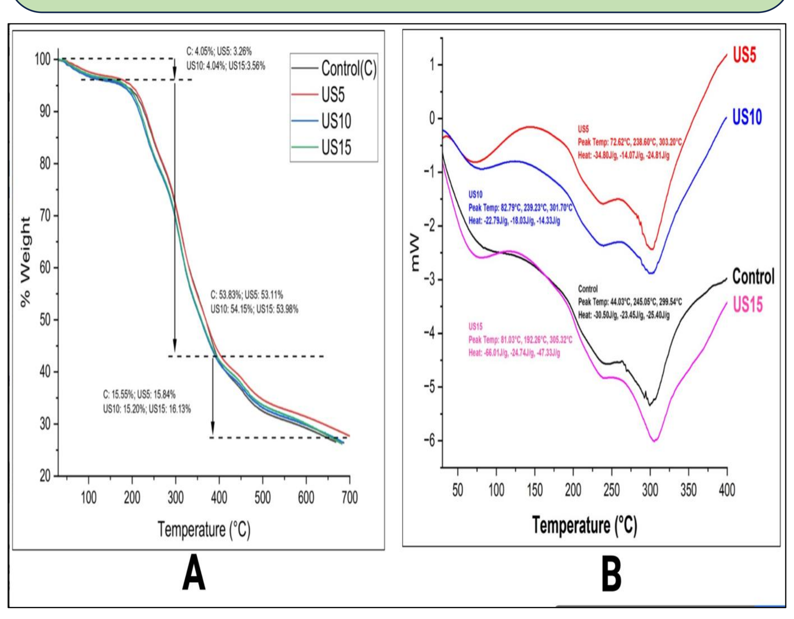


Figure 6: (A) Thermogravimetric analysis, and (B) Differential scanning calorimetry analysis of control (untreated) and ultrasound-assisted PMPs (US5, US10 and US15).

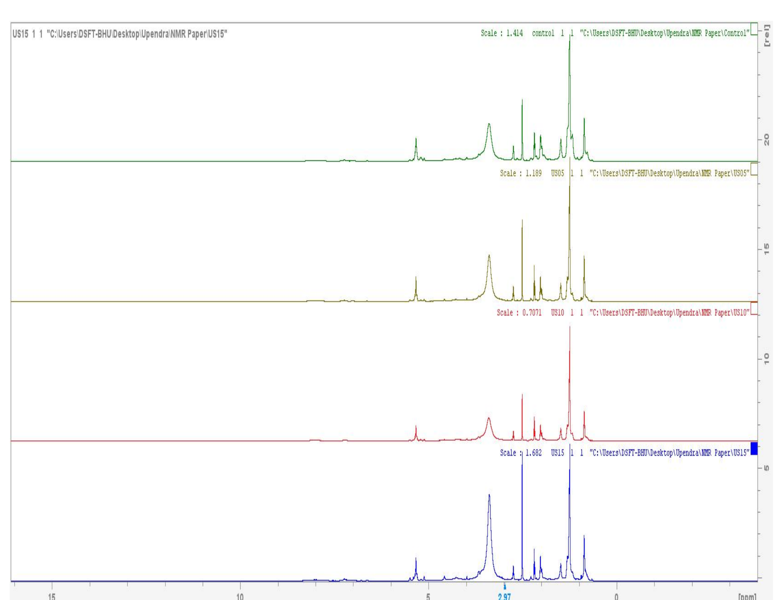


Figure 7: NMR spectra of control (untreated) and ultrasound-assisted PMPs (US5, US10 and US15).

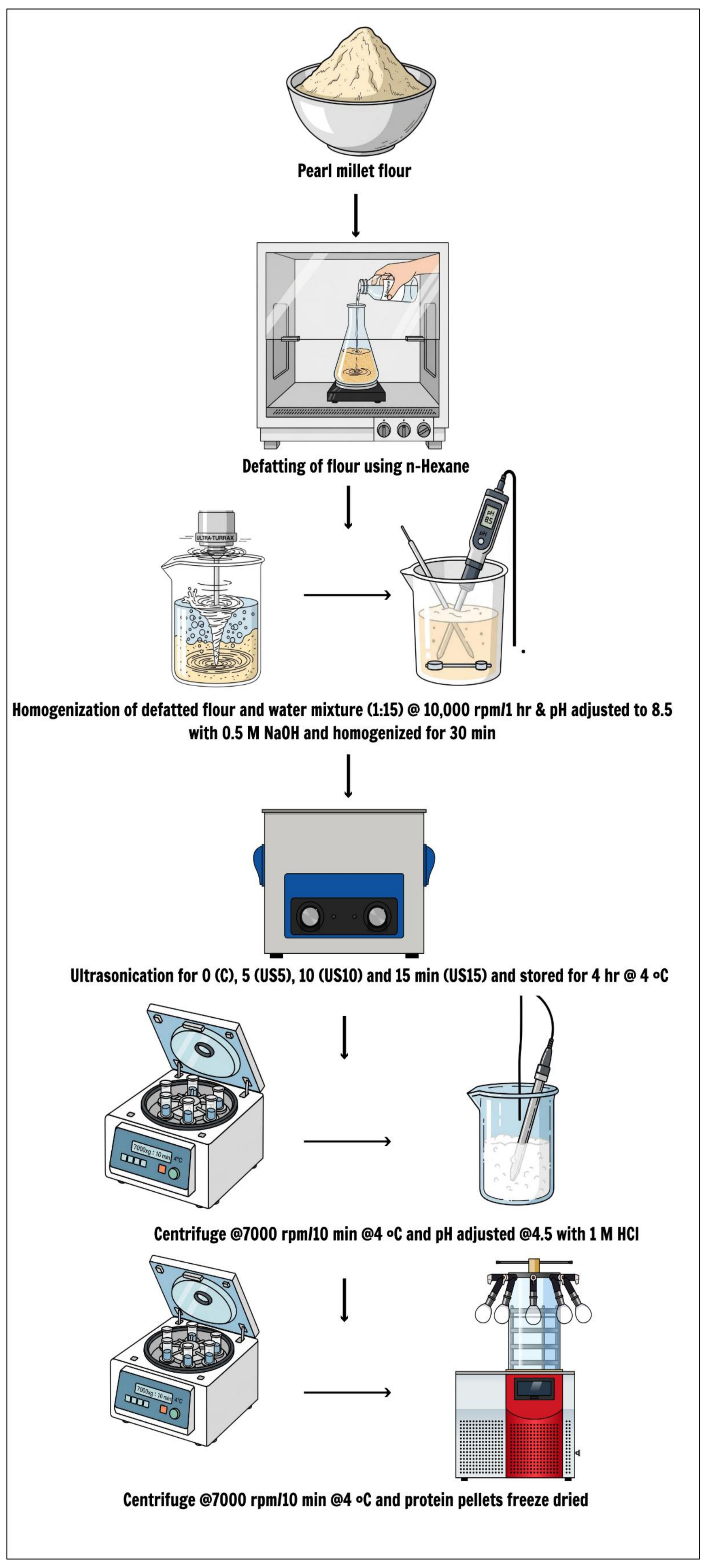


Fig 1. Flow diagram of extraction of pearl millet protein (PMP) by intervention of ultrasound treatment.

Results and Discussion

- **Enhanced Protein Recovery:** US treatment improved protein recovery, peaking at 58.81% in the 15-minute treatment, demonstrating resource efficiency.
- **Improved Functional Properties:** Solubility, water/oil holding capacity, and foaming characteristics were significantly enhanced, indicating improved functional potential for food applications.
- **Nano-Structural Refinement:** Particle size reduction and increased negative zeta potential suggested better colloidal stability, essential for food formulations.
- **Structural Transformations:** FTIR and XRD analyses confirmed disruption and rearrangement of chemical bonds, enhancing protein bioavailability.
- **Conformational Modifications:** CD spectra showed a shift from α-helix and turns to β-sheets and random coils, reflecting higher interaction potential in food systems.
- **Thermal Behavior:** Lower denaturation temperatures and enthalpy changes (via DSC/TGA) indicated easier processing adaptability-vital for energy-efficient food manufacturing.
- **Molecular Composition:** ^{1}H NMR revealed consistent profiles of amino and organic acids, maintaining nutritional integrity post-US treatment.

Conclusion

Ultrasound treatment significantly enhanced the structural and functional characteristics of pearl millet protein, offering promising potential for its application in food formulation and as a functional ingredient. The advanced characterization techniques provided valuable insights into the physical, structural, thermal, and metabolic properties of US-assisted PMPs.

Acknowledgement

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