Nutraceutical Loaded Hydrogels with Enhanced Water Retention for Functional Food Delivery

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Abstract. Developing sustainable biopolymer matrices is essential for advancing functional food innovation. This study optimizes composite hydrogels formulated from sodium alginate and silk cocoon extract by comparing aqueous and hydro-ethanolic extraction systems. A comparative analysis of methodologies for extracting silk cocoon bioactive compounds revealed that aqueous extraction yielded a superior profile to hydro-ethanolic methods. The aqueous extract achieved a protein concentration of 30.41 ± 1.54 mg/L and demonstrated a DPPH radical scavenging capacity of 32.91 ± 7.26%, linking the functional bioactivity performance to its specific phytochemical composition. The macro-structural performance of formulated hydrogels—plain hydrogel (PH) versus extract-loaded hydrogel (EH) was evaluated via swelling, syneresis, and cryo-stability assays. Network hydration behavior was sensitive to environmental pH; at neutral pH (7.0), extract-loaded variant (EH) exhibited a controlled expansion with a maximum swelling ratio of 67.85 ± 4.89%, lower than that of the plain hydrogel (PH). Syneresis analysis revealed a stabilized liquid-phase retention in EH formulation (57.23 ± 1.46%) compared to PH formulation (48.91 ± 15.10%) demonstrating superior stability against syneresis of EH. Following freeze-thaw cycles, both hydrogels exhibited high structural integrity, with recovery rates of 84.35 ± 3.94% and 97.68 ± 0.28% for EH and PH, respectively. These findings establish a scalable framework for the effective formulation of nutraceutical composite hydrogel with superior water retention, reduced syneresis, and enhanced cryo-stability, demonstrating the potential of aqueous extraction to boost nutritional density and shelf-stability in health-promoting food products.

1 Introduction

The global food industry is currently undergoing a transformative shift toward functional foods, driven by consumers who increasingly demand health-promoting benefits beyond basic caloric intake. This paradigm shift has accelerated the development of nutraceutical delivery designed to protect and deliver bioactive compounds into the human body. However, a major hurdle remains: many high-value bioactive, such as antioxidants and

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proteins are inherently sensitive to light, oxygen, and extreme pH fluctuations encountered during industrial processing and gastrointestinal transit [1].

To mitigate these issues, natural polymer-based hydrogels have emerged as one of the most promising macroscopic carriers for the food sector. Unlike microscopic delivery systems, hydrogels offer a three-dimensional, water-swollen network that provides high loading capacity, adjustable viscoelasticity, and a protective spatial structure for embedding sensitive nutraceuticals [2]. Among biopolymers, sodium alginate-a linear polysaccharide derived from brown algae-is widely used for its egg-box gelation mechanism, biocompatibility, and non-toxicity. Despite its versatility, pure alginate hydrogels often exhibit limitations such as low mechanical strength, rapid disintegration in physiological environments, and high syneresis rates, which can compromise the shelf-stability of functional food products [3, 4].

Recent innovations focus on formulating composite hydrogels to overcome these structural deficiencies. A burgeoning area of research involves the integration of silk proteins, specifically those derived from the silk cocoon of *Bombyx mori.* Beyond its traditional use in textiles, the silk cocoon is a rich reservoir of bioactive proteins (fibroin and sericin) and diet-derived phytochemicals, including flavonoids like quercetin and kaempferol [5]. Sericin, once considered as a waste byproduct of the silk industry, is now recognized for its exceptional antioxidant, anti-inflammatory, and cytocompatibility, making it an ideal candidate for nutraceutical enrichment [6].

The functional performance of silk-based extracts is heavily determined by the extraction methodology. High -impact research conducted between 2021 and 2026 highlights a shift toward green extraction techniques to ensure food safety. While various solvents are available, simple aqueous methods at elevated temperatures have proven effective for recovering sericin while preserving its antioxidant capacity [6, 7].

Furthermore, recent studies suggest that combining silk fibroin/sericin with alginate can create a synergistic network that avoids the need for chemical crosslinkers, potentially improving macro-structural performance like liquid-phase retention and freeze-thaw stability [3, 4].

This research aims to optimize the development of a nutraceutical-loaded hydrogel by incorporating silk cocoon extracts into a sodium alginate matrix. By evaluating protein yield, DPPH radical scavenging capacity, and macro-structural assays (swelling, syneresis, and cryo-stability), this research seeks to establish a scalable framework for health-promoting food products with enhanced shelf-stability and nutritional density.

2 Materials and Methods

2.1 Materials

The *Bombyx mori* cocoon was obtained from local community in Thailand. Sodium alginate is a food grade purchased from LANA GROW Co., Ltd. (Thailand). Deionized (DI) water was used in all experiments, and other chemicals were analytical reagent grade.

2.2 Methods

2.2.1 Preparation of Silk Cocoon Aqueous Extract and Ethanolic Extract

Aqueous extraction was carried out by 4.15 g of silk cocoons in 400 mL of distilled water. The mixture was then heated on a hot plate at 90°C for 60 minutes. The resulting solution was thermally concentrated to reduce its volume to 250 mL. To remove any insoluble

residues and ensure purity, the extract was filtered through Whatman No. 1 filter paper. The hydro-ethanolic extract was prepared using a solvent system composed of distilled water and ethanol in a 1:1 (v/v) ratio. In the same procedure, 4.15 g of silk cocoons were added to 400 mL of the water-ethanol mixture.

2.2.2 Physiochemical Characterization

The physiochemical properties of silk cocoon aqueous extract and hydro-ethanolic extract were evaluated by determining pH, Total Dissolved Solids (TDS) and Electrical Conductivity (EC). The pH was measured using a digital pH meter, while TDS and EC were quantified using standardized multi-parameter probes to assess the mineral and ionic concentrations within the extracts. The functional groups of the hydrogel materials were examined by Fourier Transform Infrared Spectroscopy using the attenuated total reflection mode (ATR–FTIR, VERTEX 70, Bruker, Billerica, MA, USA).

2.2.3 Determination of Protein Content

Total protein concentrations of silk cocoon aqueous extract and hydro-ethanolic extract were quantified via the Lowry method [8]. Bovine Serum Albumin (BSA) was utilized as a standard. BSA concentrations ranged from 30 to 480 µg/mL. The extract was treated with alkaline copper reagent followed by Folin-Ciocalteu reagent. After incubation, the absorbance of sample and standards were measured at 660 nm using spectrophotometer (UV 2600, Shimadzu).

2.2.4 DPPH Radical Scavenging Activity

The antioxidant capacity of silk cocoon aqueous extract and hydro-ethanolic extract was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay [8]. A 1 mL of the extract was added to 3 mL of ethanolic DPPH solution. The mixture was shaken and incubated in the dark for 30 minutes. The reduction of the DPPH radical was monitored by measuring the absorbance at 517 nm. The percentage of antioxidant activity (%AA) was calculated as shown.

$$\% \text{ of antioxidant activity} = \frac{Ac - As}{Ac} \times 100 \qquad (1)$$

where Ac = Absorbance of control, As = Absorbance of sample

2.2.5 Preparation of Hydrogel

Plain hydrogel (PH) and Extract loaded-hydrogel (EH) were prepared using a sodium alginate-based matrix. The ingredients were mixed using a magnetic stirrer for 1 hour to ensure the formation of a uniform polymer network. The resulting solution was then dispensed into molds in 10 mL aliquots. To induce gelation, 20 mL of a 0.5% (w/v) $CaCl_2$ solution was added slowly to each mold using a pipette.

Table 1. Composition of Plain Hydrogel and Extract Hydrogel Formulation

Materials	Plain Hydrogel (PH) (%w/w)	Extract Hydrogel (EH) (%w/w)
Sodium Alginate	1.9	1.9
Glycerol	2.4	2.4
Allantoin	0.5	0.5
Sodium Benzoate	0.1	0.1
Water/ Extract	95.1	95.1

2.2.6 Moisture Content Determination

Moisture content of the hydrogels was determined by the weight-loss method. Hydrogel samples were placed in a laboratory oven and dried at 40°C for 4 hours. After drying, the containers were immediately covered, cooled to room temperature in a desiccator, and re-weighed. All measurements were performed in triplicate. The percentage of water content was calculated as shown.

$$\text{Moisture content (\%)} = \frac{m_1 - m_2}{m_1} \text{ x } 100 \qquad (2)$$

where m_1 = mass of sample before drying, m_2 = mass of the sample after drying

2.2.7 Swelling Test

The swelling capacity was evaluated by immersing pre-weighed hydrogel samples in buffer solutions at specified levels (pH 5.5 and pH 7) at room temperature. At designated time intervals of (15 mins to 7 days), samples were removed, blotted to remove surface water, and re-weighed. The Swelling Ratio (SR%) was calculated as:

$$\text{SR (\%)} = \frac{M_s - M_d}{M_d} \text{ x } 100 \qquad (3)$$

where M_s = mass of swollen hydrogel, M_d = mass of initial hydrogel

2.2.8 Syneresis Test

Syneresis was measured by keeping the hydrogel samples kept at 25°C and weighed on designated days (Day 1, Day 2, Day 3, and Day 7). Syneresis rate (S%) was calculated as:

$$\text{S (\%)} = \frac{W_o - W_d}{W_o} \text{ x } 100 \qquad (4)$$

where W_o = mass of initial hydrogel, W_d = mass of hydrogels after storage

2.2.9 Freeze-Thaw Stability Test

Freeze-thawing stability was carried out on hydrogel samples that were kept in the refrigerator of -18°C for 24 hours. It was then thawed at 25°C for 4 hours. The samples were then kept at 25°C and weighed for designated days of (Day 1, Day 2, Day 3, and Day 7). The syneresis rate was then calculated using the equation as indicated in 2.2.8.

3 Results and discussion

3.1 Extraction Yield of Silk Cocoon Aqueous and Hydro-ethanolic extract

The extraction process aimed to isolate soluble sericin proteins and secondary metabolites from the *Bombyx mori* silk cocoons. The appearance of the silk cocoon and its extract at different processing solvent is presented in Fig. 1. The raw starting material (A) consists of intact, fibrous silk cocoons. Following aqueous (B) and the hydro-ethanolic extract (C), the resulting extracts appeared as a distinct yellow coloration, indicating a set of compounds from the silk cocoons. The yield for both the aqueous and hydro-ethanolic extractions was determined by the gravimetric difference between the initial cocoon mass and the dried insoluble residue. The aqueous extraction yielded 20.58 %w/w. The hydro-ethanolic extraction resulted in a slightly lower yield of 17.78 %w/w. The higher recovery observed in the aqueous system is consistent with the known solubility characteristics of sericin, which readily dissolves under thermal aqueous conditions due to disruption of intermolecular hydrogen bonding and structural relaxation of amorphous domains [9].

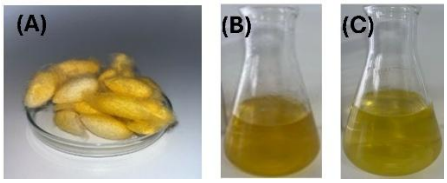

Fig. 1. Visual appearance of (A) Silk cocoon, (B) Silk cocoon aqueous extract and (C) Silk cocoon hydro-ethanolic extract

3.2 Physiochemical Analysis

The chemical profiles of the extracts are characterized by measuring pH, TDS, and EC (Table 2). These provide insight into the concentration of the dissolved proteins and minerals liberated from the silk matrix.

Table 2. Physiochemical Properties of Silk Cocoon Extracts

Parameter	Aqueous Extract	Hydro-ethanolic Extract
pH	7.41 ± 0.01	7.32 ± 0.02
TDS (ppm)	97 ± 1	97 ± 2
EC (µS/cm)	524.0 ± 3.6	280.0 ± 1.7

Both extracts-maintained a near-neutral pH, with aqueous extract at 7.41 ± 0.01 and hydro-ethanolic extract at 7.32 ± 0.02. This alkalinity is typical for sericin-rich solutions, as the amino acid composition of silk protein often exerts a buffering effect [10]. The slight decrease in pH in the hydro-ethanolic sample may be attributed to the presence of ethanol, which can slightly alter the ionization of organic acids present in silk cocoon. Interestingly, both extraction methods resulted in an identical TDS value of 97 ± 1ppm. This indicates that the total mass of dissolved inorganic and organic substances remained consistent across both solvent systems. However, a significant divergence was observed in Electrical Conductivity (EC). The aqueous extract showed an EC of 524 ± 3.6 µS/cm, nearly double of hydro-ethanolic extract at 280.0 ± 1.7 µS/cm. The higher EC in aqueous extract suggests a greater concentration of mobile ions and electrolytes. Pure water at 90°C is highly efficient at leaching ionic minerals (such as potassium or calcium) and charged amino acid fragments from the silk.

3.3 Protein Content

The aqueous extraction yielded a higher protein content of 30.41 ± 1.541 mg/L, whereas the hydro-ethanolic extraction produced a lower concentration of 17.925 ± 8.470 mg/L. Protein recovery in the aqueous medium is likely due to the high-temperature conditions, which facilitate the thermal degradation and solubilization of the sericin layer into the polar phase. The utilization of ethanol in hydro-ethanolic extraction may have induced partial protein denaturation or reduced the solubility of high-molecular weight proteins, resulting in the lower observed yield and the higher standard deviation which suggests less consistent extraction performance in the hydro-ethanolic extraction.

3.4 DPPH Radical Scavenging Activity

For antioxidant properties, scavenging activity of silk cocoon aqueous extract and hydro-ethanolic extract was determined by DPPH assay. DPPH free radicals have ability to receive electron or bind with hydrogen from antioxidant molecules becoming a stable molecule. The aqueous extract exhibited a higher DPPH scavenging activity (32.91 ± 7.26%) compared to the hydro-ethanolic extract (28.37 ± 27.24%). However, the large standard deviation associated with the hydro-ethanolic extract suggests considerable variability in its antioxidant activity. This antioxidant potential suggests that aqueous extraction was more effective at liberating and stabilizing heat-stable bioactive compounds, such as sericin associated flavonoids and phenolic acids compared to the hydro-ethanolic extract [11].

3.5 Material Characterization

Plain hydrogel (PH) and Extract-loaded hydrogel (EH) were prepared by Ionotropic Gelation. The extract-loaded hydrogel formulation comprises five key components: silk cocoon extract, sodium alginate, glycerol, allantoin, and sodium benzoate. Each ingredient serves a distinct physicochemical or biological function essential to the formulation's structural integrity, stability, and nutraceutical properties. The functional performance of silk-based extract was determined as previously described. Sodium Alginate serves as the primary gelling agent and structural polymer. biocompatibility and mild gelation conditions make it particularly suitable for pharmaceutical and food applications. Glycerol functions as a plasticizer and humectant. Allantoin acts as a bioactive therapeutic agent with keratolytic, moisturizing, and tissue-regenerating properties. Despite its widespread use in dermatology and cosmetics industry, allantoin is an endogenous substance in humans, also present in medicinal plants, and constitutes a normal component of the human diet [12]. While allantoin is not a standard food ingredient, its oral safety is supported by its use in oral pharmaceutical products and toxicity studies. However, further research is needed to fully investigate the bioavailability and safety of allantoin as food ingredient. For sodium benzoate, sodium benzoate (0.1% w/v) was incorporated solely as a preservative to ensure microbiological stability of the hydrogel system. While sodium benzoate is widely recognized as safe for food applications, its interaction with bioactive compounds may depend on environmental factors such as pH and composition. In acidic conditions, benzoic acid formation can potentially influence the stability of sensitive compounds; however, the present system maintained a near-neutral pH (7.3-7.4), where sodium benzoate predominantly exists in its ionized form, reducing its reactivity with biomolecules. Furthermore, no observable changes in physiochemical properties or functional performance (e.g., antioxidant activity and structural integrity) were detected, suggesting minimal or negligible interaction with the silk-derived bioactive components. These findings indicate that, at the applied concentration,

sodium benzoate does not adversely affect the stability or functionality of the formulated hydrogel.

As shown in Fig. 2, FTIR spectra of PH and EH revealed key absorption bands corresponding to functional groups sodium alginate. The broad peak at 3200–3600 cm^{-1} indicated O–H stretching band of hydrogen-bonded hydroxyl groups. The distinctive peak at around 1810 cm^{-1} indicated the C=O stretching vibration of carbonyl or carboxylate groups. The asymmetric and symmetric carboxylate (COO^-) stretching vibrations observed at approximately 1510 cm^{-1} and 1370 cm^{-1}, respectively. Additional bands include weak C–O stretching vibrations at 1010–1080 cm^{-1} region, corresponding to the ether linkages and saccharide structure of the polysaccharide backbone.

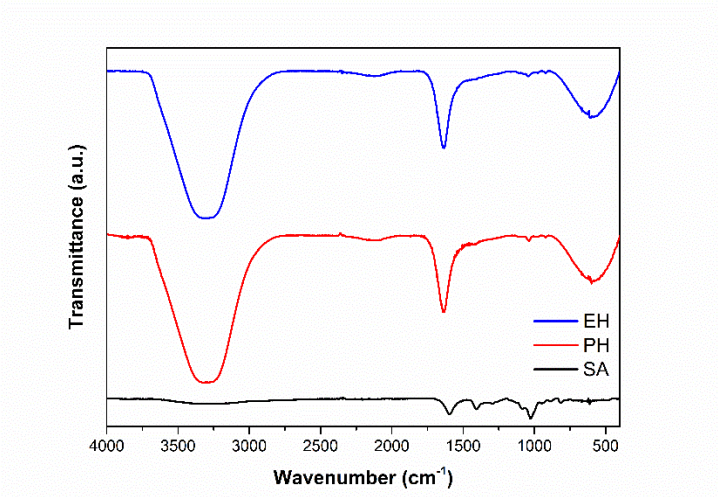

Fig. 2. FTIR spectra of sodium alginate (SA), alginate-based hydrogels as plain hydrogel (PH) and extract-loaded hydrogel (EH)

3.6 Moisture content of hydrogels

The water content of the hydrogels was quantified using the weight loss method to evaluate the influence of silk cocoon extract on the polymer network's moisture-holding properties. As illustrated in Fig.3, PH exhibited a slightly higher water content compared to EH. Quantitative analysis showed that the PH formulation possessed a water content of approximately 11.5%, while the EH formulation remained around 7.8%. This observed difference can be attributed to alterations in the polymeric network structure upon the incorporation of the silk cocoon extract. The extract has various phytochemical or proteinaceous compounds that may compete with water molecules for hydrogen bonding sites within the polymer matrix. By occupying these sites, the extract effectively reduces the overall water-binding capacity of the hydrogel, leading to a lower equilibrium moisture content. Furthermore, the introduction of the extract increases the density of the polymer network or promotes additional crosslinking, resulting in a more compact structure with reduced free volume for water entrapment. This phenomenon is commonly observed in loaded hydrogel systems, where the presence of active compounds displaces water and modifies the hydrophilic-hydrophobic balance of the formulation.

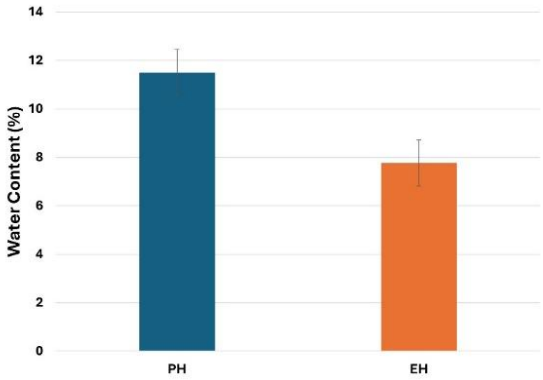

Fig. 3. Comparative water content (%) of plain hydrogel (PH) and extract-loaded hydrogel (EH)

3.7 Swelling Kinetics and pH-Responsiveness

The swelling behaviour of the hydrogels was evaluated to understand their hydration capacity and network stability under varying environmental conditions summarized in Fig. 4. At a neutral pH (7.0), PH and EH exhibited a maximum swelling ratio of 78% and 68%, respectively. The swelling ratio of EH was lower than that of the PH variant. This reduction in swelling capacity suggests that the integration of silk cocoon extract specifically the sericin and fibroin components creates a more compact and densely cross-linked synergistic network within the alginate matrix. Under acidic conditions, protonation reduces electrostatic repulsion and contracts the network, whereas neutral pH increased ionization promotes osmotic swelling stress [13].

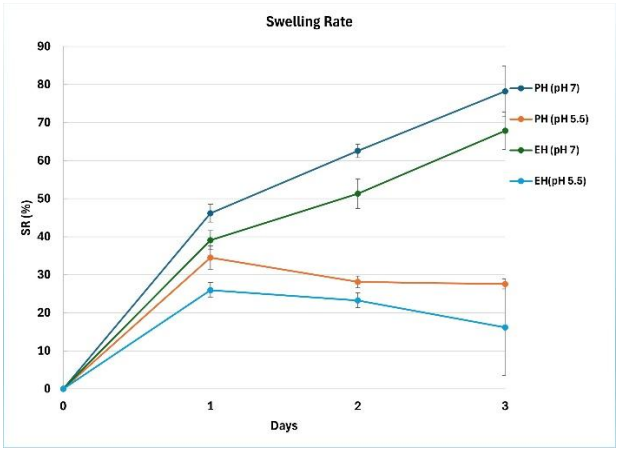

Fig. 4. Swelling ratio (SR%) of plain hydrogel (PH) and extract-loaded hydrogel in different pH environments (pH 5.5 and pH 7.0) for 3 days period

The swelling behavior of the hydrogels at pH 5.5 shows a complex two-phase kinetic profile characterized by initial network expansion followed by significant contraction. Both PH and EH formulations exhibited a decline in swelling ratio (SR%) over time, with the EH reaching a minimum of approximately 16% on Day 3. This initial contraction observed at pH 5.5 is likely attributed to the protonation of carboxyl groups within the sodium alginate matrix. The reduction in electrostatic repulsion between polymer chains facilitates the formation of a more compact "egg-box" structure, thereby limiting water uptake. Throughout this period,

the EH consistently maintained a lower SR% compared to the PH, further supporting the role of incorporated silk proteins as structural stabilizers that reinforce the gel network. Interestingly, this contractive behavior at pH 5.5 stands in contrast to the hydrogel performance at pH 7.0, where excessive swelling stress resulted in complete structural degradation and dissolution by Day 3.

The macro-structural integrity and visual transition of hydrogels are shown in Fig.5. At a neutral pH 7.0, both the PH and EH variants exhibited progressive expansion within the first 48 hours. However, by 72 hours, noticeable structural failure was evident, characterized by surface fracturing and loss of spherical integrity. This physical deterioration confirms that the extensive swelling stress at neutral pH exceeded the mechanical tolerance of the hydrogel network, ultimately leading to the complete degradation observed by Day 7. Such behavior is typically associated with ion-exchange processes and alterations in polymer chain relaxation mechanisms inherent to alginate-based matrices [14].

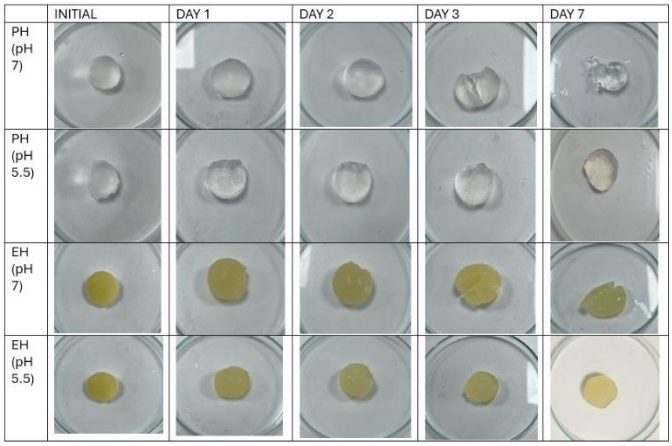

Fig. 5. Visual morphological changes of plain hydrogel (PH) and extract-loaded hydrogel (EH) during a 72-hour swelling study at pH 7.0 and pH 5.5

3.8 Syneresis and Liquid-Phase Retention

The liquid phase retention and structural stability of hydrogels were evaluated through a syneresis assay over a 7-day period at 25°C. As illustrated in Fig.6, both PH and EH exhibited a progressive increase in syneresis rate over time, which corresponds to the spontaneous expulsion of water from the polymer matrix. On Day 1, the EH formulation demonstrated superior initial stability, retaining 57.23 ± 1.46% of the liquid phase, compared to the PH formulation, which exhibited a lower retention rate of 48.91 ± 15.10%. The markedly lower standard deviation associated with the EH further indicates more consistent and reproducible performance. The visual transition showed a gradual reduction in hydrogel volume and decreased translucency as storage progressed Day 7. While both types of hydrogels eventually reached high syneresis levels, the significantly lower standard deviation found in the EH samples during the early stages suggested that the silk cocoon extract-comprised of sericin and fibroin acts as a structural stabilizer that enhances the initial water-binding capacity of the alginate ‘egg-box’ network [15]. This reduced syneresis in the composite EH network is critical for maintaining the shelf-stability and nutritional density of functional food products, preventing the premature leakage of encapsulated bioactive compounds.

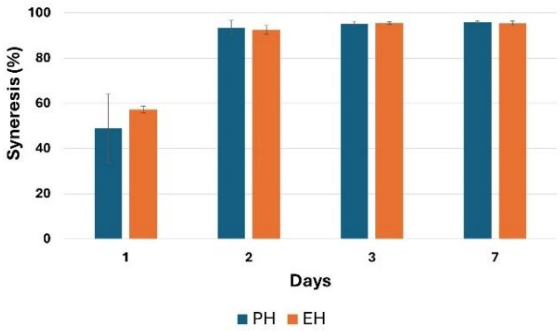

Fig. 6. Comparative syneresis rates (%) of Plain Hydrogel (PH) and Extract-loaded Hydrogel (EH) during storage at 25°C over 7-day period

3.9 Freeze-thawing Stability and Structural Integrity

The cryo-stability of the hydrogels was evaluated to determine their capacity to maintain structural integrity after extreme thermal fluctuations, a critical factor for functional foods requiring cold-chain distribution. As illustrated in Fig. 7(A), both the plain hydrogel (PH) and extract-hydrogel (EH) demonstrated high macro-structural resilience following a 24-hour freezing cycle at -18°C. The PH formulation exhibited a substantially higher recovery rate (97.68 ± 0.28%) compared to the EH formulation (84.35 ± 3.94%), indicating greater structural resilience during analysis. The slightly lower recovery rate in the EH samples may be attributed to the increased complexity of the composite matrix; the integration of silk cocoon proteins creates a more densely cross-linked network that may experience minor stress during ice crystal formation. The visual progression in Fig. 7(B) confirms these quantitative findings. Upon thawing, both hydrogels successfully regained their original shape. The EH samples retained their characteristic yellow hue, indicating that the silk extract remained securely encapsulated within the matrix.

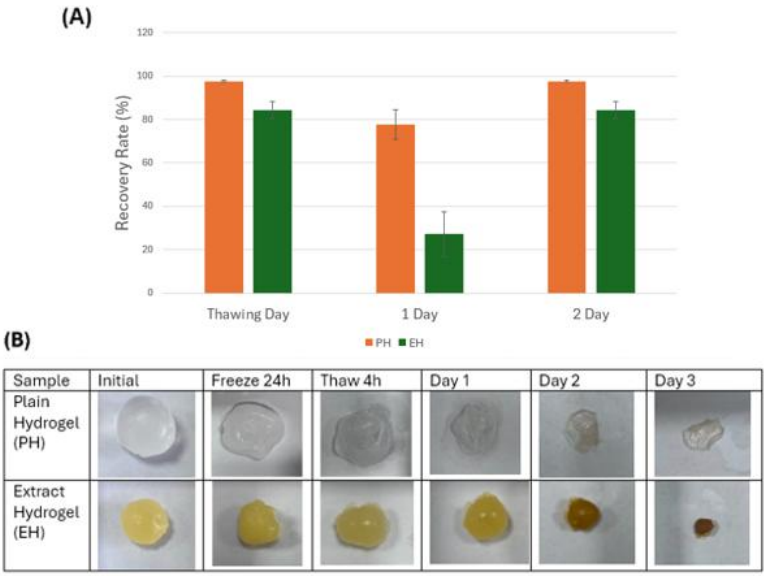

Fig. 7. Freeze-thaw stability of PH and EH. (A) Quantitative Freeze-thaw recovery rates. (B) Macroscopic appearance of PH and EH samples at different stages of the freeze-thaw process

4 Conclusion

This study revealed that the extraction system significantly influenced the bioactive profile of *Bombyx mori* silk cocoon extracts. Incorporation of the silk cocoon extract into alginate-based hydrogel reduced swelling stress and enhanced shape retention of the hydrogel, attributing to protein–polyphenol interactions with alginate chains that reinforced the polymer network. The silk cocoon extract-loaded hydrogel exhibited an enhanced structural integrity, which limited excessive water uptake within the alginate matrix. This reinforcement conferred superior stability against syneresis and improved freeze-thaw resistance. Our study suggests additional benefits of incorporating silk cocoon extract into alginate hydrogel to enhance the functional and structural properties of the hydrogel, supporting its potential application in nutraceutical delivery and functional food systems.

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