Development and Characterization of Whey Protein-Based Hydrogels for Sugar-Free Protein-Fortified Rose Jam Applications

Janjira Tangsanthatkun[1], *Peeranut* Kaimook[1], and *Kanokwan* Kingphadung[1*]

[1]Department of Food Technology, Faculty of Engineering and Industrial Technology, Silpakorn University, Nakhon Pathom, Thailand

Abstract. The growing demand for sugar-free and protein-enriched fruit preserves has driven interest in alternative structuring systems development that provide both desirable texture and nutritional benefits. This study aimed to develop and characterize whey protein isolate (WPI)–based hydrogels in combination with low methoxyl pectin (LM-pectin) and to evaluate their potential application in sugar-free, protein-fortified rose jam. WPI was incorporated at various concentrations (1, 3, and 5% w/v), and the effects on gel strength, water-holding capacity (WHC), and syneresis (%) were systematically investigated. The results demonstrated that increasing WPI concentration significantly enhanced gel strength and WHC, indicating improved network formation and water retention within the hydrogel matrix. The synergistic interaction between WPI and LM-pectin contributed to improved structural integrity. Application in sugar-free rose jam demonstrated that WPI-based hydrogels successfully provided jam-like texture, acceptable spreadability, and enhanced protein content without added sugar. These findings highlight the potential of WPI–LM pectin hydrogels as a functional and sustainable ingredient for the development of sugar-free fruit-based products, supporting innovation in health-oriented food formulations.

1 Introduction

The global food industry is experiencing a paradigm shift toward healthier, functional food products that address consumer demands for reduced sugar content and enhanced protein fortification. Excessive sugar consumption has been strongly associated with non-communicable diseases (NCDs), prompting regulatory pressures and consumer demand for sugar-free or low-sugar alternatives. However, in fruit preserves such as jams, the high sugar content (typically 55–70%) plays a critical multifunctional role, not only as a sweetener but also as a key structural component contributing to gel formation, texture, water activity control, and product stability [1]. Therefore, the reduction or removal of sugar presents significant technological challenges, particularly in maintaining desirable rheological and

* Corresponding author: kingphadung_k@su.ac.th

sensory attributes. Alternative structuring systems capable of replacing the functional role of sugar are therefore essential for next-generation fruit-based products.

Hydrogel-based structuring systems have emerged as promising alternatives for designing sugar-free fruit products. Food hydrogels are three-dimensional polymeric networks capable of entrapping large amounts of water while maintaining structural integrity as widely reported in protein-based food hydrogel systems [2]. Among various protein sources, whey protein isolate (WPI) has attracted considerable interest due to its high nutritional value, excellent gelation ability, and well-documented functional properties, including water binding, emulsification, and viscoelastic behavior [3, 4]. The gelation of whey proteins involves the transformation of native, soluble protein molecules into a three-dimensional network capable of entrapping water and other components [5, 6]. Three primary gelation mechanisms are relevant to jam and jelly applications, including heat-induced, acid-induced and cold-set gelation. When whey proteins are heated above their denaturation temperature (typically 70–80°C), causing unfolding of the native structure, exposure of hydrophobic groups and reactive thiol groups, and subsequent aggregation through hydrophobic interactions, hydrogen bonding, and disulfide bond formation [7]. The resulting gel structure depends on pH, ionic strength, protein concentration, and heating rate [8]. In addition, acid-induced gelation of whey proteins is particularly relevant for jam applications with acidic pH values of 3.0–3.5. At pH values near the isoelectric point of whey proteins (pH 4.6–5.2), electrostatic repulsion is minimized, promoting protein aggregation and gel network formation. and cold-set gelation. Moreover, cold-set gelation is relevant to initial protein denaturation followed by gelation triggered at ambient or refrigeration temperatures through addition of salts (calcium chloride, sodium chloride) or pH adjustment [9]. This mechanism offers processing advantages for heat-sensitive ingredients common in fruit-based products. Thus, whey protein-based hydrogels offer unique advantages for jam and jelly applications. They form thermally reversible or irreversible networks depending on processing conditions, exhibit excellent water-binding properties, provide desirable textural attributes, and contribute to nutritional enhancement.

Rose jam represents a value-added traditional fruit-based product with unique aroma and bioactive compounds, yet conventional formulations rely heavily on high sugar content for texture and preservation. The development of WPI–based hydrogels as alternative structuring matrices offers the dual benefit of texture replacement and protein fortification, aligning with current trends in functional, protein-enriched, and health-oriented foods. Despite extensive studies on whey protein gels, limited research has explored their integration with LM-pectin as sugar-structure replacers in acidic fruit jam systems.

Therefore, this study aimed to develop and characterize WPI-based hydrogels incorporated at three concentrations (1, 3, and 5% w/v). The effects of protein concentration on gel firmness, water holding capacity (WHC), and syneresis were systematically investigated. Furthermore, the potential application of the developed hydrogels in sugar- free, protein-fortified rose jam was evaluated to assess their feasibility as an alternative structuring system for reduced-sugar fruit-based products.

2 Materials and Methods

2.1 Materials

Whey protein isolate (WPI, ≥90% protein), citric acid, inulin, maltitol, sucralose and gellan gum were purchased from Krungthepchemi Co., Ltd., Bangkok, Thailand. Low methoxyl pectin (LM-pectin, degree of esterification < 50%) was purchased from Baker Street Co., Ltd., Bangkok, Thailand. Calcium chloride ($CaCl_2$) was used as a crosslinking agent,

obtained from S M L Chamrich Co., Ltd., Bangkok, Thailand. Dried rose and lychee powder were sourced from A.P. Siamfood Co., Ltd., Chiang Mai, Thailand. It should be noted that the dried rose powder used in this study contributed primarily to flavor and bioactive properties rather than color. A food-grade colorant was added to achieve an acceptable appearance. Therefore, the final product color was not solely dependent on native anthocyanin content. Rose flavor was provided from Aromatech Flavours Co., Ltd., Samut Prakan, Thailand.

2.2 Preparation of WPI-LM pectin hydrogels

WPI solutions were prepared at concentrations of 1, 3, and 5% (w/v) by dispersing the powder in distilled water under continuous stirring and mixed with 0.5% (w/v) LM-pectin at a predetermined ratio. The LM-pectin concentration was fixed at 0.5% (w/v), which was selected based on preliminary optimization and literature reports indicating that this range is sufficient to promote effective calcium-mediated gelation, allowing the observed changes in gel properties to be primarily attributed to variations in protein content and ionic crosslinking density. The mixture was then heated at 85 ºC for 15 min, and then cooled to room temperature to produce heat-denatured WPI [7]. For acidic conditions, the pH of the WPI–LM pectin mixture was adjusted using citric acid solution to achieve a target pH range of approximately 3.5–4.3. For neutral conditions, no pH adjustment was applied, and the system remained at its inherent pH (~6.4). $CaCl_2$ solution was then added to promote ionic crosslinking of LM-pectin and induce the gelation with final concentrations of 5, 7.5 and 10 mM using a magnetic stirrer. The formed hydrogels were stored at 4 °C for 24 h to allow network stabilization prior to analysis.

2.3 Determination of pH

The pH of each hydrogel and rose jam sample was determined by using a digital pH meter (MP 220, Mettler Toledo, Thailand).

2.4 Gel Firmness Measurement

The determination of gel firmness was based on the protocol described previously with some minor modifications [10]. Initially, hydrogels were prepared in 25 mL beakers and then maintained at room temperature for 24 h. Then the hardness was measured using an instrumental texture analyzer (Texture Analyser, Model TA-XT2i, Stable Micro System, UK) equipped with a p/0.5s stainless sphere probe. The measurement parameters used were: pre-test speed, 1.0 mm/s; test speed, 1 mm/s; trigger force, 5 g; post-test speed, 1.0 mm/s; puncture distance, 10.0 mm; and, measurement temperature, 25 °C. The firmness of the gels was referred to the hardness calculated using the software provided with the instrument. At least triplicate tests were carried out on each sample.

2.5 Water holding capacity (WHC)

The water-holding capacity (WHC) of the hydrogels was determined using a centrifugal method as previously described [10]. Briefly, 10 g of gel sample was transferred into a 50 mL centrifuge tube and centrifuged at 4,000 rpm at 25 °C for 20 min. Following centrifugation, the released water was carefully drained off, and the surface of the gel was gently blotted with filter paper to remove residual moisture. The centrifuge tube containing the gel sample was accurately weighed before and after centrifugation, respectively. The WHC of the gel was then calculated using the following equation:

$$WHC\ (\%) = (W_t - W_r)/W_t \times 100 \quad (1)$$

Where W_t is the total water weight in the gels, and W_r is the weight of water released.

2.6 Syneresis (%)

Syneresis (%) of the hydrogels was determined using a centrifugation method. Approximately 10 g of gel sample was transferred into a 50 mL centrifuge tube and centrifuged at 4,000 rpm at for 20 min at 25 °C. After centrifugation, the released water was carefully decanted without disturbing the gel matrix and weighed the tube containing the remaining gel. The syneresis of the gels was then calculated using the following equation:

$$Syneresis\ (\%) = (W_r/W_i)\ x\ 100 \quad (2)$$

Where W_r is the weight of expelled liquid, and W_i the initial weight of hydrogel sample.

2.7 Application in sugar-free rose jam

Sugar-free rose jam was prepared by dispersing dried rose and lychee powder in water and heating with 0.5% LM-pectin. The developed WPI-based hydrogel was incorporated as a structuring and protein-fortification component. Maltitol and sucralose were used as sugar substitutes to provide sweetness, while inulin was incorporated as a bulking and texturizing agent in the sugar-free formulation. The mixture was heated to approximately 85 °C with continuous stirring until a jam-like consistency was obtained, followed by hot filling into sterilized containers before evaluation.

2.8 Sensory evaluation

Sensory evaluation was conducted using a 9-point hedonic scale to assess consumer acceptance of the developed sugar-free rose jam. Panelists were asked to evaluate appearance, color, flavor, texture, spreadability and overall liking, where 1 = extremely dislike; 5 = neither like nor dislike; 9 = extremely like. The evaluation was carried out using 50 untrained panelists under controlled conditions.

2.9 Statistical Analysis

All experiments were conducted in triplicate. Results were expressed as mean ± standard deviation. Statistical differences among treatments were analyzed using one-way ANOVA, followed by Duncan's multiple range test at a significance level of $p < 0.05$.

3 Results and Discussion

3.1 The effect of pH on gelation behavior of WPI-based hydrogels

Table 1 presents the pH analysis of all treatments, revealed distinct differences between acidic (pH 3.5–4.3) and neutral conditions, particularly in response to increasing WPI concentration. Under acidic conditions, a significant increase ($p < 0.05$) in pH was observed as WPI concentration increased from 1% to 5%. Specifically, the pH values rose progressively from approximately 3.50 (1% WPI) to 4.34 (5% WPI). In contrast, under

neutral conditions, increasing WPI concentration did not significantly affect pH, with values remaining relatively constant (6.40–6.44) across all treatments.

Table 1. pH values of WPI-based hydrogels with different concentrations of $CaCl_2$

Treatments	pH	
	acidic condition	neutral condition
1% WPI + 5.0 mM $CaCl_2$	3.50 ± 0.03^{a}	6.40 ± 0.02^{a}
1% WPI + 7.5 mM $CaCl_2$	3.52 ± 0.03^{b}	6.41 ± 0.02^{a}
1% WPI + 10.0 mM $CaCl_2$	3.55 ± 0.03^{b}	6.41 ± 0.03^{a}
3% WPI + 5.0 mM $CaCl_2$	3.76 ± 0.02^{c}	6.41 ± 0.03^{a}
3% WPI + 7.5 mM $CaCl_2$	4.00 ± 0.02^{d}	6.42 ± 0.03^{a}
3% WPI + 10.0 mM $CaCl_2$	4.03 ± 0.03^{d}	6.42 ± 0.02^{a}
5% WPI + 5.0 mM $CaCl_2$	4.23 ± 0.01^{e}	6.43 ± 0.02^{a}
5% WPI + 7.5 mM $CaCl_2$	4.31 ± 0.03^{f}	6.43 ± 0.03^{a}
5% WPI + 10.0 mM $CaCl_2$	4.34 ± 0.03^{f}	6.44 ± 0.03^{a}

The observed pH increase in acidic systems can be attributed to the buffering capacity of whey proteins. Whey proteins contain ionized amino acid residues capable of interacting with hydrogen ions, thereby moderating the acidity of the system. As the protein concentration increased, the buffering effect became more pronounced, leading to a measurable elevation in pH. This effect was not evident under neutral conditions because the system was already distant from the isoelectric point (pI) of whey proteins (~4.5–5.0), and the net charge distribution remained relatively stable.

From a gelation perspective, pH plays a critical role in governing protein–protein interactions. Under mildly acidic conditions approaching the pI, electrostatic repulsion between protein molecules is reduced, promoting aggregation and network formation [11]. The gradual increase in pH with higher WPI levels may shift the system slightly away from the pI, potentially influencing aggregation kinetics and gel microstructure. However, the acidic range (3.5–4.5) remains close enough to the pI to favor enhanced intermolecular association compared to neutral conditions. This distinction is particularly important for optimizing WPI-based hydrogels intended for sugar-free jam applications, where pH control critically determines texture, stability, and structural integrity.

3.2 Visual appearance of WPI-based hydrogels

To complement quantitative measurements, visual assessment was conducted as a qualitative indicator of macroscopic gel integrity. To determine the hydrogel used for product application was prepared with 7.5 mM $CaCl_2$, which was selected as the optimal condition based on a balance between physicochemical and practical textural properties. The visual appearance of WPI-based hydrogels after 1 and 24 h of storage provided qualitative evidence supporting the mechanical and physicochemical analyses. When compared with the control sample (without WPI), hydrogels containing WPI exhibited progressively stronger structural integrity as protein concentration increased from 1% to 5% (w/v) as shown in Fig 1. The control gel displayed a relatively weak and soft structure with the lowest firmness of 21.79 ± 1.22 N, as evidenced by partial deformation when inverted in the vial. In contrast, WPI-containing samples maintained their shape more effectively upon inversion, indicating enhanced gel firmness and structural cohesion related to the texture analysis.

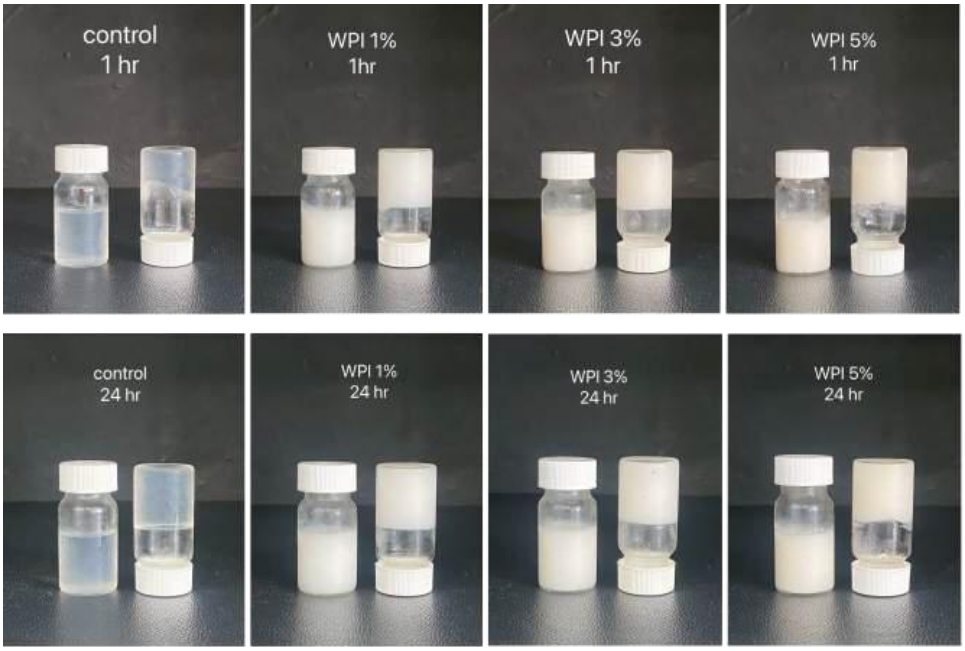

Fig.1. Visual appearance of WPI-based hydrogels after 1 and 24 h storage at 4 ℃.

The texture analysis results revealed that gel firmness increased significantly ($p < 0.05$) with increasing WPI concentrations (22.58 ± 3.01, 25.53 ± 3.44, and 28.72 ± 4.30 N for 1%, 3% and 5% WPI addition, respectively). At 1% WPI, the gel showed moderate body formation but still exhibited slight structural relaxation under gravitational stress. Increasing the concentration to 3% resulted in a visibly firmer and more self- supporting structure, while the 5% WPI gel demonstrated the highest rigidity, retaining its original geometry without deformation when inverted. This concentration-dependent strengthening can be attributed to the increased availability of whey protein molecules participating in heat-induced aggregation and network formation. Upon thermal denaturation, unfolded WPI molecules interact through hydrophobic interactions and disulfide bond formation, leading to a denser and more interconnected three-dimensional network. The increased protein content enhances crosslink density and reduces network mobility, thereby improving gel firmness and resistance to gravitational flow [12, 13].

3.3 The effect of $CaCl_2$ concentrations on the structural strength of WPI-based hydrogels

Following confirmation of protein-induced gel formation, calcium-mediated crosslinking was investigated to further reinforce the composite hydrogel network. The addition of calcium chloride ($CaCl_2$) at different concentrations (5, 7.5, and 10 mM) significantly influenced the structural integrity of 3% WPI–LM pectin hydrogels, as reflected by changes in WHC and syneresis ($p < 0.05$) (Table 2). As $CaCl_2$ concentration increased from 5 to 10 mM, WHC improved markedly from 94.98% to 99.03%, respectively, while syneresis decreased significantly from 1.92% to 0.36%. The gel containing 10 mM $CaCl_2$ exhibited the highest WHC and lowest serum release, indicating superior water immobilization and network stability. In contrast, the 5 mM system showed comparatively higher water expulsion, suggesting a less compact gel structure. This behavior strongly supports the calcium-mediated crosslinking mechanism consistent with the classical egg-box model of

LM-pectin gelation. Calcium ions promote the formation of junction zones between homogalacturonan chains following the "egg-box" model, where Ca^{2+} bridges adjacent carboxyl groups, generating a three-dimensional ionic network [12]. At higher Ca^{2+} concentrations, increased ionic crosslink density enhances the formation of a more cohesive and interconnected gel matrix. In composite systems, the LM-pectin network may form an interpenetrating or coupled structure with heat-denatured whey protein aggregates. Protein–pectin interactions are governed primarily by electrostatic attraction, hydrogen bonding, and physical entanglement, depending on pH and ionic strength [13].

Table 2. Water holding capacity (WHC) and syneresis (%) of WPI-based hydrogels with different concentrations of $CaCl_2$

Treatments	Water holding capacity (WHC)	Syneresis (%)
5.0 mM	94.98 ± 0.10^{a}	1.92 ± 0.38^{a}
7.5 mM	96.10 ± 0.27^{b}	0.68 ± 0.10^{b}
10.0 mM	99.03 ± 0.21^{b}	0.36 ± 0.08^{c}

At lower $CaCl_2$ concentration (5 mM), insufficient crosslinking led to a looser network with reduced ability to retain immobilized water, leading to higher syneresis. Increasing $CaCl_2$ to 10 mM significantly reduced serum separation, indicating that a threshold crosslinking density was reached, beyond which structural reinforcement became more pronounced. The inverse relationship between WHC and syneresis further confirms that enhanced calcium- induced crosslinking improves water entrapment efficiency within the composite hydrogel matrix [14]. Reduced syneresis reflects improved network contraction resistance and structural homogeneity, both critical parameters for sugar-free jam applications where water separation negatively affects product quality and consumer acceptance.

Although microstructural characterization techniques such as scanning electron microscopy (SEM) or confocal laser scanning microscopy (CLSM) were not employed in this study, the observed improvements in water-holding capacity and reduction in syneresis suggested a more compact and interconnected network structure. These findings are consistent with previous reports on whey protein–pectin composite gels, where calcium-mediated crosslinking enhanced network density [14].

3.4 Application in sugar-free rose jam

Although the 10 mM $CaCl_2$ treatment exhibited the highest WHC and the lowest syneresis, formulation selection for practical application must also consider textural attributes and spreadability. Thus, in this study, the WPI-based hydrogel containing 7.5 mM $CaCl_2$ was selected as the optimal condition for rose jam application due to its more desirable textural characteristics and handling properties required for consumer acceptance.

This application step bridges fundamental hydrogel design with practical food system implementation. The developed WPI-based hydrogel was incorporated into sugar-free rose jam, resulting in improvements in gel strength compared to the control formulation without hydrogel addition (Fig 2). The hydrogel-fortified jam exhibited a firmer and more cohesive texture, with enhanced shape retention and structural stability during storage. No visible syneresis or serum separation was observed during storage, indicating effective water immobilization within the composite gel matrix. In contrast, the control sample (without WPI-based hydrogel) demonstrated comparatively weaker body structure and a higher tendency toward moisture migration, highlighting the critical role of the protein–pectin hydrogel network in stabilizing the product. The improved stability can be attributed to the

formation of an interpenetrating network between heat-denatured whey protein aggregates and LM-pectin chains, reinforced by calcium-mediated crosslinking as previous mentions.

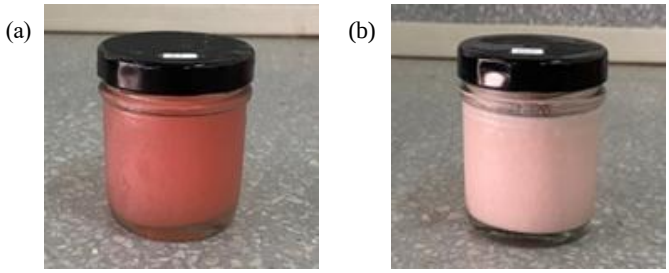

Fig.2. Visual appearance of sugar-free rose jam (a) without and (b) with 3% WPI-based hydrogel

3.5 Sensory evaluation

The sensory evaluation of the developed product was conducted using a 9-point hedonic scale presented in Fig.3. The results revealed moderate overall consumer acceptance with the overall liking score of 6.17 (slightly like to moderately like), indicating that the formulation was generally acceptable.

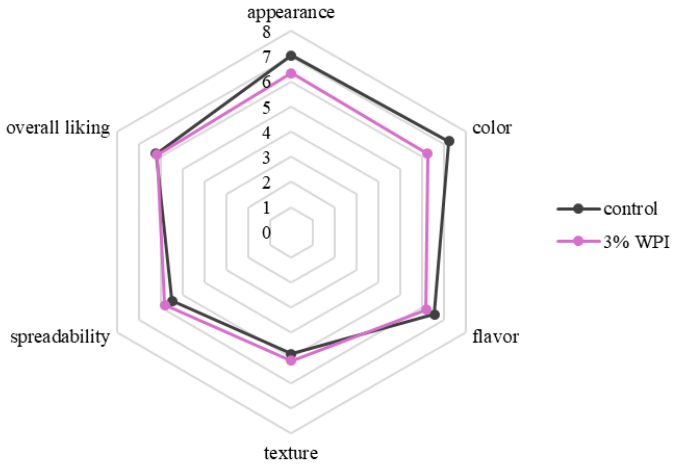

Fig.3. Sensory attributes of sugar-free rose jam with 3% WPI-based hydrogel compared to control sample

Among the evaluated attributes, appearance (6.34) and color (6.26) received relatively higher scores, suggesting that the product exhibited a visually appealing and acceptable appearance. While the texture score (5.11) and spreadability score (5.81) indicated moderate acceptability. This may be associated with the structural characteristics of the WPI–LM pectin hydrogel system, where calcium-mediated crosslinking and protein aggregation contribute to increased gel firmness, limiting ease of spreading compared to conventional jam systems. Although the addition of WPI increased the turbidity of the jam due to light scattering from protein aggregates [15], the modified formulation maintained acceptable spreadability, exhibiting a smooth and uniform consistency suitable for consumer application.

From a functional perspective, the integration of WPI-based hydrogel not only improved textural stability but also enhanced the nutritional profile by increasing protein content, supporting the development of value-added, sugar-free fruit preserves. Overall, the sensory results demonstrated that while the developed formulation achieved acceptable visual and general liking attributes, further optimization is required to improve textural properties and spreadability. These results provided quantitative confirmation of the macroscopic observations and supports the proposed gelation mechanism. Moreover, the approach supports sustainable food innovation by enabling reduced sugar formulations without synthetic texturizers. Future optimization may focus on refining optical clarity and microstructural homogeneity, potentially through adjustments in protein concentration, or homogenization conditions, to further enhance product quality.

4 Conclusion

This study demonstrated that WPI-based hydrogels, formulated in combination with LM-pectin and optimized calcium concentration, can effectively serve as alternative structuring systems in sugar-free rose jam. Increasing WPI concentration influenced gel formation under acidic conditions, while calcium-mediated crosslinking significantly enhanced water holding capacity and reduced syneresis. The incorporation of the optimized hydrogel improved textural stability, prevented water separation during storage, while maintaining desirable spreadability compared with the control. Although the developed hydrogel system demonstrated promising short-term stability, as evidenced by high water-holding capacity and low syneresis, extended storage studies are required to confirm its suitability for commercial applications. These findings highlight the potential of protein–polysaccharide hydrogels as sustainable and functional sugar replacers in fruit-based preserves, supporting the development of health-oriented, protein-fortified formulations.

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