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Trehalose–whey protein conjugates prepared by structural interaction: Mechanisms for improving the multilevel structure and their water solubility and protein digestibility

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ORIGINAL ARTICLE

Abstract

Whey proteins (WPs) are the most widely used protein supplements worldwide. This study investigates the impact of incorporating trehalose into WPs at different ratios ranging from 1% to 5% (w/v) on the structural characteristics, surface properties, and functionality of trehalose (T) conjugated to WPs (T-WP). The T-WP conjugate was produced using the pH-shifting technique. Our findings demonstrated that conjugating trehalose into WPs significantly altered the Fourier-transform infrared (FTIR) spectrum, tertiary structure, and protein conformation. The surface charge and hydrophobicity of T-WPs were changed significantly ($p < 0.05$). Structural modifications had a notable effect on the solubility and digestibility of T-WPs. The water solubility of T-WPs increased from 88.12% to 95.53% when conjugated with 5% (w/v) trehalose. Furthermore, the impact of the development of T-WPs on FTIR spectrum was investigated. The β-sheet, random coil, α-helix, and β-turn were changed significantly from 36.17% to 45.21%, 12.38% to 16.39%, 10.69% to 13.44%, and 40.76% to 24.94%, respectively. The results presented in this study offer structural information to enhance the creation of WP products with improved functional properties.

Keywords: disaccharide; tertiary structure; water solubility; whey proteins

Introduction

The increasing demand for whey proteins (WPs) among bodybuilders has prompted the development of new dry formulations of protein supplements. These formulations include protectants to preserve functional properties during storage (Alrosan *et al.*, 2023). WPs have high-quality protein derived from milk during the production of cheese (Bondoc, 2007; Bondoc and Şindilar, 2002). The most functional property of proteins is water solubility; other functional properties depend on their water solubility (Alrosan *et al.*, 2022). The water solubility of WPs

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is approximately 89.4% (Alrosan *et al.*, 2023), which is considered to have excellent water solubility, compared to other proteins, such as casein (~84%), lentil (~58%), and quinoa (~76%) (Alrosan *et al.*, 2024a, 2024b; Wang *et al.*, 2023). Water solubility and digestibility are important considerations for bodybuilders because the digestibility of a protein is a crucial factor in determining its quality. Protein quality is a measure of a protein that can be digested, absorbed, and used by the body for various functions, particularly for tissue growth and repair (Adhikari *et al.*, 2022; Bondoc, 2007).

The digestibility of WPs is ~88.4% (Alrosan *et al.*, 2023). Numerous studies have reported that pH shifting is a technique used to modify protein structure by altering the pH of the surrounding alkaline environment (Jiang *et al.*, 2017; Li *et al.*, 2020; Wang *et al.*, 2023; Alrosan *et al.*, 2022). The pH-shifting method is a technique used to alter the pH environment of proteins to induce changes in their structure, solubility, and functionality (Alrosan *et al.*, 2022; Figueroa-González *et al.*, 2022; Jiang *et al.*, 2022). The pH shifting method can cause proteins to unfold in an alkaline environment and refold the conformation protein (Alrosan *et al.*, 2024a). This property is applied to expose reactive sites on protein for conjugation with other molecules, including disaccharides (Alrosan *et al.*, 2024a; Chen *et al.*, 2019; Cui *et al.*, 2023) and polysaccharides (Yang *et al.*, 2020; Yildiz *et al.*, 2018). It was discovered by Yildiz *et al.* (2018) that processing plant protein with pH-shifting could enhance water solubility, surface hydrophobicity, and emulsion properties if combined with polysaccharides. Moreover, the interaction between proteins and disaccharides is extensively studied concerning their effect on the surface characteristics of proteins. A recent study conducted by Alrosan *et al.* (2024a) discovered that trehalose interacts with the surface of protein molecules, altering their hydration shell and reducing intermolecular interactions that lead to aggregation. This interaction prevents proteins from forming insoluble aggregates, thereby increasing their solubility. On the other hand, noncovalent bonds play a crucial role in modifying the conformation of proteins (Dai *et al.*, 2022). These interactions are essential for maintaining protein structure and functionality (Alrosan *et al.*, 2024a; Hao *et al.*, 2022; Ke *et al.*, 2023). Hydrogen bonds are crucial to form secondary structures, such as α-helices and β-sheets. In α-helices, hydrogen bonds are formed between the carbonyl oxygen of one amino acid and the amide hydrogen of other amino acids. In β-sheets, hydrogen bonds are formed between carbonyl and amide groups of adjacent strands (Tan *et al.*, 2021). The main objective of this research is to find an innovative method that combines WPs with trehalose based on pH-shifting to prepare a composite of trehalose-conjugated WPs (T-WP) with high solubility and digestibility. In addition, this study also evaluates the properties of the surface, particle size, and protein structures, including secondary and tertiary structures.

Materials and Methods

Whey protein (protein: ~83.4%, fat: ~4.6%, moisture: ~8%, and ash: ~4%) was obtained from Now Food (IL, USA), while trehalose with a molecular weight of 378.33 was purchased from Sigma-Aldrich (CA, USA). All additional chemicals or materials utilized in this research were of reagent grade and obtained from Sigma-Aldrich.

Preparation of trehalose-conjugated WPs (T-WPs)

Trehalose solution was produced at different concentrations (0%, 1%, 2%, 3%, and 5% w/v) in a solution buffered with phosphate. A magnetic stirrer (Toanlab, SH-4, Arizona, USA) agitated trehalose solutions of different concentrations for 2 h at room temperature (21°C). T-WPs were produced using the pH-shifting method at a pH of 12. This method involved combining 1 g of WP with various concentrations of trehalose (Alrosan *et al.*, 2024a). The samples were labeled as 0T-WP (control), 1T-WP, 2T-WP, 3T-WP, and 5T-WP, indicating different concentrations (1–5%, w/w) of trehalose.

Percentage of water solubility

The water solubility of T-WPs was measured based on the procedure used by Alrosan et al. (2024a) and Wang *et al.* (2019) with slight modifications. In brief, 200 mg of sample and 18 g of distilled water were dissolved in a 50-mL glass beaker. Following that, the pH values of suspensions were adjusted to pH 7.0 using 0.2-M NaOH and again stirred for 60 min. After 10 min of rest, the suspensions were adjusted to 1% (w/v). The water solubility percentage of the sample was determined using Equation (1). Soluble nitrogen in whole sample (N_W), supernatant (N_S), and blank sample (N_B) was determined based on the Kjeldahl method (AOAC Method 930.29) (AOAC, 2012):

$$\text{Water solubility } (\%) = \frac{(N_S - N_B)}{N_W} \times 100\%. \quad (1)$$

Fourier-transform infrared (FTIR) analysis

The FTIR analysis was conducted using an FTIR spectrophotometer (Shimadzu, IRAffinity-1S, Kyoto, Japan). The FTIR spectrum was analyzed between 400 cm^{-1} and 4000 cm^{-1} based on the procedure followed by Alrosan *et al.* (2023). The FTIR analysis provided valuable

insights into the molecular structure and composition of samples by measuring the absorption of infrared light at various wavelengths based on the procedure described by Alrosan *et al.* (2023).

ζ-potential and particle size

The surface charge (ζ) of the samples was determined using the method described by Alrosan *et al.* (2023). In brief, the sample was dissolved in distilled water at a final ratio of 1 mg/mL. The pH was adjusted to 7.0, and the solution was analyzed using the zetasizer particle size analyzer (Malvern Panalytical, Nano-ZS, Malvern, UK). The particle size of the sample was studied using the Zetasizer Nano-ZS based on the procedure adopted by Wang *et al.* (2023). Prior to the scans, the samples were dissolved in distilled water for a final concentration of 0.01% and pH 7.0.

Surface hydrophobicity

1-Anilino-8-naphthalenesulfonate (ANS) was used to measure the surface hydrophobicity of T-WPs using a fluorescence spectrometer (Agilent, Cary Eclipse, Santa Clara, USA) based on the procedure mentioned by Alrosan et al. (2024b) Phosphate-buffered solutions with a pH of 7.0 were used to dissolve samples to produce diluted samples. The concentration of samples ranged from 0.01% to 0.1%. ANS (20 µL, 8 mM) was added to each sample (4 mL). The emission and excitation wavelengths were measured within a range of 470–390 nm, with slits of 1 nm width. The surface hydrophobicity of WPs and T-WPs was evaluated by determining the gradient of graphs showing the correlation between relative fluorescence intensity and protein quantity.

Intrinsic fluorescence

The tertiary protein structure of T-WPs was evaluated using the Cary Eclipse fluorescence spectrometer with emission and excitation at a respective wavelength of 300–450 nm and 280 nm. The samples were diluted to a concentration of 0.001% (w/v) at pH 7.0.

Ultraviolet (UV) spectrum

The conformation of T-WPs was caused using a UV-visible spectrophotometer (Shimadzu, UV-3600, Kyoto, Japan) with a UV-spectrum of 190–350 nm, and the peak was observed at approximately 280 nm, indicating the presence of tryptophan. The samples were evaluated at a concentration of 0.01 mg/mL at pH 7.0.

Noncovalent interactions

Molecular forces governing protein interactions were investigated using the Santa Clara Fluorescence spectrometer with an emission of 300–450 nm and an excitation of 280 nm. Before scanning, the sample was treated with 10-mM NaCl, thiourea, and sodium dodecyl sulfate (SDS) to evaluate various contributing forces of interaction, such as electrostatic interaction, hydrogen bonding, and hydrophobic interaction. The samples were diluted to a concentration of 0.001% (w/v) and at pH 7.0.

Digestibility

The digestibility of T-WPs was evaluated according to the procedure described by Alrosan *et al.* (2023). Samples (250 mg) were mixed in pepsin solution (1.5 mg/mL) and the suspension was dispersed in 15-mL 0.1-M HCl solution containing 1-mL 0.005-M sodium azide. The suspension was placed in a water bath (Memmert, WB22, Schwabach, Germany) at 37°C for 3 h. Subsequently, a solution containing 7.5 mL of 0.5-M NaOH and 10 mg of pancreatin was added to the suspension and placed in a water bath at 37°C for 24 h. Throughout the incubation period, the mixtures were centrifugated (CN Meditech, CNME060222, Nanjing, China) at 10,000 ×*g* for 20 min. Equation (1) was used to determine the protein digestibility of WPs and T-WPs.

Denaturation temperature

A differential scanning calorimeter (Mettler-Toledo, DSC 3, Greifensee, Switzerland) was used to measure denaturation temperature (T_d) of T-WPs according to the procedure described by Alrosan *et al.* (2024a). A 5-mg sample was placed on aluminum crucible pots. Nitrogen gas was used as a carrier with a 100-mL/min flow rate. The temperature range was set at -70 to 150°C, with a heating rate of 20°C/min. The DSC software was employed to determine the T_d of T-WPs based on the thermograms.

Statistical analysis

The statistical analysis of the study was performed using SPSS version 23.0 (IBM, Chicago, US).

Results and Discussion

Solubility and digestibility of trehalose–whey protein conjugates

Soluble T-WPs were generated using the pH-shifting method. The effect of pH on the solubility of T-WPs is

shown in Figure 1A. The highest level of solubility was observed as ~95.5%, higher than the percentage of WPs, which was ~88%. Our observation about the solubility of WPs was according to a computed result of Alrosan *et al.* (2023). There was a significant difference ($p < 0.05$) in the influence of pH on the conjugates of 1T-WP, 2T-WP, and 3T-WP. Meanwhile, the trehalose ratio significantly influences ($p < 0.05$) the water solubility of T-WPs. Figure 1A illustrates a significant rise ($p < 0.05$) in the solubility of 5T-WP, approximately 95.5%, compared to the control (0T-WP). This substantial increase suggests that trehalose conjugation markedly improves the solubility of WPs, potentially enhancing their functional properties for various applications. It was reported by Yildiz *et al.* (2018) that soy protein isolate's water solubility was affected by pH shifting after it was conjugated with polysaccharides because of the factors related to protein and polysaccharide interactions, protein's isoelectric point, and the structural changes that occurred during the formation of T-WPs.

The digestibility of T-WPs significantly increased ($p < 0.05$) from 88.16% to 94.89% after the conjugation of trehalose with WPs during the pH-shifting process (Figure 1B).

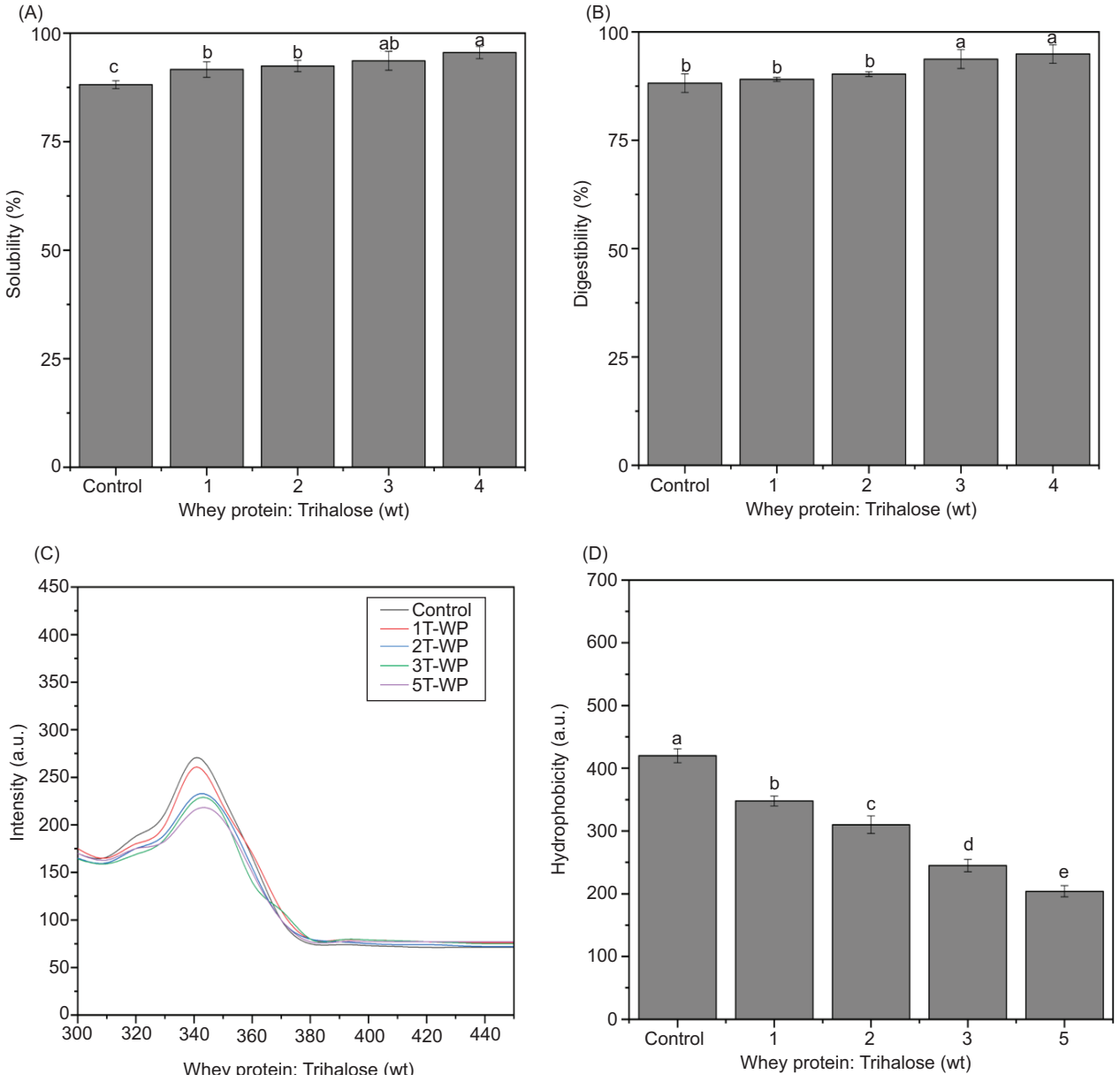

Figure 1. Changes in (A) average water solubility, (B) protein digestibility, (C) fluorescence intensity, and (D) UV absorbance of trehalose-whey protein conjugates (T-WPs). Control (0T-WP) represents the absence of trehalose, while 1T-WP, 2T-WP, 3T-WP, and 5T-WP represent trehalose conjugation with WPs at 1%, 2%, 3%, and 5% (w/v) concentration of trehalose, respectively. Different lowercase letters represent significant differences ($p < 0.05$).

The increased concentration of trehalose ameliorated the digestibility of T-WPs. This increase was due to the formation of whey protein–trehalose complexes, increasing protein solubility. This allowed enzymes (e.g., pepsin and protease) to have easier access to active sites on proteins for digestion (Alrosan *et al.*, 2024b; Liu *et al.*, 2023), compared to intact WPs, which had less surface area or sites for the enzymes to bind and breakdown of proteins (Nourmohammadi *et al.*, 2024).

Effect of trehalose conjugation on surface hydrophobicity and intrinsic fluorescence

The surface polarity and microdomain environment of T-WPs could additionally affect their protein digestibility. An investigation of intrinsic fluorescence and surface hydrophobicity was conducted (Figure 1C). The T-WP's emission spectrum peaked at 335 nm approximately, suggesting that the tryptophan residues were partially visible on the protein surface (Alrosan *et al.*, 2024a; Nourmohammadi *et al.*, 2024). The pre-digestibility of T-WPs resulted in notable alterations in the protein's absorption characteristics and the wavelength at which the highest intensity took place. These changes provided insights into the micro-environment surrounding tryptophan residues. The fluorescence intensity of T-WPs was compared to the control. Furthermore, additional research documented similar results to WPs (Alrosan *et al.*, 2024a; Nourmohammadi *et al.*, 2024) Recently, studies conducted by Alrosan *et al.* (2024b) and Nourmohammadi *et al.* (2024) reported that certain buried tryptophan residues had a modest quantum yield but could boost fluorescence intensity by more than 10-fold. Pre-digestibility of T-WPs could potentially cause the unfolding of protein and reveal previously hidden tryptophan residues (Alrosan *et al.*, 2023). The presence of trehalose caused a decrease in the fluorescence intensity of T-WPs, which was dependent on trehalose concentration in T-WPs. It could improve the local electrostatic area, leading to interactions between the side chain group and the excited indole rings of tryptophan residues, consequently decreasing the fluorescence intensity of proteins.

The surface hydrophobicity of T-WPs decreased dramatically following hydrolysis, regardless of trehalose concentration (Figure 1D), implying increased polarity of T-WPs. The surface hydrophobicity of T-WPs declined significantly ($p < 0.05$) by two-fold, 420 arbitrary units approximately, compared to that of the control of 204 a.u. approximately. Our results were similar to the findings reported by Alrosan *et al.* (2024a), Liu *et al.* (2023), and Nourmohammadi *et al.* (2024). The generated peptides also formed aggregates through hydrophobic interactions, which occurred if the solvent was exposed and increased hydrophilic groups (Chen and Campanella, 2022). It is essential to mention that intrinsic fluorescence and surface hydrophobicity were investigated in the trehalose range of 1–5%. Several studies discovered that coating specific hydrophobic regions with trehalose decreased the surface hydrophobicity of proteins (Liu *et al.*, 2024; Yue *et al.*, 2021). In the present study, this phenomenon could have happened, and the process of pre-digestibility revealed some internal areas of WPs, thereby enabling trehalose to attach through hydrophobic interactions.

Effect of trehalose conjugation on protein conformation and secondary protein structure

The influence of conjugating disaccharides, specifically trehalose, on the structure of WPs was investigated using FTIR (Figure 2A). Peaks at 1236 cm^{-1} and 1535.3 cm^{-1} in the FTIR spectrum were observed to change positions, indicating the influence of trehalose in the pH-shifting technique (Jiang *et al.*, 2022). In addition, the peak at 3300.2 cm^{-1} experienced a shift, which was attributed to the vibrational stretching of the hydroxyl group. Furthermore, the stretching vibrations of C–O and C–C bonds were affected by a moved peak at 1031 cm^{-1}. The observed absorption peaks provided precise information for carbohydrates. These are organic molecules consisting of oxygen, hydrogen, and carbon. The presence of peaks at 3300 cm^{-1} and 3388 cm^{-1} suggested that trehalose was associated with WPs, as evidenced by the shift to 3308 cm^{-1} and the quantity of trehalose in the conjugated state.

The secondary protein structure components of T-WPs exhibited a substantial difference ($p < 0.05$) (Table 1). The protein's secondary structure was analyzed by examining the FTIR spectrum of amide II in a range of 1600–1699 cm^{-1} (Table 1). This analysis aimed to understand the conformational changes that occurred in case WPs interacted with trehalose. The secondary structure components, such as β-sheet, random coil, and α-helix, increased significantly ($p < 0.05$) from 36.16% to 45.21%, 12.38% to 16.39%, and 10.69% to 13.44%, respectively. The most crucial β-sheet conformation was frequently located within the hydrophobic interior of proteins, thus decreasing protein solubility. On the other hand, the percentage of random coil typically exposed more hydrophilic and polar residues to the solvent, enhancing water solubility. Previous investigation showed that alterations in the secondary protein structure were the result of changes in molecular forces, which included electrostatic interactions, hydrogen bonds, and hydrophobic interactions (Alrosan *et al.*, 2022, 2024a; Ducel *et al.*, 2008; Soltanizadeh *et al.*, 2014). The percentage of β-turn decreased significantly ($p < 0.05$) from 40.76% to 24.95%. The findings suggested that trehalose had a

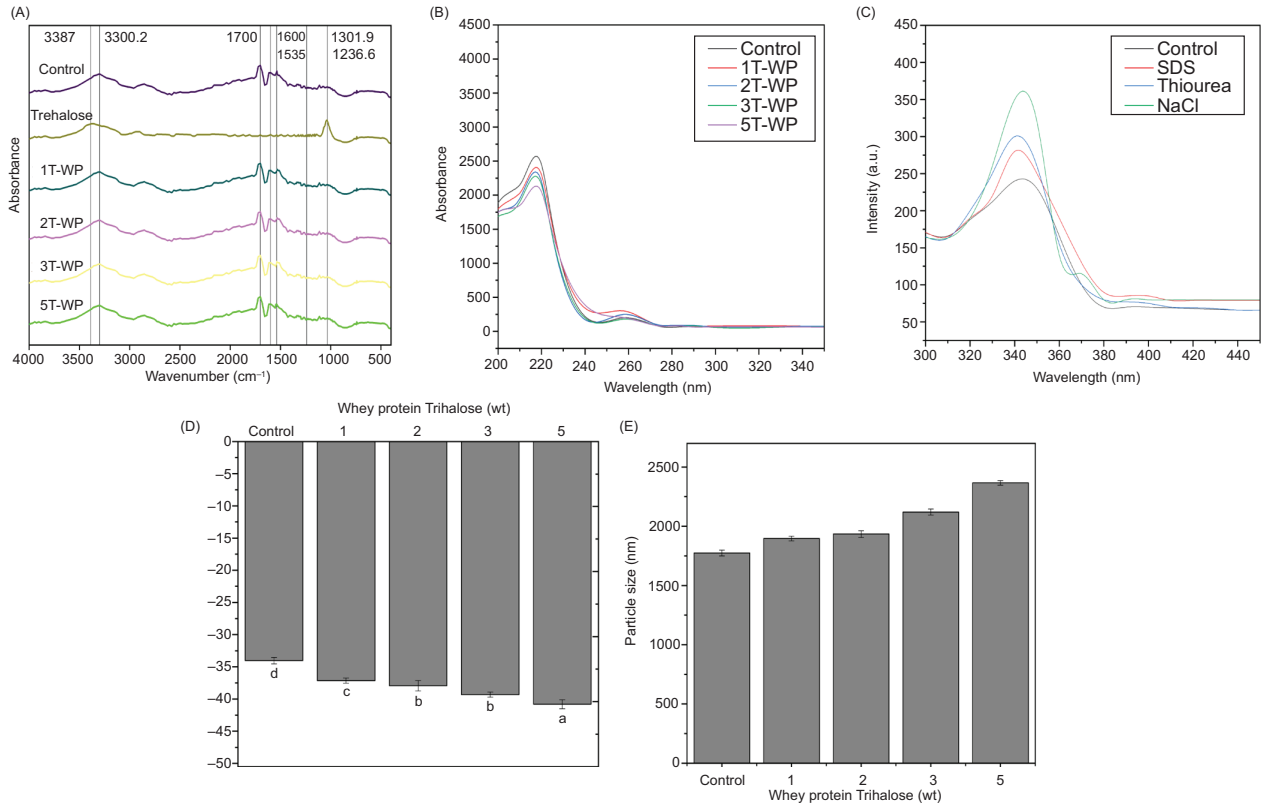

Figure 2. Changes in (A) FTIR absorbance, (B) fluorescence intensity (molecular forces), (C) surface charge, (D) surface hydrophobicity, and (E) particle size of trehalose–whey protein conjugates (T-WPs). Control (0T-WP) represents the absence of trehalose, while 1T-WP, 2T-WP, 3T-WP, and 5T-WP represent trehalose conjugation with WPs at 1%, 2%, 3%, and 5% (w/v) concentration of trehalose, respectively. Different lowercase letters represent significant differences ($p < 0.05$).

Table 1. The proportion of secondary protein components detected in complex protein structures derived from trehalose-whey protein conjugates at different concentrations of trehalose (0–5% w/w).

Secondary protein components	Trehalose–whey protein conjugates (T-WPs)					*p* value
	0T-WP	1T-WP	2T-WP	3T-WP	5T-WP	
β-sheet (Σ)	36.17^{d}	38.15^{c}	39.11^{c}	42.78^{b}	45.21^{a}	<0.05
Random Coil (Σ)	12.38^{c}	13.68^{b}	13.94^{b}	16.00^{a}	16.39^{a}	<0.05
α-Helix (Σ)	10.69^{e}	11.82^{d}	12.12^{c}	13.15^{b}	13.44^{a}	<0.05
β-turn (Σ)	40.76^{a}	36.33^{b}	34.81^{c}	28.05^{d}	24.94^{e}	<0.05
T_d	82.5^{d}	84.5^{c}	85.5^{b}	86.1^{b}	87.0^{a}	<0.05

Mean values (n = 3) with different superscripts in the same row differ significantly ($p < 0.05$). Control (0T-whey protein [WP]) represents the absence of trehalose conjugation with WPs. Meanwhile, 1T-WP, 2T-WP, 3T-WP, and 5T-WP represent WPs conjugated with trehalose at 1%, 2%, 3%, and 5% (w/w), respectively. T_d: denaturation temperature.

critical role in modifying the secondary structure of proteins. Specifically, the conjugation of trehalose with WPs affected the proportion of α-helices and random coils, influencing the protein's functional properties, such as solubility and digestibility.

Ultraviolet spectroscopy is a powerful tool for analyzing protein conformations because the absorption characteristics of proteins in the UV region were susceptible to their secondary and tertiary structures (Alrosan *et al.*, 2022). Aromatic amino acids, that is, phenylalanine, tyrosine, and tryptophan, had a crucial role in the UV absorption characteristics of proteins and showed distinct absorption properties at 280 nm approximately. The interaction between trehalose and WPs decreased absorbance at 230 nm (A_{230}), as shown in Figure 2B. This increase indicated protein refolding processes. Changes in pH could lead to the protonation or deprotonation of amino acid side

chains, particularly those with ionizable groups, disrupting existing interactions and promoting new ones, driving conformational changes because of conjugated trehalose with WPs. It was evident that conjugation did not occur because of WP coating on the trehalose surface. Instead, it happened because of synergistic structural interactions between WPs and trehalose (Alrosan et al., 2024a). These results intended that the structures of WPs and trehalose probably interacted through the interaction of peptide chains or secondary structures, rather than higher structures, that is, tertiary structures.

The role of noncovalent bonding in the molecular binding of T-WPs and the emission spectrum demonstrated molecular forces within or between T-WPs. Remarkably, the presence of each foreign component improved the F_{max} of the emission spectrum (<340 nm) by 10 mM. NaCl, thiourea, and SDS affected electrostatic, hydrogen bonds, and hydrophobic interactions. In Figure 2C, electrostatic interactions showed a higher peak than hydrogen bonds and hydrophobic interactions, suggesting that electrostatic interactions contributed more to the formation of conjugates between trehalose and WPs than hydrogen bonds and hydrophobic interactions.

Effects of trehalose conjugation on ζ-potential

ζ-potential measures the particle's overall charge in a particular medium of T-WPs. It reflects the net surface charge influenced by the ionizable groups present and increases water solubility (Asen and Aluko, 2022). The 5T-WP shows a significantly higher ($p < 0.05$) surface charge level of approximately -34 mV, compared to that of the control of approximately -40.8 mV (Figure 2D). This indicates that ζ-potential regulates the electrostatic stability of protein solutions. Various studies have reported that higher absolute values suggest greater repulsion between protein molecules, leading to increased solubility (Gao *et al.*, 2020; Alrosan et al., 2024a). Cui *et al.* (2021) reported that the surface charge of pea proteins is increased after their conjugation with disaccharides, specifically sucrose and trehalose. A substantial electrostatic repulsion is anticipated between the molecules, thereby preventing their aggregation. Overall, manipulating surface charge through conjugation with disaccharides can significantly impact the electrostatic stability of protein solutions. This finding has important implications for enhancing solubility and preventing aggregation in various applications of protein-based products.

Effect of trehalose conjugation on thermal stability

The thermal stability of T-WPs was determined by measuring their denaturation temperature (T_d) as shown in Table 1. Our findings revealed a significant increase ($p < 0.05$) in the thermal stability of WPs, increasing the concentration of T-WPs. The highest thermal stability of T-WPs is 87°C approximately, while the control has a lower thermal stability of 82.5°C approximately. Trehalose prevents denaturation, which increases the structural resistance of WPs. This impediment is significant under stress conditions, such as pH changes or thermal treatment. In addition, trehalose improved the solubility and digestibility of WPs with combined pH-shifting techniques, which further modulate the protein's surface charge and structure.

Trehalose-conjugated whey proteins are formed by many noncovalent interactions, which play a vital role in enhancing their activity. The interactions observed in this study encompass quantified hydrogen bonds, electrostatic interactions, and hydrophobic interactions. In a study conducted by Alrosan *et al.* (2024a), molecular forces, such as hydrogen bonding, electrostatic interaction, and hydrophobic interaction, might enhance the thermal stability of structured protein. Understanding these molecular forces can provide valuable insights for optimizing protein-based formulations in various applications.

Effect of trehalose conjugation on particle size

Particle size determines the degree of protein breakdown (Sun *et al.*, 2023). The particle size of T-WPs with varied proportions of trehalose during conjugation based on the pH-shifting process is shown in Figure 2E. The conjugation of trehalose increased the higher concentration of trehalose reacted with WPs. Furthermore, the diameter of particles in digestive products grew gradually during gastrointestinal digestion (Huang *et al.*, 2024), suggesting that greater quantities of trehalose conjugating during pH shifting can enhance the digestion of proteins in WPs, which aligns with the observed digestion rate. In addition, a notable difference ($p < 0.05$) in the particle size of T-WPs was observed compared to the control. Previous studies indicated that the particle sizes of complex compounds increased due to interactions between disaccharides or polysaccharides and proteins (Alrosan *et al.*, 2024a; Wang *et al.*, 2023), as disaccharides formed hydrogen bonds and hydrophobic interactions with protein molecules (Alrosan *et al.*, 2024a). These interactions could lead to the aggregation of protein molecules, resulting in the formation of larger particles. Moreover, polysaccharides have multiple binding sites that interact with proteins (Liu *et al.*, 2023), leading to cross-linking, where a single polysaccharide molecule binds to multiple protein molecules, thereby increasing the overall size of the complex. Proteins and polysaccharides often carry charges on their surfaces

(Xue and Luo, 2023). Electrostatic attractions between oppositely charged regions lead to the formation of larger aggregates (Alrosan *et al.*, 2024a).

Conclusions

This study investigated the effect of different trehalose (as conjugates) concentrations combined with WPs on secondary protein structure, protein conformation, tertiary structure, particle size, surface charge, and surface hydrophobicity. The results demonstrated that water solubility and digestibility of WPs improved by adding trehalose in the pH-shifting process. On the other hand, noncovalent bonds synthesized trehalose conjugates with WPs in the pH-shifting process. The ratio of trehalose in T-WPs exhibited significant variations in changing WP structure and surface properties. Furthermore, the study found that incorporating trehalose into WPs resulted in protein conformation and changes in tertiary structure. These alterations could improve water solubility and digestibility.

Conflicts of Interest

The authors stated that they had no conflict of interest.

Data Availability

Data are available on request.

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Author Contributions

Mohammad Alrosan: Supervision, Funding acquisition, Conceptualization, Writing – review & editing, Writing – original draft, Project administration, Formal analysis, Resources, Software, Investigation, Methodology, Validation, Visualization, and Data curation. Motasem Al-Massad: Software, Writing – review & editing. Ali Al-Qaisi: Investigation, Writing – original draft. Sana Gammoh: Project administration, Writing – review & editing. Muhammad H. Alu'datt: Formal analysis, Writing – review & editing. Farah R. Al Qudsi: Investigation, Writing – original draft. Thuan-Chew Tan: Methodology, Project administration, Software, Supervision, Writing – review & editing. Ammar A. Razzak Mahmood: Writing – review & editing. Ali Madi Almajwal: Writing – original draft, Resources, Methodology, and Investigation.

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