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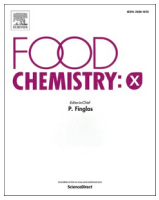

Evaluation of digestibility, solubility, and surface properties of trehalose-conjugated quinoa proteins prepared via pH shifting technique

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ABSTRACT

Soluble trehalose-conjugated quinoa proteins (T-QPs) were effectively prepared using the pH-shifting mechanism. The structural properties of the T-QPs were evaluated using a comparative evaluation, which included analyzing the amide I, surface charge and hydrophobicity, protein conformation, thermal stability, and protein structures. The results suggested that the development of the T-QPs was influenced mainly by no-covalent bonds. These interactions significantly influenced ($P < 0.05$) the quinoa proteins' conformation and higher-protein structure. T-QP had significant ($P < 0.05$) surface properties. Furthermore, the T-QPs exhibited improved solubility (79.7 to 88.4%) and digestibility (79.8 to 85.1%). Therefore, quinoa protein proved an excellent plant-based protein for conjugation with disaccharides. These findings provide significant insight into the potential development of modified proteins with enhanced solubility and digestibility by creating trehalose-conjugated plant-based proteins.

1. Introduction

Quinoa has been cultivated for thousands of years, primarily in South America. Its protein is considered an excellent protein source due to the presence of all nine necessary amino acids that the human body cannot synthesize individually (Alrosan, Tan, Easa, Gammoh, & Alu'datt, 2022a; Zhong, Wang, & Shen, 2024). These amino acids are essential for various physiological processes, such as protein synthesis, enzyme generation, and overall health maintenance (Yang et al., 2023). The quinoa contains a protein level of 14 to 18% (Alrosan et al., 2023). The protein digestibility of quinoa is ~78% (Elsohaimy, Refaay, & Zaytoun, 2015; Alrosan, Tan, Easa, Gammoh, & Alu'datt, 2022a; Srinivasu & Eligar, 2024), and water solubility is ~75% (Elsohaimy et al., 2015). The digestibility of quinoa proteins (QPs) is lower than that of some animal-based proteins, including whey protein, at around 89% (Almeida, Monteiro, da Costa-Lima, Alvares, & Conte-Junior, 2015). These are due to the structural composition of quinoa-based proteins, which can make them less easily solubilized in water and digestible in the stomach. The solubility of proteins is influenced by the hydrophilic (water-attracting) and hydrophobic (water-repelling) properties of the protein structure (amino acid) (Al-Qaisi et al., 2024; Elsohaimy et al., 2015) and the QPs have a rigid structure that greatly influences the

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hydrolysis of proteins (Elsohaimy et al., 2015; Niu et al., 2024). Consequently, the main factors that contribute to limitations in the food sector.

The protein-disaccharide interactions have been successfully investigated through different methods to enhance the functionality of conjugates or complex proteins (Alrosan et al., 2024; Jiang et al., 2022; Wang et al., 2023). The alterations of the higher protein structure have been reflected in the digestibility and solubility (Alrosan et al., 2024; Jiang et al., 2022). A study by Wang, Ghosh, and Nickerson (2019) demonstrated that anionic polysaccharides may influence the structure of lentil protein, leading to improved emulsion. Additionally, it was reported by Zhang et al. (2023) that the functional properties were enhanced by the conjugation of lactoferrin with chlorogenic acids. These studies focused on the potential of using various methods, such as protein structure alterations and conjugation with bioactive compounds, to enhance the functionality of proteins and improve their properties for various applications.

Trehalose's ability to protect macro-molecules, including proteins, is advantageous in various applications, including fresh food preservation and moisture retention. Trehalose plays a crucial role in the crystallization of sugars. It is a product of biological fermentation resulting from starch's enzymatic breakdown (Olsson & Swenson, 2019). The presence of dextran particles, which are a by-product of this process, has an essential effect on the structure of the crystal of trehalose (Taglieri et al., 2021) trehalose (two glucose molecules) and contains hydroxyl groups (-OH) on each glucose unit. These hydroxyl groups can assist the trehalose in hydrogen bonding with macro-compounds, including protein. Trehalose can preserve the typical shape of proteins by forming hydrogen, consequently preventing protein denaturation. A study by Han, Liu, and Tang (2023) demonstrated that the water solubility of the soy protein increased from 71 to 80% after the interaction with trehalose, leading to a decrease in aggregation.

Although there are some investigations regarding the conjugation of proteins to enhance their functionality using nonreducing sugars, to our best understanding, no existing research on improving quinoa protein solubility and digestibility using disaccharides based on pH-shifting techniques has been reported. Therefore, the objectives of this study are (1) to study the potential production of trehalose-conjugated quinoa protein complexes through disaccharide and protein interaction, (2) to clarify the relationship between the secondary, tertiary, and conformation of quinoa protein structures with digestibility and solubility, and (3) to prepare conjugates with high digestibility and solubility. This study aims to promote the development of QPs with high added value using non-thermal technique (pH-shifting).

2. Materials and methods

2.1. Preparation of freeze-dried quinoa proteins

Quinoa grains (15% protein, 7.5% fat, and 65% carbohydrates) from a local market (Irbid, Jordan) were from the *Chenopodium quinoa* Wild. An alkaline extraction method (pH 9.0) was used to prepare the QPs (Abugoch, Romero, Tapia, Silva, & Rivera, 2008). Following that was precipitate at pH 5.0 and centrifugation at 9000 $\times g$ for 20 min. The QPs were collected and then subjected to freeze-drying to obtain the freeze-dried QPs. The QPs (84.33% $\pm$ 2.1, N $\times$ 6.25) were determined using the AOAC Method 930.29 (AOAC, 2012).

2.2. Preparation of trehalose-conjugated quinoa protein

The T-QP solutions were prepared using phosphate-buffered solution in various levels ranging from 0 to 5 g. The mixtures were subsequently put on a magnetic stirrer for 2 h at a temperature (21 °C). T-QP solutions were created by combining QPs (1 g) trehalose (molecular weight of 378.33) solutions (1, 2, 3, and 5%, *w*/w) and stirring at pH 7.0 for 4 h. The resulting solutions had a concentration of 1% (*w*/*v*) and were left overnight at 4 °C. Sodium azide solution (0.002%) was added to each solution. Subsequently, each T-QP solution was stirred for 60 min in a highly alkaline environment (pH 12.0 using 1 M NaOH). This process is vital for the complete hydration of the solution (Han et al., 2023). After 1 h, the pH of the T-QP solutions was then corrected to a neutral pH of 7.0. Following that, the T-QP solutions were kept at 4 °C overnight. The supernatants were then collected by centrifugation (7000*g* for 10 min). Finally, the top layers were collected and freeze-dried to obtain the T-QP granules. The control group (untreated quinoa proteins without trehalose) was subjected to the same treatment as the previously mentioned. The control was then analyzed and compared to the trehalose-conjugated samples.

2.3. Water solubility

The solubility of QPs and T-QPs has been measured using the method according to Alrosan et al. (2023). The analysis is specifically on the ratio of dissolved protein at pH 7.0 to the total protein content. Distilled water (18 g) was used to dissolve the samples (0.20 g), placed on the stirrer for 1 h, and then the suspension was adjusted to pH 7.0. After stirring for 50 min, the volumes were adjusted to 20 mL (1%, *w*/*v*) before centrifuging at 10,000*g* for 20 min to separate insoluble components from soluble ones. Samples before centrifugation and supernatants were transferred into micro-Kjeldahl digestion flasks. The Kjeldahl method 930.29 (AOAC, 2012) was used to measure the nitrogen content in the samples. The water solubility of QPs and T-QP was calculated according to Eq. (1).

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

where, N_S, N_T, and N_B represent the nitrogen content of the samples before and after centrifugation and blank, respectively.

2.4. FT-IR spectroscopy

The Fourier-transform infrared (FT-IR) spectrophotometer (Shimadzu, IRAffinity-1S, Kyoto, Japan) was used to measure components of the amide I region, including the α-helix (1650–1660 cm^{-1}), β-sheet (1600–1639 cm^{-1}), β-turn (1661–1699 cm^{-1}), and random coil (RC, 1640–1649 cm^{-1}) (Alrosan et al., 2022). The diamond was placed in the middle of the FT-IR scanners, and 60 mg of samples were placed on it for scanning. The baseline adjustment was carried out in the 1600–1700 cm^{-1} (amide I region). This step removes unwanted variations or fluctuations in the baseline of the spectrum, improving the signal-to-noise ratio by isolating the actual features of interest. Additionally, FTIR spectra were analyzed with the support of the IR solution software according to the method presented by Alrosan et al. (2023c). Three scans for each protein sample and 15 spectrum scans were used in this study.

2.5. Particle size

The average measurement of each particle for QPs and T-QPs was determined using a zetasizer (Malvern Panalytical, Mastersizer 2000, Malvern, UK). Samples were diluted with distilled water to achieve 1 mg/mL with pH 7.0, and then 1 mL was injected into the zetasizer. The results values were derived by calculating the average of three measurements.

2.6. Surface hydrophobicity

The average measurement of each surface hydrophobicity of QPs and T-QPs was measured using a fluorescence spectrophotometer (Agilent, Cary Eclipse, Santa Clara, USA) according to the method used by Johnston, Nickerson, and Low (2015) with slight modifications. Protein samples (1%, *w*/*v*) were dissolved using a phosphate buffer solution (pH

7.0). The samples were placed on a stirrer (1000 rpm) for 2 h. protein samples-phosphate solution was used to generate the calibration curve, which was generated between the percentages of 0.01 and 0.1%. The sample dilution (0.1%, *w/v*) was prepared by mixed sample (4 mL) and 20 μL of 8 mM ANS dye at pH 7.0. Before analysis, the samples were placed in an area without light for 15 min. The emission wavelength of the fluorescence spectrophotometer was 390 nm, the excitation wavelength was 470 nm, and the slit width was 1 nm. The measurement of the surface hydrophobicity of QPs and T-QPs was determined according to the slope of the graphs plotted (relative fluorescence intensity against protein concentration).

2.7. Surface charge

The average surface particle size of QPs and T-QPs was determined using the Mastersizer 2000 zeta sizer, following the method outlined by Alrosan et al. (2023). Sample solutions were prepared by diluting protein in distilled water until 1 mg/mL of protein per mL, then injecting 1 mL into the device. The results values were derived by calculating the average of three measurements.

2.8. UV-spectrometry

The conformations of QPs and T-QPs were measured using a UV spectrophotometer (Shimadzu, UV-3600, Kyoto, Japan) according to the method mentioned by Liu et al. (2023) sample (0.01, w/v, pH 7.0) was dissolved using distilled water at room temperature (21 °C). The range-wavelength UV spectroscopy of protein samples was between 190 and 350 nm (Alrosan et al., 2023).

2.9. Intrinsic fluorescence

The intrinsic fluorescence of tryptophane was used to investigate the tertiary structures of the QPs and T-QPs. Distilled water was used to dissolve the protein sample, resulting in a concentration of 0.001% (*w/v*) at a pH of 7.0 and a temperature of 21 °C. A spectrophotometer probe was excited at a wavelength of 280 nm, and the emission measurements were conducted in the wavelength range of 300 to 450 nm (Wang et al., 2023).

2.10. Non-covalent interactions

Non-covalent bonds that contribute to T-QPs were determined based on the methods mentioned by Wang, Xu, Chen, Zhou, and Wang (2019) and Alrosan, Tan, Easa, Gammoh, Kubow, and Alu'datt (2022). Thiourea (10 mM), NaCl (10 mM), and sodium dodecyl sulfate (10 mM) were dissolved in the interaction between the QPs and trehalose as a represented non-covalent bond including hydrogen bonds, hydrophobic interaction, and electrostatic interaction, respectively (Alrosan et al., 2024).

2.11. In vitro protein digestibility (IVPD)

The IVPD of QPs and T-QPs was determined using the method described by Almeida et al. (2015) with slight modifications. A 0.1 M hydrochloric acid solution had been used to dissolve a protein sample (0.25 g). The solution consisted of 15 mL of pepsin (to hydrolyze proteins into smaller peptides) and was heated to approximately 37 °C for 3 h using a water bath (Memmert, WB22, Schwabach, Germany). After that, A 0.2 mol/L phosphate buffer (10 mL, pH 8.0) solution was added to the mixture, along with 1 mL of 0.005 M sodium azide and 10 mg of pancreatin (containing trypsin, chymotrypsin, and carboxypeptidase) and then left overnight (37 °C). Following the pancreatic hydrolysis, 10% trichloroacetic acid (1 mL, 10%) was added to the mixture before the centrifugation (503 ×*g*, 20 min). The IVPD proportion was determined by dividing the nitrogen content proportion of proteins in the supernatant by the total nitrogen content and multiplying the outcome by 100.

2.12. Differential scanning calorimetry (DSC)

The average measurement of the thermal behavior of QPs and T-QPs was determined using a differential scanning calorimeter (Mettler-Toledo, DSC 3, Greifensee, Switzerland) according to the procedure stated by Alrosan et al. (2024). The DSC pans were filled with 5 mg of protein samples and then sealed and left to rest for 3 min at 25 °C to stabilize the samples or initiate a specific reaction or phase transition. After the 3-min resting period, the samples were cooled to −70 °C for 20 min. This step is used to "freeze" biological or chemical reactions quickly, preserving the state of the sample at that moment. After that, the temperature of the samples was raised to 150 °C at a rate of 20 °C/min. The temperature at which denaturation occurs (T_d) obtained from the thermal spectrum investigation was examined using the DSC program.

2.13. Statistical analysis

The data analysis was performed utilizing SPSS version 23.0 (IBM, Chicago, USA). The statistical analyses carried out in this study included Duncan's multiple-range test and one-way ANOVA. Statistical significance was determined at a *p*-value of <0.05. The results showed a significant difference between the groups.

3. Results and discussion

3.1. Effects of trehalose conjugation on the digestibility and solubility of QPs

In general, the poor solubility of QPs restricts the food industry. This limitation hinders the development of protein-based food products and formulations. Consequently, alternative methods, such as interaction proteins, improve their functionality in food applications (Alrosan, Tan, Easa, Gammoh, & Alu'datt, 2022b; Liu et al., 2023; Alrosan et al., 2024). As shown in Fig. 1A, the water solubility of 0 T-QP was ~81.12%. The lower solubility of plant-based proteins is attributed to the rigid structure (Al-Qaisi et al., 2024). The solubility of 5 T-QP exhibited a significant rise ($P < 0.05$), reaching around 88.53%. This finding indicates that adding trehalose at a concentration of 5% (*w*/w) might substantially enhance the water solubility of the QPs. Moreover, a study by Wang et al. (2023) obtained that functional properties of egg yolk protein can be influenced by structural interactions with trehalose, leading to increase the water solubility from 14 to 62%. A recent study by Liu et al. (2023) found that modifying a chickpea protein's secondary and tertiary structure may influence its digestibility and water solubility. It was suggested that these alterations occurred due to the capacity of trehalose to create molecular forces (such as electrostatic interaction, hydrogen bonding, and hydrophobic interaction) with other compounds, enhancing the solubility of egg yolk protein.

The hydrolysis of proteins indicates the body's enhanced absorption and use of amino acids. This process involves breaking down proteins into constituent amino acids by adding water molecules. Once hydrolyzed, these amino acids can be readily absorbed by the body and utilized for various physiological functions such as muscle repair and hormone synthesis. The digestibility of QPs exhibited a comparable pattern to the solubility that was found at different levels of trehalose (Fig. 1B). The digestibility of 0 T-QPs (control) is around 79.7%, which aligns with the outcomes obtained by Elsohaimy et al. (2015), which was reported to be ~78.37%. The digestibility of 5 T-QP conjugates containing trehalose exhibited a considerable improvement ($P < 0.05$) to approximately 85.15%. This improvement suggests that trehalose can contribute to enhancing the digestibility of QPs. The findings indicate the potential of trehalose as a valuable ingredient in improving the

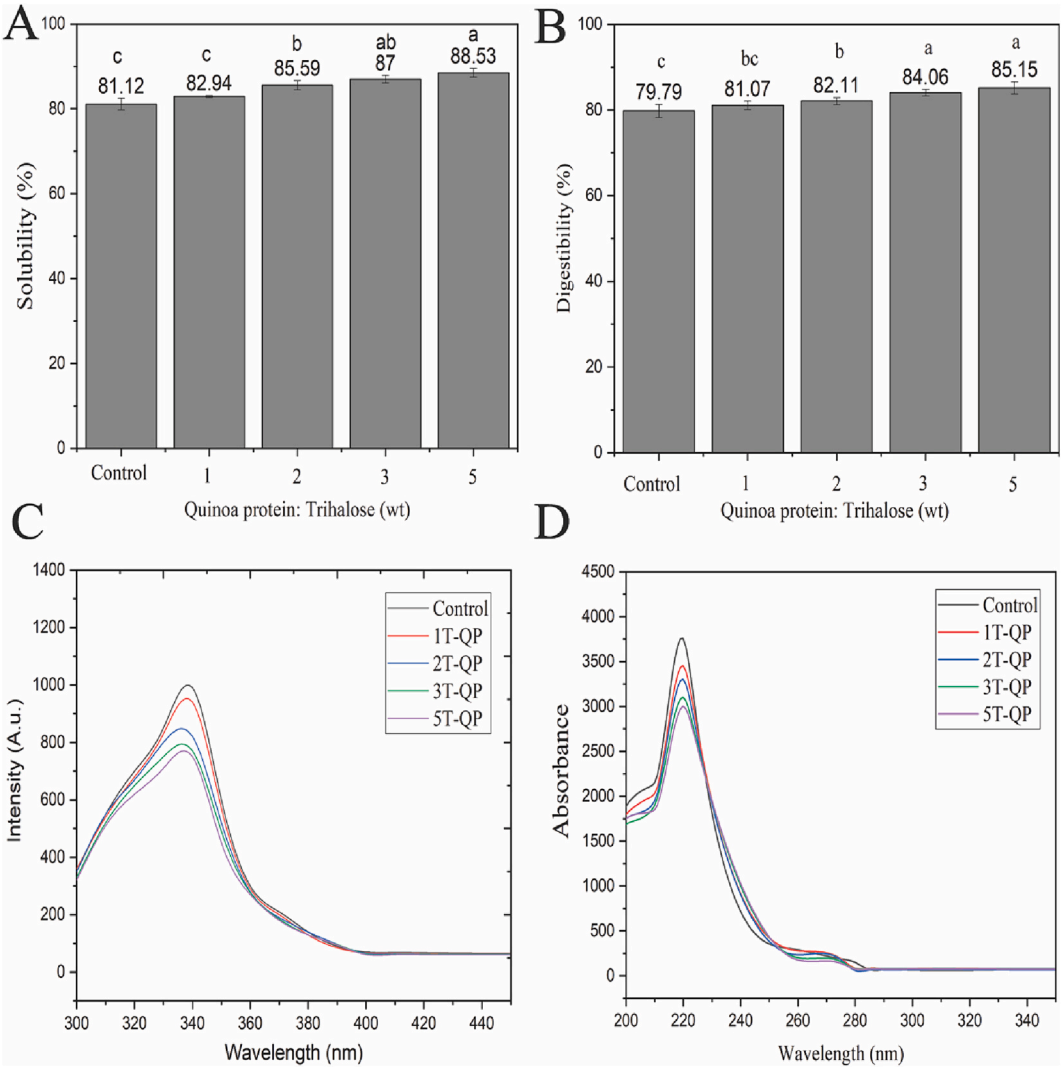

Fig. 1. Changes in the (A) water solubility, (B) protein digestibility, (C) fluorescence intensity of tryptophane, and (D) UV absorbance of trehalose-conjugated quinoa proteins (T-QPs). Control (0 T-QP) represents the absence of trehalose conjugation with the QP. Meanwhile, 1 T-QP, 2 T-QP, 3 T-QP, and 5 T-QP represent QP conjugated with trehalose at 1, 2, 3, and 5% (w/w), respectively.

overall nutritional value of these conjugates.

Nevertheless, no significant variation ($P > 0.05$) was noted across the other 4 samples. Trehalose has been documented to improve the solubility of proteins, thus impacting their ability to be absorbed (Alrosan et al., 2024). Both Alrosan et al. (2024) and Han et al. (2023) contributed major contributions that trehalose might cause dehydration of proteins, which suggests trehalose (2%, *w*/w) can play on modifications in the molecular forces firing throughout the conjugated protein and trehalose. The hypothesis suggests that larger quantities of trehalose in QPs are mainly attributed to non-covalent bonds, including electrostatic interactions between the trehalose and QPs. Moreover, trehalose could influence non-covalent bonds within the structure of proteins, specifically hydrogen bonds and electrostatic interaction (Alrosan et al., 2024).

3.2. Protein structure of QPs

3.2.1. Secondary protein structure

The FT-IR spectra (between 1600 and 1699 cm^{-1}) of T-QPs were analyzed to understand the alterations in β-sheet, RC, α-helix, and β-turn (Table 1). The findings of this research demonstrate that trehalose had a significant ($P < 0.05$) impact on amide group I of the conjugated proteins. The alterations in the secondary protein structure may have important implications for these T-QPs' functionality and nutritional values. The mechanisms of the relationships between molecules, which include hydrogen bonds, can be evaluated using the FT-IR spectra.

The percentage of α-helix conformation in 0 T-QP was at 21.44%. Subsequently, a significant decrease ($P < 0.05$) in the α-helix content was observed by adding trehalose. The α-helix content reached 16.74% at a trehalose concentration of 5% (*w*/w). The results indicate that the trehalose concentration influences the α-helix conformation in QPs. The decrease in α-helix content with higher trehalose concentrations indicates a potential stabilizing effect of trehalose on the protein structure. It was reported by Wang et al. (2023) that the addition of trehalose at 5% (w/w) altered the amide group I in egg protein, from 13.01 to 14.61% (α-helix) and from 13.13 to 12.99% (random coil).

This study found noteworthy changes ($P < 0.05$) in the percentage of β-sheet (increasing trend) and β-turn (decreasing trend) at various concentrations of trehalose. One potential explanation for the phenomenon that has been observed is that the unfolding of proteins in QPs during lyophilization could lead to the exposure of hydrophobic residues (Blume, Dietrich, Lilienthal, Ternes, & Drotleff, 2015). This exposure, in turn, could have increased intermolecular forces, leading to a greater formation of intermolecular β-sheet and β-turn (Wang et al., 2023). On the other hand, the percentage of the random coil conformation of the QPs showed a notable rise after the formation of conjugates with

Table 1
Thermal protein stability and proportion of secondary protein components detected in quinoa protein structure conjugated with trehalose (T-QP) at various concentrations.

Secondary Protein Components	Concentration of Trehalose					*P*-Value
	0 T-QP	1 T-QP	2 T-QP	3 T-QP	5 T-QP	
β-Sheet (Σ)	41.8 ± 0.90^{d}	43.7 ± 1.00^{c}	44.6 ± 1.00^{c}	48.64 ± 1.20^{b}	50.1 ± 1.60^{a}	< 0.05
RC	14.55 ± 0.40^{d}	16.91 ± 0.2^{c}	17.24 ± 0.20^{b}	18.24 ± 1.10^{a}	18.44 ± 0.50^{a}	< 0.05
α-Helix	21.44 ± 0.50^{a}	19.99 ± 0.90^{b}	19.07 ± 0.30^{c}	17.49 ± 0.21^{d}	16.74 ± 0.22^{e}	< 0.05
β-Turn (Σ)	22.12 ± 0.42^{a}	19.39 ± 0.10^{b}	19.08 ± 0.22^{b}	15.62 ± 0.20^{c}	14.64 ± 0.50^{d}	< 0.05
T_d	97.6 ± 0.25^{d}	99.3 ± 0.30^{c}	99.9 ± 0.30^{b}	100.5 ± 0.40^{a}	100.9 ± 0.20^{a}	< 0.05

Means ($n = 3$) with different superscripts in the same row differ significantly ($P < 0.05$). Control (0 T-QP) represents the absence of trehalose conjugation with the QPs. Meanwhile, 1 T-QP, 2 T-QP, 3 T-QP, and 5 T-QP represent QP conjugated with trehalose at 1, 2, 3, and 5% (*w*/w), respectively. Denaturation temperature (T_d).

trehalose (Table 1). This increase in the percentage of random coil conformation suggests that the conjugation with trehalose has induced structural changes in the QPs. These changes could have important implications for their functionality.

The interactions between proteins (primarily amino acids) and sugars can lead to various chemical changes that are observable through FT-IR spectroscopy. The alterations in FT-IR spectra resulting from the interactions can provide insights into the formation of new functional groups, such as the amide II band (N—H bending and C—N stretching) around 1535.3 cm^{-1} and amide III bands (N—H bending and C—N stretching) around 1236 cm^{-1} (Alrosan et al., 2024). The QPs compound exhibited a broad absorption peak at around 3300.2 cm^{-1}, which can be attributed to the vibration stretching of the hydroxyl group (Fig. 2A). The peaks observed at around 1031.9 cm^{-1} in the protein were identified due to the stretching vibrations of C– O and C– C bonds and the bending of C– H bonds (Alrosan et al., 2024). Fig. 2A illustrates the shared characteristics between the QPs and trehalose FT-IR spectroscopy. The identified absorption peaks were derived from the presence of trehalose. Identifying C—C and C—O bonds in the absorption peaks indicates the existence of trehalose in the QPs.

Additionally, the bending of C—H bonds further supports this conclusion as it is a characteristic feature of trehalose. The absorption peaks of QPs and T-QPs at 3300.4 and 3388 cm^{-1}, respectively, shift to 3308 cm^{-1} upon incorporation. The peak detected at 3308 cm^{-1} in the T5-QP may be attributed to the development of hydrogen bonds. The absorption peaks observed at around 1031.9 cm^{-1} in the T-QPs were attributed to the stretching vibrations of C—C and C—O bonds and the bending of C—H bonds. The amide I and II bands shifted due to the interaction between the proteins and carbohydrates (Chen et al., 2019).

3.2.2. Tertiary protein structure

Flexible regions or exposed loops in the protein structure may have more accessible amino groups and thus be more susceptible to glycation (Kan, Chen, Zhou, & Zeng, 2021). Additionally, proteins' tertiary structure can affect the spatial arrangement of reactive amino groups (Al-Qaisi et al., 2024). Fluorescence spectroscopy is a widely used technique to study proteins' structure and conformational changes (Alrosan et al., 2024; Wang et al., 2023). Tryptophan is an amino acid commonly found in proteins. It exhibits intrinsic fluorescence properties that can be used as a probe to monitor protein tertiary and quaternary structure changes. This amino acid can absorb light most efficiently at an excitation wavelength of around 280 nm (Alrosan, Tan, Easa, Gammoh, & Alu'datt, 2022b). Upon excitation, tryptophane can undergo several electronic transitions and emit fluorescence in 300–450 nm (Wang et al., 2023), with the maximum emission typically occurring between 340 and 350 nm (Han et al., 2023). The fluorescence intensity of the QPs was measured at various concentrations of trehalose, which resulted in alterations to the tertiary protein structure (Fig. 1C) because the fluorescence intensity of tryptophan decreased as the level of trehalose rose in the QPs. This observation indicates that modifications occurred, resulting in an alteration in the tertiary protein structure of QPs. Furthermore, the fluorescence spectra showed a noticeable shift towards longer wavelengths, suggesting a potential change in the chromophore environment. To confirm these findings, additional experiments were conducted to investigate the effect of varying concentrations of 2 T-PQ on the secondary structure and conformations of QP.

Our findings are consistent with various research studies by Wang, Xu, et al. (2019) and Alrosan, Tan, Easa, Gammoh, Kubow, and Alu'datt (2022), whereby the incorporation of exogenous substances within proteins significantly impacts their tertiary structure. It was reported by Han et al. (2023) that trehalose could play a significant role in altering the tryptophane residue in the soy protein nanoemulsion. Overall, the fluorescence emission spectra of tryptophane at an excitation wavelength of 280 nm offer a means to probe protein conformational changes. The tertiary structure of QPs was altered and significantly affected depending on the trehalose concentration in the solution during conjugation. This significant alteration to the protein structure suggested that trehalose plays a crucial role in modulating the conformational dynamics of the QPs, highlighting its potential as a stabilizing agent for protein structure.

Recent research conducted by Okagu, Abioye, and Udenigwe (2023) revealed that pea glutelin interacts with lipophilic bioactive compounds, significantly impacting the modification of protein structure in an alkaline solution. Additionally, Dufour and Dangles (2005) utilized fluorescence spectroscopy to investigate the interaction between serum albumin and flavonoids. They found that this complexation provides a crucial role in the binding sites of serum albumin.

3.2.3. Protein conformation

UV spectra typically range from 190 to 350 nm. This range is suitable to be used to study proteins because of its response to the aromatic side chains in many amino acids (Alrosan, Tan, Easa, Gammoh, & Alu'datt, 2022b), including tryptophan, tyrosine, and phenylalanine, all capable of absorbing the UV light within this region (Han et al., 2023). These aromatic amino acids contain conjugated π-electron systems, which give rise to characteristic absorption peaks in the UV spectrum (Kan et al., 2021). These absorption peaks can provide valuable information about the structure and environment of quinoa protein (Fig. 1D). Our findings indicate that the conformation of the protein structure changed based on the concentration of trehalose, suggesting that trehalose contributes to modulating the protein's structural integrity. The decrease in absorbance of the peak (around 220 nm) is associated with the presence of the bonds of peptides inside the protein structure, and the presence of phenylalanine residues that contribute to the formation of the absorption peak was decreased (around 260 nm). This conformational change could affect the protein's function and ability to interact with other molecules within the proteins (Alrosan, Tan, Easa, Gammoh, Kubow, & Alu'datt, 2022; Wang et al., 2023).

Furthermore, a recent study by Bhat, Mir, Singh, Hussain, and Dar (2023) observed that modifications of the protein conformation indicated improved functionality and behavior of the protein. The modified conformation protein showed promising results in preclinical studies, demonstrating improved efficacy and reduced side effects compared to the original form. However, Jiang et al. (2022) reported that modifications to the higher and secondary protein structures of pea protein improved the solubility and nutritional value of the protein, including a higher amount of antioxidants and greater thermal stability. The protein

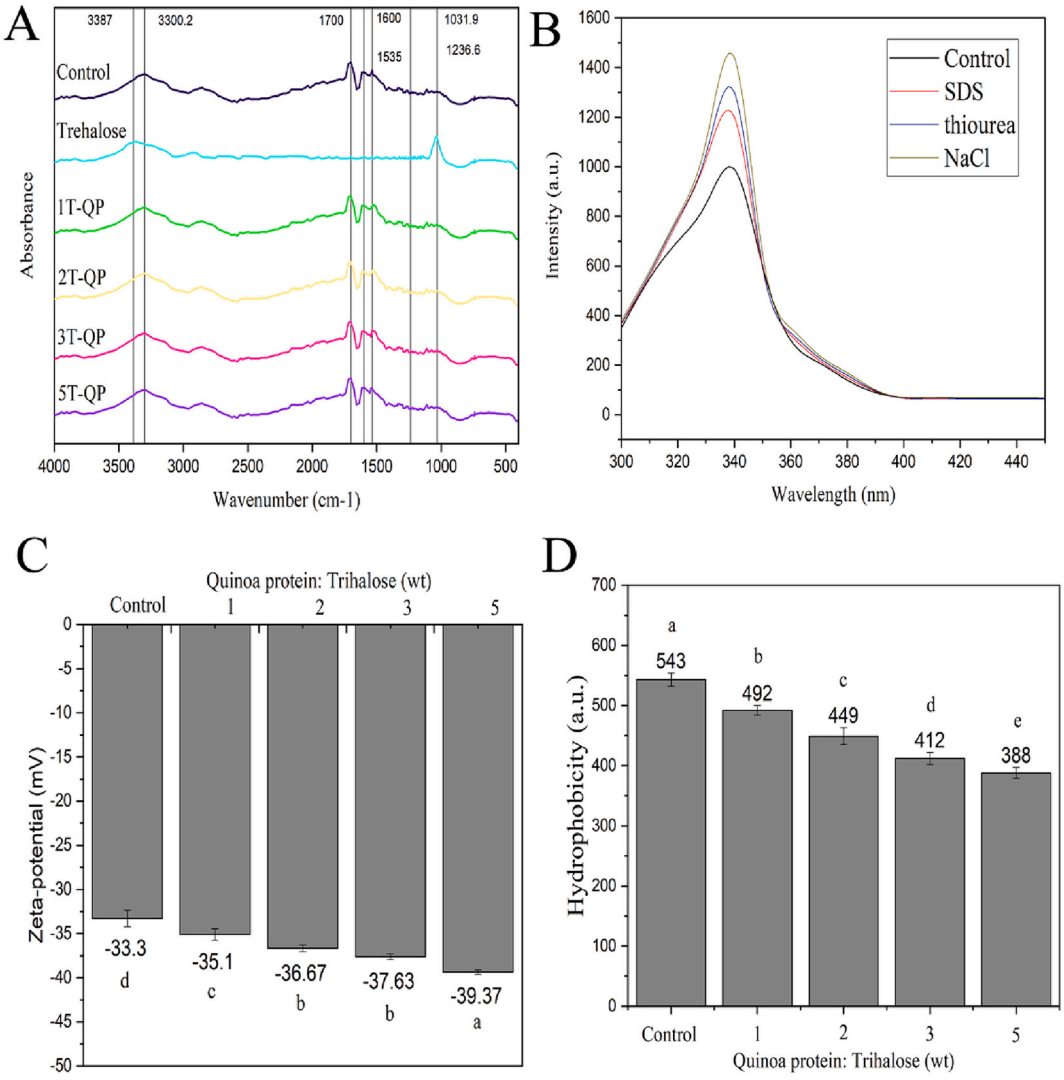

Fig. 2. Changes in the (A) fluorescence intensity (molecular forces), (B) surface charge, (C) surface hydrophobicity, and (D) FTIR absorbance of trehalose-conjugated quinoa proteins (QPs). Control (0 T-QP) represents the absence of trehalose conjugation with the QPs. Meanwhile, 1 T-QP, 2 T-QP, 3 T-QP, and 5 T-QP represent QP conjugated with trehalose at 1, 2, 3, and 5% (w/w), respectively.

is subjected to partial unfolding and refolding in the alkaline environment (Alrosan, Tan, Easa, Gammoh, & Alu'datt, 2022b), which results in structural and functional modification because of the treatment. Changes in pH generate a conformational change in proteins because of increased intramolecular forces, including hydrogen bonds, hydrophobic contact, and electrostatic attraction within the protein structure and polypeptide chain (Alrosan, Tan, Easa, Gammoh, & Alu'datt, 2022b). Protein-molecule interaction methods have shown that pH-shifting treatment can allow the unfolding and re-engagement of protein structure-driven non-covalent forces (Alrosan, Tan, Easa, Gammoh, & Alu'datt, 2022b), which can enhance the functionality of the proteins.

3.3. Molecular forces governing QPs

Numerous non-covalent forces govern protein interactions and are critical in regulating proteins' structure and functional properties (Wang, Xu, et al., 2019). NaCl, thiourea, and SDS were used to examine electrostatic interaction, hydrogen bonding, and hydrophobic interaction, respectively (Alrosan, Tan, Easa, Gammoh, Kubow, & Alu'datt, 2022). These forces within the QPs were analyzed using a fluorescence probe. The results shown in Fig. 2A revealed the conjugation of trehalose with the QPs. The disruption of hydrogen bonds, as evidenced by the exposure of hydrophobic residues, resulted in a partial instability of protein structure in the QPs. Hence, the elongated structures of the T-QPs interaction produced enhanced protein digestibility, coordinating with the findings of the protein digestibility (Fig. 1A). The fluorescence intensity reaches its maximum value due to the interaction between the conjugated trehalose and the QPs. The dominant factors contributing to this trend are mainly likely to be hydrogen bonds and electrostatic interactions, which have a greater influence compared to hydrophobic interaction (Fig. 2B). The results indicate the reaction of the T-QPs with thiourea and NaCl, followed by SDS. These results are consistent with the findings of FT-IR spectra (Fig. 2A).

Recently, Liu et al. (2023) reported that when hydrophobic residues become exposed, they interact with water molecules, causing the protein structure to become looser. The exposure of hydrophobic residues and the activation of hydrogen bonds and electrostatic interaction can change the protein's overall conformation and reflect on the solubility and digestibility. On the other hand, in a hydrogen bond, the hydrogen atom involved typically has a partial positive charge due to the unequal sharing of electrons in the covalent bond with the electronegative atom. This partial positive charge allows the hydrogen atom to be attracted to another electronegative atom nearby, resulting in an electrostatic interaction between the partial positive charge on the hydrogen atom

and the partial negative charge on the electronegative atom (Alrosan, Tan, Easa, Gammoh, & Alu'datt, 2022b). Hydrogen bonding stabilizes protein secondary structures, especially α-helix and β-sheet (Alrosan, Tan, Easa, Gammoh, & Alu'datt, 2022b; Wang, Xu, et al., 2019). It also contributes to protein-ligand and protein-protein interactions (Alrosan et al., 2024). Trehalose's hydroxyl groups can shift water molecules, resulting in the formation of strong hydrogen bonds with proteins and other substances, including fats; this interaction contributes to preserving the integrity of the structure of the protein, thus avoiding aggregation and denaturation (Butreddy, Janga, Ajjarapu, Sarabu, & Dudhipala, 2021). At low concentrations (<5%, *w*/w), trehalose has no substantial influence ($P > 0.05$) on the solubility of the QPs, indicating the lack of hydrogen bonds to build strong networks within the protein molecules of protein complexes.

3.4. Surface charge and hydrophobicity of QPs

The surface charge of proteins can be crucial in determining their ability to bind to and interact with disaccharides, including trehalose (Wang et al., 2023). In this study, the surface charge of the control (0 T-QP) was found to be approximately −33.3 mV (Fig. 2C). However, the surface charge of T-QPs raised considerably ($P < 0.05$), reaching −39.36 mV. This change in the surface charge suggests that electrostatic interactions greatly influence the surface charge, resulting in an increased negative charge of QPs following their conjugation with trehalose. As a result, it is expected that there might be a significant electrostatic repulsion among the molecules, consequently impeding their aggregation. Recently, Jiang et al. (2022) reported that an attraction between proteins and inulin was hypothesized to be caused by electrostatic interactions between the proteins and inulin. Contrary, the surface charge of quinoa protein was not altered ($P > 0.05$) at low trehalose concentrations, i.e., <3%, w/w (Fig. 2C). The lack of alteration in this scenario indicates that the hydroxyl group of trehalose is insufficient to adequately replace water molecules and establish robust hydrogen bonding with the quinoa protein. The lack of strong hydrogen bonding may limit the ability of trehalose to stabilize protein structures and protect them from denaturation.

Surface hydrophobicity reflects the presence and exposure of hydrophobic groups on the protein's surface and is closely related to the stability of protein, conformation, and function. Changes in surface hydrophobicity can impact protein stability, conformation, interactions, and solubility, ultimately influencing its overall functionality. The surface hydrophobicity of QPs, upon conjugation with trehalose, exhibited a substantial reduction ($P < 0.05$) as the concentration of trehalose rose (Fig. 2D). Trehalose acts as a protein stabilizer by selectively attaching to hydrophobic areas on the surface of proteins (Alrosan et al., 2024; Bhat et al., 2023). The interactions between trehalose and the hydrophobic residues protect them from exposure to the nearby polar molecules, hence decreasing the hydrophobic property of the surface.

The surface hydrophobicity of 0 T-QP showed a vital reduction around ~449 at the percentage of trehalose was 2% (*w*/w). Subsequently, it was decreased until ~388 at a higher trehalose concentration (5%, w/w). This reduction in surface hydrophobicity suggests that the presence of trehalose significantly impacts the water-repelling properties of the quinoa protein. Hence, this reduction in surface hydrophobicity may prevent protein aggregation and denaturation, ultimately enhancing stability. Additionally, trehalose has been found to promote proper protein folding by stabilizing the native conformation and preventing misfolding or unfolding events (Al-Qaisi et al., 2024).

3.5. Particle size of QPs

The size of the trehalose-conjugated quinoa protein showed a substantial increase with the concentration of trehalose 1% (*w*/w) (Fig. 3). It has been hypothesized that the reaction sites on the surfaces of the protein molecules have reached a state of saturation with trehalose. The particle size of the conjugates showed a substantial increase ($P < 0.05$) with an increased ratio of trehalose-incorporated quinoa protein. The increase can be attributed to the increase in solution viscosity, which reduced the frequency of encounters between proteins and polysaccharides (Jiang et al., 2022). Previous studies have indicated that the interaction between polysaccharides/disaccharides with proteins might increase the size of conjugates (Alrosan et al., 2024; Jiang et al., 2022). Furthermore, the increase in particle size could significantly affect the functionality of conjugated proteins. Additionally, it has been suggested that the conjugation of disaccharides with whey and lentil proteins may also affect their solubility and bioavailability (Alrosan et al., 2024).

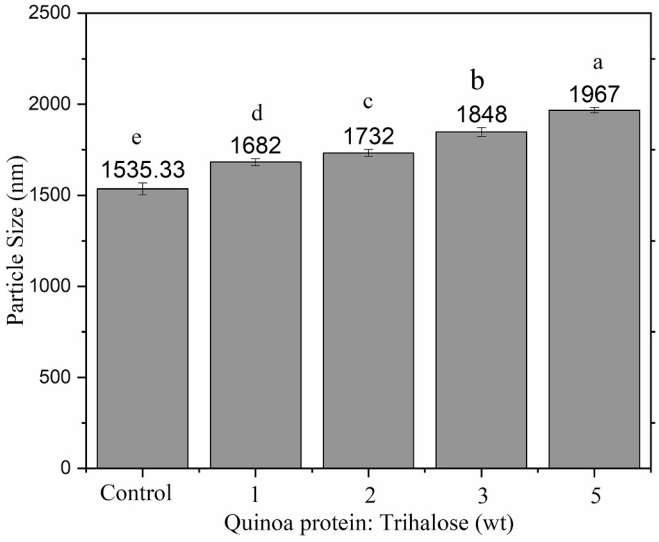

Fig. 3. Changes in the particle size of trehalose-conjugated quinoa proteins (QPs). Control (0 T-QP) represents the absence of trehalose conjugation with the QPs. Meanwhile, 1 T-QP, 2 T-QP, 3 T-QP, and 5 T-QP represent QP conjugated with trehalose at 1, 2, 3, and 5% (w/w), respectively.

3.6. Thermal stability of QPs

The denaturation temperature range of the trehalose-conjugated QPs (82.5–87 °C) was higher than that of the control (0 T-CP, 97.7 °C). Liu et al. (2023) observed that the thermal stability of QPs is around 99. This result agrees with our findings. On the other hand, the thermal stability of T-QPs increased significantly to reach 101.0 °C at a ratio of 5% (w/w) of trehalose. This finding supported the hypothesis that the protein-disaccharides interaction could substantially influence increases in protein structures' resistance against heat (Alrosan et al., 2024). The improved stability could be attributed to the glycosylation procedure, which might have impeded the unfolding of proteins through steric effects (Kan et al., 2021). Furthermore, the glycosylation process could have created a protective barrier around the proteins, preventing denaturation and maintaining their structural integrity. These results suggest that the protein-trehalose interaction and glycosylation could play crucial roles in enhancing the thermal stability of QPs. Previous studies have also proven that glycosylation can improve protein structures' resistance against heat (Alrosan et al., 2024). Furthermore, in contrast to other conjugates, the highest conjugates showed a higher denaturation temperature, indicating better thermal stability. This observation suggests that there might be a connection between the grafting degree of the conjugates and their thermal stability. Consequently, the interaction between complexation proteins and conjugated trehalose mixtures is highly successful in enhancing the resistance of heat stability in QPs.

4. Conclusion

The study attempted to modify the secondary and tertiary structures of quinoa proteins, which resulted in improved solubility and digestibility through the conjugation of trehalose at various concentrations ranging from 1 to 5% (*w*/w). By pH-recycling and trehalose conjugation, the results in this study revealed significant changes in the amide I region and intensity, protein conformation, particle size, surface charge, and hydrophobicity. Furthermore, the trehalose-conjugated quinoa protein's digestibility and solubility increased after the trehalose conjugation. The study demonstrates a successful approach for turning quinoa protein-conjugated trehalose into a soluble complex protein composite. This composite consists of plant proteins and shows high solubility and digestibility. These properties allow it to be an appropriate candidate for various food applications.

CRediT authorship contribution statement

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

Declaration of competing interest

The authors assert that no conflicts of interest exist.

Data availability

Data will be made available on request.

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