Research article

Effect of ultrasonic cavitation on the formation of soy protein isolate – rice starch complexes, and the characterization and prediction of interaction sites using molecular techniques

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ABSTRACT

Protein-carbohydrate interactions occur naturally in glycoproteins which are highly stable in nature and are involved in various food complexes and can enhance the quality and functional properties of foods. In the current study, we characterized the protein-carbohydrate complex formed between commercial soy protein isolate and rice starch using different treatments namely heat treatment alone, ultrasound treatment alone, combination of ultrasound and heat treatment and mixing alone. The structural data obtained using circular dichroism indicated that during the complex formation, the α-helix values were reduced by a maximum of 67% compared to soy protein isolate alone. The crystalline nature of the complexes formed by ultrasound treatment preserved the techno-functional properties as compared to complexes formed by heat treatments. The FTIR analysis of the complexes formed indicated the formation of glycosidic bond. Molecular docking analysis revealed the interaction between the complexes occurred due to hydrogen bonds which make the proteins more stable in nature thus enhancing their denaturation temperature. Glutamine, Proline and Arginine present in the D subunit of 7S 3AUP interacts with the starch molecule. The obtained results suggest that sonication combined with heat treatment led to higher interaction between the soy proteins isolate and rice starch.

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1. Introduction

Food proteins have numerous applications in food systems like, stabilization and formation of films, foams, emulsions, gel networks and have a significant effect on the rheological, textural and sensory attributes of foods (Nooshkam et al., 2020). The study of interactions occurring between the proteins and carbohydrates at molecular levels using molecular docking can lead to the designing of highly effective and stable protein-carbohydrate complexes with a wide range of applications. The formation of a protein-carbohydrate complex can occur due to the formation of hydrogen bonds, and hydrophobic or electrostatic interactions occurring between them. Numerous factors like molecular weight, nature of the polymer, temperature of the medium, ionic strength and pH influence the formation and stability of the protein (+ve) and carbohydrates (−ve) complexes (Gosh and Bandyopadhyay, 2009). Though proteins have numerous applications and functionalities they are highly heat-sensitive in nature which makes them of low utilization for commercial purposes. To overcome this, glycation of the proteins with carbohydrates to form protein-carbohydrate complexes is carried out. The various methods adopted for the formation of protein-carbohydrate complexes are Maillard reaction, glycation, physical mixing, coacervation, chemical cross-linking and enzymatic cross-linking (Wei and Huang, 2019).

Protein-carbohydrate complexes can occur due to covalent or non-covalent interactions which can be irreversible or non-specific in nature (Wijaya et al., 2017). The nature of the bonding between the protein-carbohydrate complexes determines the functionality of the complexes (Gentile, 2020). Apart from food applications, protein-carbohydrate interactions have a wide range of applications in biological processes such as molecular transport, cell-cell adhesion, and antigen-antibody recognition. In the pharmaceutical field, it helps in targeted drug delivery, drug design and also in encapsulation of bioactive components (Williams and Davies, 2001). The interaction between proteins and carbohydrates plays a key role in the biomedical field by aiding in the early detection and prevention of harmful diseases like diabetes, cancer and neurodegenerative diseases (Pérez et al., 1995). Though protein-carbohydrate complexes have numerous advantages the precise determination of the site of interaction still remains a challenge.

During the protein–carbohydrate interaction, the amino acids present in proteins like ASP, ASN, GLU, ARG, HIS, TRP, LYS, GLN, and THR involve in formation of a hydrogen bond between the carbohydrates. However, aromatic amino acids with aromatic side chains like TYR interacts with the carbohydrate molecules by van der Waals interaction (Poveda and Jim, 1998). Traditionally the protein-polysaccharide complexes are formed by applying wet heating treatment which requires a lot of energy and time for the interactions. Various novel technologies or combined technologies are being researched currently which can enhance the protein-carbohydrate interaction with minimal energy requirements and make it more stable in nature.

Ultrasonic cavitation has been applied for a range of food processing applications like enzyme activation, microbial inactivation, extraction and food matrix modification (Choudhary and Rawson, 2021; Naik et al., 2022; Sengar et al., 2020; Venkateswara Rao et al., 2021). The increase in functionality of the biomolecules like proteins and carbohydrates by inducing a structural change using ultrasonic cavitation technology is an emerging field of study in recent days (Wang et al., 2021). The ultrasonic treatment modifies the spatial arrangement of carbohydrates and secondary and tertiary structure of proteins due to the cavitation produced which causes high-velocity inter-particle collisions and high shear stress (Naik et al., 2021). The cavitation produced during ultrasound treatment induces structural change which increases the glycation of carbohydrates with proteins (Lin et al., 2020). The application of ultrasound treatment to the protein-carbohydrate complexes preserves the native structure of proteins from denaturation and increases their thermal stability.

Though there are several studies (Li et al. 2021; Liu et al., 2021; Zhao et al. 2021), the application of ultrasound treatment in protein-carbohydrate complex formation is not very well realized. And further studies are warranted to understand the interaction of protein carbohydrates due to the cavitations which can possess numerous applications in food systems. The current research mainly focuses on the modification of the protein-carbohydrate complex subjected to different treatments viz., mixing alone (without any treatment), ultrasound; heat and combination of both ultrasound and heat. Further to characterize the soy protein rice starch complex formed and to elucidate the structural changes that occur due to the above treatments.

Soy Protein Isolate (6 g) + Rice Starch (3 g) in 100 ml of water
Magnetic stirring at 28°C for 2 hrs
Stored overnight in refrigerator at 4°C

| Treatment Conditions | Heating Alone | Ultrasound Alone | Ultrasound + Heating | Mixing Alone |
|----------------------|---------------|-----------------|---------------------|-------------|
| Heating at 90°C for 1 hr | Ultrasound treatment at 450 W for 5 min | Ultrasound treatment at 450 W for 5 min and heating at 90°C for 1 hr | No heating or ultrasound treatment |

Cooled to room temperature and refrigerated at 4°C for 24 hr

Freeze Dried and stored

Figure 1. Flowchart of treatment conditions used.
2. Materials and methods

2.1. Materials

The high-quality spray-dried Soy Protein Isolate (SPI) with a protein content of more than 99% was purchased from Natures Velvet Life Care (Hyderabad, India). Rice Starch (RS) used for the study was procured from Urban Platter (Mumbai, India). The SPI and RS were stored appropriately in a dry place in airtight containers and used for the entire study.

2.2. Formation of soy protein – rice starch (SPI-RS) complexes

The Soy Protein Isolate and Rice Starch (SPI-RS) complexes were prepared according to the method described by Zhao et al. (2021) with minor modifications. The soy protein and rice starch were added in a ratio of 6:3 in 100 ml of distilled water and mixed at room temperature. Then the mixture was stirred at 28 °C for 60 min using a magnetic stirrer operated at 1200 rpm for 2 h. After stirring the mixture was stored overnight in a refrigerator at 4 °C for enhancing the hydration and homogeneous dissolution of soy protein and rice starch. The refrigerated samples were divided into four equal portions and subjected to different treatment conditions (Figure 1). Two sample portions were subjected to ultrasound treatment using a probe-type sonication system, with a 19 mm probe which was operated at 20 kHz with a maximum power output of 750 W. The treatment time of the samples was 5 min and it was maintained as constant. The pulsation during the treatment was maintained as 3 s ON and 1 s OFF. The ultrasound treatment intensity used for treatment was 60% i.e. 450 W. Among the two ultrasound treated sample portions, one was heated at 90 ± 1 °C for 1 h to determine the combined effect of ultrasound and heating on the interaction between the proteins and carbohydrates. The initial trials were conducted at four ultrasound treatment power levels (150 W, 300 W, 450 W, 600 W and 750 W) and heating temperature (80 °C and 90 °C) at two different time levels (30 min and 60 min) for formation of SPI-RS complex. Based on the initial trials, the final optimized condition of 450 W ultrasound power level and 90 °C heat treatment for 60 min was given to the samples, such that the final obtained SPI-RS possess good stabilizing and thickening nature. However, the ultrasound treatment time and pulsation used for the study were maintained as given by Zhao et al. (2021). A portion of sample was subjected heating alone to elucidate the effect of ultrasound in complex formation. Followed by heat treatment for 1 h, all the sample portions were cooled down to room temperature and refrigerated at 4 °C for 24 h to enable complete interaction between soy protein and rice starch. Another sample portion was used without any treatment i.e. ultrasound or heat treatment, to understand the effect of both heating and ultrasound. Further, the samples were lyophilized and stored for characteristic analysis. The glycation of protein with carbohydrates is generally mediated through heating. So to evaluate the effect of ultrasound treatment in formation of protein carbohydrate complex the samples were subjected to heat treatment and combination of heat and ultrasound treatments.

2.3. Circular dichroism (CD) spectroscopy

Circular Dichroism (CD) spectroscopy is a widely used rapid detection method to study the binding and fold recognition properties of proteins and to determine the secondary structure. Though CD doesn’t provide details about the specific residues and structural arrangement like XRD and NMR the short analysis time makes it a widely used technology in structural proteomics (Greenfield, 2007). The SPI-RS conjugates prepared by wet heating, ultrasound, a combination of ultrasound and heating and freeze-drying were analyzed using Circular Dichroism spectroscopy (JASCO J1500, Tokyo, Japan). The proteins samples for analysis were prepared at a concentration of 0.25 mg/ml in a 0.01 M PBS buffer solution of pH 7. The spectra of the samples were obtained at an ultraviolet wavelength region of 190–250 nm. The different parameters involved in CD spectroscopy are bandwidth 2 nm, data pitch 1 nm, scanning speed 100 nm/min, data accumulation 3 times and path length of the cuvette is 1 cm. The PBS buffer solution is used as a blank. The mean ellipticity values were obtained and they are converted to the secondary structural values using the BESTSEL online server (Wien et al., 2018).

2.4. Fourier transform infrared spectroscopy (FTIR)

FTIR spectroscopy is a widely applied rapid technique to study the nature and formation of protein-carbohydrate complexes (Zhang et al., 2020). The freeze-dried samples were analyzed in an FTIR (Nicolet iS50, Thermo Fisher Scientific, USA) spectrophotometer with a DTGS KBr detector controlled by Omnic 9.9.549 software. The samples were mixed with FTIR grade KBr to form a pellet and analysed to determine the characteristic peaks. The different parameters involved in FTIR analysis are a resolution of 4 cm⁻¹, with a total of 16 scans and at a scanning wavelength range of 400 cm⁻¹–4000 cm⁻¹ for each sample.

2.5. X-ray diffraction (XRD)

To determine the structural information and determine the nature of the proteins and starch molecules XRD analysis was performed (Dong and Cui, 2021). The diffraction patterns of soy protein–rice starch complexes were obtained from the freeze-dried powders using the XRD system (PANalytical Xpert 3 Powder, Malvern, United Kingdom) following the method adopted by Dong and Cui (2021) with slight modifications. The crystalline nature of the SPI-RS complexes obtained by different treatments can be determined by using Cu Kα radiation of 1.5418 Å in a continuous mode without spinning. The XRD data of the samples were obtained at a constant temperature of 25 °C in the region between 5° and 60°, at a scan rate of 2°/min, scan angle of 20° and at 30 mA and 45 kV working conditions. The diffraction data obtained were analyzed using High Score Plus Version 5.0 PANalytical Inc. (Malvern, United Kingdom) software to compare the data obtained for different treatments.

2.6. Differential scanning calorimetry (DSC)

The thermal stability and denaturation or gelatinization temperatures of the protein and starch under the controlled condition with a constant heating rate and nitrogen gas flow were determined using a DSC system (Li et al., 2014). The denaturation temperature of the SPI-RS complexes was obtained using the modified method of Li et al. (2014) and Dong and Cui (2021) in a DSC (NETZSC DSC 204 F1 Phoenix, Germany). The thermogram obtained from the samples was evaluated for the peak denaturation temperature using the software OriginPro version 8.5. The samples were heated in a Nitrogen atmosphere with a flow rate of 50 ml/min, heating range of the sample is 20–150 °C and the heating rate is maintained as 10 °C/min. 2 mg of the sample is used for analysis and the empty pan is used as reference.

2.7. Molecular docking

The molecular docking study was carried out to identify the binding between the soy protein and rice starch. To evaluate the nature of the interactions and the binding energy involved in the formation of protein-carbohydrate complex. The three-dimensional characteristic structure of the major protein fraction available in soybean 7S (Globulin, 3AUP) (Yoshikawa et al., 2011) was obtained from RCSB Protein Data Bank. The 3-D conformational structure of the rice starch was obtained from PubChem (“National Center for Biotechnology Information (2021), PubChem Compound Summary for CID51003661, Starchsoluble. Retrieved September 14, 2021 from https://pubchem.ncbi.nlm.nih.gov/compound/Starchsoluble,” n.d.) and the structures of protein and starch were converted to the suitable format for docking in AUTODOCK software (Morris et al., 2012). To
determine the binding site of the rice starch in 3AUP protein blind docking was carried out using the Lamarckian Genetic Algorithm with total genetic algorithm runs of 50 for a population size of 300 while the energy evaluation and the number of generations were maintained at a medium level to obtain the results for interaction between soy protein and rice starch. The conformation with the least binding energy is selected and analyzed for the presence of various bonds using PLIP (Protein-Ligand Interaction Profiler) (Salentin et al., 2015) an online server. Further, PyMOL software was used for the visualization of docking and interaction sites of the SPI-RS complex. The 2D representations of the amino acids involved in the interaction were determined using Discovery Studio visualizer 2019 (Dassault Systemes Biovia Corp®; California; USA).

3. Results and discussion

3.1. Circular dichroism (CD) spectroscopy

The CD spectroscopy of the SPI-RS complexes revealed that the interaction between the soy protein and rice starch modified the secondary structure of the proteins. The application of heat energy protein without the addition of polysaccharides reduced the α-helix content by 49% and increased the β-sheets by 322% and unordered content by 45% indicating, the α-helix is converted to the unordered and β-sheets (Liu et al., 2020). Figure 2 indicated the structural changes that occurred in the soy protein rice starch complexes obtained from the mean ellipticity values using circular dichroism spectroscopy. The cavitation produced during ultrasound treatment disrupted the sequential arrangement of the amino acids which leads to the structural change of protein. The results obtained suggest that the structural change is achieved maximum using ultrasound combined with heat treatment which proves to be the efficient method for glycation of soy protein with rice starch (Table 1). The formation of SPI-RS complex reduced the α-helix and unordered content, whereas the β-sheets contents were increased compared to the raw protein. The α-helix values were reduced to a maximum of 67% compared with raw soy protein for the SPI-RS complexes formed using a combination of ultrasound, heat treatment, and mixing treatment. During the SPI-RS interaction, protein structural changes were observed, indicating the conversion of α-helix to β-sheets. Due to this phenomenon, the values for β-sheets reached a maximum of 28% in the complexes after the interaction of soy proteins with rice starch. The protein to polysaccharide ratio present in the mixture, reaction parameters and the nature of proteins are the factors that attribute to the secondary structural changes of proteins being analyzed (Shen and Li, 2021). The α-helix and β-sheets of the protein decreased while the random coils increased upon the complex formation. The complex formed using wet heating and along with ultrasound treatment decreased the α-helix and increased the β-sheets of soy protein (Ma et al., 2020). The applied external energy disrupted the protein secondary structure and thus it can be presumed that the various treatment conditions used in this study may also exhibit similar change in the samples used for this study. During the complex formation α-helix to β-type structural transformation occur due to the binding between the reduced carbonyl groups present in the carbohydrate molecule with the amino acids in the α helix region, which may cause the reduction of α-helix content of soy protein (Zhao et al., 2021).

3.2. Fourier transform infrared spectroscopy (FTIR)

The changes in the FTIR spectra obtained for the samples indicated the glycation between SPI-RS. The glycation of SPI-RS complexes can reduce the NH2 functional group of soy protein and can lead to the emergence of some distinct functional groups due to the formation of Schiff base and Amadori compounds (Chen et al., 2019). The characteristic peaks of SPI which correspond to the Amide I, Amide II and Amide III regions were obtained at 1647 cm⁻¹, 1540 cm⁻¹ and 1396 cm⁻¹. Generally, the Amide I region occurs between 1600 cm⁻¹ and 1700

| Sample       | Helix | Anti parallel | Turns | Others | Total |
|--------------|-------|---------------|-------|--------|-------|
| HEATING ALONE | 2.4   | 34.3          | 16    | 47.3   | 63.3  |
| US ALONE     | 1.4   | 37.2          | 15.4  | 46     | 61.4  |
| US + HEATING | 1.3   | 37.2          | 15.6  | 45.9   | 61.5  |
| MIXING ALONE | 1.3   | 37.1          | 15.6  | 46     | 61.6  |
| SOY PROTEIN  | 3.9   | 29.1          | 15.5  | 51.5   | 67    |

Figure 2. Circular Dichroism Data obtained for Soy Protein Rice Starch complexes.
cm⁻¹ which corresponds to the vibrations occurring due to the C=O stretching of peptides and free carboxyl groups, N–H bending vibrations of the proteins generate the absorption peaks between 1500 cm⁻¹–1600 cm⁻¹ in the Amide II region and the peaks in 1200 cm⁻¹–1450 cm⁻¹ reveals the C–N stretching and N–H vibrations in Amide III region of proteins (Figure 3) (Dong and Cui, 2021; Boostani, Aminlari, Moosavi-nasab, Niakosari and Mesbahi, 2017). The absorption peaks obtained for rice starch at 900 cm⁻¹–1200 cm⁻¹ are termed as saccharides bands that occur due to C–O and C–C bonds stretching and the bending of C–H bonds, which forms the fingerprint region of polysaccharides (Mummaleti et al., 2020; Rohiwal et al., 2021). The FTIR peaks for the SPI-RS complexes in the region of 1647 cm⁻¹ for the control, US alone and mixing alone samples and in the region of 1658 cm⁻¹ for ultrasound combined with heat treatment sample reduces compared to raw soy protein isolate due to the reduction in amino groups present in them which indicates the interaction between the soy protein isolate rice starch complexes (Mao et al., 2018). The spectra peaks obtained in 1647 cm⁻¹ and 1540 cm⁻¹ for ultrasound combined with heat treatment and ultrasound treatment alone had decreased intensity compared to the mixing and heating treatments which prove that the ultrasound treatment enhances the glycation between the SPI-RS (Chen et al., 2019). The spectral peaks obtained for SPI-RS complexes using various treatments showed a new peak in 1022 cm⁻¹ or 1023 cm⁻¹ which occurs as a result of stretching vibrations produced by C–O–C
glycosidic bonds formed during interaction (Zhao et al., 2021). The FTIR peaks for the complexes increases in the wavelength region of 3300 cm\(^{-1}\)–3450 cm\(^{-1}\) compared to SPI except for sample treated with ultrasound alone which indicates the complex formation can be due to hydrogen bonds (Xu and Liu, 2016). The cavitation produced during ultrasound treatment broke the hydrogen bonds which was evident by the decrease in peak intensity observed at the same wavelength, suggesting the possible complex formation through hydrophobic or

Figure 5. a: XRD diffraction pattern of soy protein rice complexes formed by various treatments. b: Stacked XRD diffraction pattern of soy protein rice complexes formed by various treatments.
electrostatic interactions (Xue et al., 2020). The FTIR spectra obtained for the SPI-RS complexes formed using various treatments show that the complex formed has the characteristic peaks of both proteins and carbohydrates (Figures 4, S1, S2, S3 and S4). The intensity of the peak obtained in the Amide III regions decreased due to the complex formation between soy protein and rice starch which changes the secondary structure of the proteins (Liu et al., 2019). This is evident from the structural change of soy proteins obtained using CD spectra. Further, the ultrasound combined with heat treatment enhanced the glycation of soy protein with rice starch compared to other methods.

3.3. X-ray diffraction (XRD)

The results obtained from XRD suggest that the rice starch is crystalline in nature with sharp peaks, whereas the SPI and SPI-RS complexes have broader amorphous peaks which indicate they are semi-crystalline in nature (Figure 5(a)). The diffraction peaks obtained around $8^\circ$ and $19^\circ$ for the soy protein isolate and the SPI-RS complexes in the 20 region are the characteristics peaks of SPI which denotes the $\alpha$-helix and $\beta$-sheets region (Gu et al., 2020). The diffraction peaks obtained for the SPI-RS complexes in the $\alpha$-helix and $\beta$-sheets region decrease as the structural changes occur during the interaction. These results differ from the results obtained using CD spectroscopy as the reduction in $\alpha$-helix and increase in $\beta$-sheets values are obtained during complex formation. The SPI-RS complexes obtained using heat treatments had a reduced crystalline nature of the complexes compared to the other two treatments without heat treatment. This indicated that the application of ultrasound treatment to the starch-modified the diffraction pattern slightly without affecting its crystalline structure. The intensity count of the diffraction peaks obtained for starch with sonication was reduced compared to untreated samples (Amini et al., 2015). The results obtained using XRD confirm the interaction between the soy protein isolate and rice starch which leads to the reduction in the intensity counts of the diffraction peaks obtained for the SPI-RS complexes compared to the native SPI and rice starch. The structural changes occurring in the soy protein and rice starch during the interaction lead to the decreased intensity of the complex. The intensity counts of the complexes formed are low compared to the raw SPI and rice starch which indicates the reduction in crystalline nature which can be due to the non-covalent interactions (Shang et al., 2021; Dong and Cui, 2021). The diffraction peaks obtained for the different treatments suggest that the application of ultrasound treatment preserved the crystalline nature of the SPI-RS complexes compared to other treatments (Figure 5(b)). Sharp diffraction peaks were obtained for SPI-RS complex treated with ultrasound treatment whereas the rest of the treatments do not show significant difference in spikes (Figures S5, S6, S7 and S8). It can be ascertained that less amount of energy is involved in the ultrasound treatment may be preserving the crystalline nature of complex with high techno functional properties. Since the functionality of the proteins are correlated with the crystalline nature, the ultrasound treatment does not alter the native form conserving the functionality of the protein.

3.4. Differential scanning calorimetry (DSC)

The thermogram obtained for SPI-RS complexes suggests that the formation of complex increases the denaturation temperature and enthalpy values compared to raw SPI. The glycation of soy protein isolate with dextran lowers the aggregation nature of proteins compared to the untreated SPI due to the covalent interactions between the proteins and carbohydrates. The formation of protein-carbohydrate complexes reduces the enthalpy; during conjugation, the exothermic reaction occurs, which leads to cross-linking of protein and carbohydrate (Boostani et al., 2017). The presence of the proteins restricts the gelatinization; similarly, polysaccharides prevent the denaturation of proteins in protein-polysaccharide complexes, which leads to the increase in the thermal stability of the complex.

### Table 2. Denaturation Temperature values obtained for Soy protein isolate and rice starch complexes.

| Sample        | Denaturation temperature (°C) |
|---------------|-------------------------------|
| HEATING ALONE | 85.8                          |
| US ALONE      | 83.7                          |
| US + HEATING  | 88.8                          |
| MIXING ALONE  | 86.2                          |
| SOY PROTEIN   | 80.7                          |

Figure 6. DSC thermogram of soy protein rice complexes formed by various treatments.
complexes formed (Li et al., 2014). The glycation of milk proteins acts as a highly efficient technique that improves the thermal stability of proteins (O’Mahony et al., 2016). The results indicate that the denaturation of the soy protein rice starch complex is maximum for ultrasound combined with heat treatment compared to other treatments (Figure 6). The denaturation temperatures of the complexes formed using all treatments increased compared to the raw SPI. The improvement of thermal stability of the protein-carbohydrate complex is influenced by the chain length, the number of carbohydrates attached and the point of interaction (Table 2). The denaturation temperature of SPI-RS complexes increased, as the rice starch, with a gelatinization temperature of 92 °C, was added to soy protein isolate. Since the freeze-drying treatment results in a marked disordering of starch structures because of the freezing of water and the sublimation of ice crystals, we assume that it has changed the gelatinization temperature of rice starch. Similarly, the addition of high molecular weight carbohydrates to whey protein isolates increases the steric repulsions compared to monosaccharides that enhance the thermal stability of proteins (Mulachy, 2017). The glycation of different proteins like canola protein, whey protein and porcine protein isolates preserves the natural structure of proteins and decreases the protein-protein interactions to improve the heat stability of proteins (Nooshkam et al., 2020). The various treatment conditions followed during the protein-carbohydrate glycation can affect the thermal stability of the complexes formed (Mahony et al., 2019). The attachment of carbohydrates increases the steric hindrance of the proteins molecules which acts as a key factor in increasing the thermal stability of proteins (Mulachy, 2017). Thus the glycation of proteins makes them more heat-stable in nature and tends to increase their application in the food industry.

Figure 7. a: Interaction between 3AUP (7S) and rice starch molecules. b: Hydrogen bonding between 7S and rice starch molecules.
3.5. Molecular docking

The molecular docking results of 7S protein (3AUP) of SPI with rice starch predicted the formation of hydrogen bonds between the amino acids and hydroxyl groups present in the rice starch (Figure 7(a)). The formation of hydrogen bonds can lead to a stable structure which provides the necessary thermal stability and texture to the soy protein and rice starch complex. The molecular docking is applied to the proteins and carbohydrates such that the docked conformation must have very low binding energy after interaction (Agarwal and Mehrotra, 2016). The results obtained during the AUTODOCK run suggested that the best run with the binding energy of $-7.79$ kcal/mol with an inhibition constant ($K_i$) of 1.95 $\mu$M is selected for further analysis and visualization of the interactions in the PLIP server. The maximum negative value obtained for the binding energy is considered the least value in which the interaction between the protein (3AUP) and rice starch can occur naturally without any applied external energy or with minimal energy requirement. The binding energy is expressed as the sum of various forces that involve in interactions like van der Waals forces, hydrogen bonding, electrostatic and internal bonds. The interaction between the hyperoside and soy protein occurs due to the hydrogen bonding or van der Waals force which increases the stability leading to the balance of the food systems (Wu et al., 2021). A complex network of hydrogen bonds is usually formed between two or three hydroxyl groups of the carbohydrates to the proteins as the carbohydrates don’t involve in charge to charge interactions with proteins (Fadda and Woods, 2010). The PLIP results indicated that the interaction between the SPI and rice starch had occurred in the D subunit of the 7S (3AUP) subunit by the formation of hydrogen bonds between the amino acids Glutamine (354, D), Proline (355, D) and Arginine (356, D) and OH groups of rice starch (Figures 7(b), S9(a), S9(b), S10(a), and S10(b)). The 2 D representations plot (Figure 8) obtained using Discovery Studio software also indicated the hydrogen bond formation between the OH group of rice starch with Arginine, Proline and Glutamine. These results suggested that the interaction between the 3AUP and rice starch is mainly mediated through hydrogen bonds. This indicated the presence of positively charged motif in the D subunit of protein which produced a conducive environment for bonding with rice starch molecule. However, similar amino acids are also present in the other sub units of the protein albeit these amino acids are distanced from each other where such positively charged motifs cannot be formed which may be the reason bonding does not happen in other subunits. The hydrogen bonds formed between the amino acids of 3AUP and the rice starch molecule are of small distances in the range of 2–4 Å, which makes the complex more stable in nature. This correlates with the results obtained for DSC suggesting the increase in denaturation temperature of the soy protein rice starch complexes compared to soy protein alone. Though molecular docking is widely applied to study molecular interaction between biomolecules often the results obtained are subjected to validation. The application of molecular docking in protein-carbohydrate interactions is limited due to the difficulty in precise estimation of energy requirements and site of interaction. The results obtained in this study using a model protein can vary case-to-case based on the processed food that have a different conformation (Zhang et al., 2021).

4. Conclusion

In summary, among the different treatments, viz., heat treatment alone, ultrasound treatment alone, a combination of ultrasound and heat treatment and simple mixing applied for the soy protein rice starch complexes; the combination of ultrasound with heat treatment proved to be an efficient method that enhanced the glycation of soy proteins with rice starch. The complex formation modified the protein’s secondary structure, as evident from the spectra obtained using CD and FTIR. The complex formation lowered the $\alpha$-helix and unordered content; however, it increased the $\beta$-sheets values, which ascertainment the interaction of SPI and RS. This interaction enhances the thermal stability and the

Figure 8. 2 D representation of 7S rice starch interaction.
amorphous nature of the complexes formed. Based on the docking studies, we presume that the interaction occurs by hydrogen bonding between the OH group of rice starch and Proline, Arginine and Glutamine present in the D subunit of 3AUP protein. The environment plays a significant role in determining the interaction. A positively charged motif in the D subunit of protein produces a conducive environment for the interaction of SPI and RS. Thus we conclude that the glycation of soy protein isolate and rice starch can lead to the development of products with modified protein structure and functionality. Such modified proteins replace commercially available stabilizers and thickeners in the food industry, primarily composed of synthetic fats.

**Declarations**

**Author contribution statement**

Nirmal Thirunavookarasu: Performed the experiments; Analyzed and interpreted the data.

Sumit Kumar: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Arunkumar Anandharaj: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Ashish Rawson: Conceived and designed the experiments; Analyzed and interpreted the data.

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**Data availability statement**

Data will be made available on request.

**Declaration of interests statement**

The authors declare no conflict of interest.

**Additional information**

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**References**

Agarwal, S., Mehostra, R., 2016. An overview of molecular simulation. JSM Chem. 4 (2), 1024–1028.
Amini, A.M., Razavi, S.M.A., Mortazavi, S.A., 2015. Morphological, physicochemical, and viscoelastic properties of sonicated corn starch. Carbohydr. Polym. 122, 282–292.
Boostani, S., Amilhari, M., Moosavi-nazab, M., Nikolou, M., Moshahi, G., 2017. Fabrication and characterisation of soy protein isolate-grafted dextrin biopolymer: a novel ingredient in spray-dried soy beverage formulation. Int. J. Biol. Macromol. 102, 297–307.
Chen, W., Ma, X., Wang, W., Lv, R., Guo, M., Ding, T., et al., 2019. Preparation of modified whey protein isolate with gum acacia by ultrasound malldar reaction. Food Hydrocolloids 95, 298–307.
Choudhary, P., Rawson, A., 2021. Impact of power ultrasound on the quality attributes of green lentil and/or guar gum conjugation enhanced protein isolate. Ultrason. Sonochem. 61, 104812.
Chowdhury, P., Sun, Q., Wang, J., Zheng, B., Guo, Z., 2021. Structural characteristics and emulsifying properties of a novel Maillard conjugate of soy protein isolates induced by ultrasound malldar reaction. Ultrason. Sonochem. 72, 105458.
Liu, D., Zhang, Q., Xiao, L., Huang, Y., Yang, Z., Wu, Z., et al., 2020. Effects of ultrasound on functional properties, structure and glycation properties of proteins: a review. Crit. Rev. Food Sci. Nutr. 1–11.
Liu, Y., Liu, J., Zhang, W., Pearce, R., Chen, M., Zhang, T., Liu, B., 2020. Effect of the degree of glycation on the stability and aggregation of bovine serum albumin. Food Hydrocolloids 108 (April), 105892.
Liu, X., Yang, Q., Yang, M., Du, Z., Wei, C., Zhang, T., et al., 2021. Ultrasound-assisted Maillard reaction of ovalbumin/xyllose: the enhancement of functional properties and its mechanism. Ultrason. Sonochem. 73 (January), 105477.
Ma, X., Hou, F., Zhao, H., Wang, D., Chen, W., Miao, S., Liu, D., 2020. Conjugation of soy protein isolate (SPI) with pectin by ultrasound treatment. Food Hydrocolloids 108 (May), 106056.
Mahony, J.A.O., Drapala, K.P., Mulchay, E.M., Mulvihill, D.M., 2019. Whey Protein - Carbohydrate Conjugates.
Mao, L., Pan, Q., Hou, Z., Yuan, F., Gao, Y., 2018. Development of soy protein isolate-Carrageenan conjugates through malldar reaction for the micromacapsulation of Bifidobacterium longum. Food Hydrocolloids 84, 489–497.
Morris, G.M., Huey, R., Lindstrom, W., Sanner, M.F., Belew, R.K., Goodsell, D.S., Olson, A.J., 2012. AutoDock4 and AutoDockTools4: automated docking with selective receptor flexibility. J. Comput. Chem. 32, 174–182.
Mulachy, E.M., 2017. Preparation, Characterisation and Functional Applications of Whey Protein-Carbohydrate Conjugates as Food Ingredients. University College Cork School.
Mummaleti, G., Sarma, C., Kalakadan, S.K., Sivanandham, V., Rawson, A., Anandharaj, A., 2020. Optimization and extraction of edible microbial polysaccharide from fresh coconut inflorescence sap: an alternative substrate. LWT - Food Sci. Technol., 110619.
Naik, M., Natarajan, V., Modupalli, N., Thangaraj, S., 2022. Pulsed ultrasound assisted protein extraction of defatted Bitter melon seeds (Momordica charantia L.) meal: kinetics and quality measurements. LWT 155 (December 2021), 112907.
Naik, R.R., Wang, Y., Selomulya, C., 2021. Improvements of plant protein functionalities by Maillard conjugation and Malldar reaction products. Crit. Rev. Food Sci. Nutr. 1–26.
National Center for Biotechnology Information, 2021. PubChem Compound Summary for CID 51003661, Starchosub. Retrieved September 14, 2021 from, https://pubch em.ncbi.nlm.nih.gov/compound/Starchosub (n.d.).
Nooshkam, M., Varidi, M., Verma, D.K., 2020. Functional and biological properties of Maillard conjugates and their potential application in medical and food: a review. Food Res. Int. 131, 109003.
Naik, M., Natarajan, V., Selomulya, C., 2021. Conventional solubility of milk proteins and peptides: functional properties. Int. Dairy J. 67 (April 2017), 16–34.
Peter, S., Meyer, C., Inmerty, A., 1995. Practical tools for molecular modeling of complex carbohydrates and their interactions with proteins. Mol. Eng. 5 (1–3), 271–300.
Poveda, A., Jim, J., 1998. NMR studies of carbohydrate – protein interactions in solution. Chem. Soc. Rev. 27, 133–143.
Rohilwal, S.S., Elledrova, Z., Tiwari, A.P., Alqarni, M., Elazab, S.T., El-Saber Batiha, G., et al., 2021. Self-assembly of bovine serum albumin (BSA)-dextrin bio-nanocojugate: structural, antioxidant and in vitro wound healing studies. RSC Adv. 11 (8), 4308–4317.
Salentin, S., Schreiber, S., Haupt, V.J., Adasme, M.F., Schroeder, M., 2015. PLP: fully atomic protein–ligand interaction profile. Nucleic Acids Res. 43 (W1), W443–W447.
Sengar, A.S., Rawson, A., Mathial, M., Kalakadan, S.K., 2020. Comparison of different ultrasound assisted extraction techniques for pectin from tomato processing waste. Ultrason. Sonochem. 61, 104812.
Shen, Y., Li, Y., 2021. Acylation modification and/or guar gum conjugation enhanced functional properties of pea protein isolate. Food Hydrocolloids 117 (November 2020), 105892.
Venkatswara Rao, M., Sunil, C.K., Rawson, A., Chidandam, D.V., Venkatabhshyapati, N., 2021. Modifying the plant proteins techno-functionalities by novel physical processing technologies: a review. Crit. Rev. Food Sci. Nutr. 1–22.
Wang, T., Chen, X., Wang, W., Liang, J., Lu, D., Xie, F., 2021. Effect of ultrasound on the properties of rice bran protein and its chlorogenic acid complex. Ultrason. Sonochem. 79, 105756.
Wei, Z., Huang, Q., 2019. Assembly of protein – polysaccharide complexes for delivery of bioactive ingredients: a perspective paper Assembly of Protein – polysaccharide complexes for delivery of bioactive ingredients: a perspective paper. J. Agric. Food Chem. 67 (5), 1344–1352.
Wien, F., Kun, J., Lee, Y., Goto, Y., Matthieu, R., 2018. BeStSel: a web server for accurate prediction of protein interactions in solution. Nucleic Acids Res. 46 (June), 315–322.
Wijaya, W., Patel, A.R., Setiowati, A.D., Van der Meer, P., 2017. Functional colloids from proteins and polysaccharides for food applications. Trends Food Sci. Technol. 68, 56–69.

Gu, W., Liu, X., Ye, Q., Gao, Q., Gong, S., Li, J., Shi, S.Q., 2020. Bio-inspired co-deposition strategy of aramid fibers to improve performance of soy protein isolate-based adhesive. Ind. Crop. Prod. 150 (March), 112424.
Li, S., Wei, Y., Fang, Y., 2014. DSC Study on the thermal Properties of Soybean Protein Isolates/Cor Starch Mixture, pp. 1633–1638.
Li, Z., Zheng, Y., Sun, Q., Wang, J., Zheng, B., Guo, Z., 2021. Structural characteristics and emulsifying properties of a novel Maillard conjugate of soy protein isolates induced by ultrasound malldar reaction. Ultrason. Sonochem. 72, 105458.
Williams, S.J., Davies, G.J., 2001. Protein-carbohydrate interactions: learning lessons from nature. Trends Biotechnol. 19 (9), 356–362.

Wu, D., Tang, L., Duan, R., He, X., Geng, F., Zhang, Y., et al., 2021. Interaction mechanisms and structure-affinity relationships between hyperoside and soybean β-conglycinin and glycinn. Food Chem. 347 (January).

Xu, Y., Liu, L., 2016. Structural and Functional Properties of Soy Protein Isolates Modified by Soy Soluble Polysaccharides.

Xue, F., Li, C., Adhikari, B., 2020. Physicochemical properties of soy protein isolates-cyanidin-3-galactoside conjugates produced using free radicals induced by ultrasound. Ultrason. Sonochem. 64 (December 2019), 104990.

Yoshizawa, T., Shimizu, T., Yamabe, M., Taichi, M., Nishischi, Y., Shichijo, N., et al., 2011. Crystal structure of basic 7S globulin, a xylloglucan-specific endo-β-1,4-glucanase inhibitor protein-like protein from soybean lacking inhibitory activity against endo-β-glucanase. FEBS J. 278 (11), 1944–1954.

Zhang, H., Zhang, Y., Huang, Y., Wu, L., Guo, Q., Wang, Q., et al., 2021. Interaction between bovine serum albumin and chitosoligosaccharides: I. Molecular mechanism. Food Chem. 358 (April), 129853.

Zhang, X., Li, X., Liu, L., Wang, L., Massounga Bora, A.F., Du, L., 2020. Covalent conjugation of whey protein isolate hydrolysates and galactose through Maillard reaction to improve the functional properties and antioxidant activity. Int. Dairy J. 102, 104584.

Zhao, C., Yin, H., Yan, J., Niu, X., Qi, B., Liu, J., 2021. Structure and acid-induced gelation properties of soy protein isolate-maltodextrin glycation conjugates with ultrasonic pretreatment. Food Hydrocolloids 112 (August 2020), 106278.