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Effect of ultrasound treatment on soy protein: Rice starch interaction

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Abstract
The protein – polysaccharide complex is formed by the interaction between protein and polysaccharides which has high techno functional properties like solubility, foaming and emulsifying nature. The traditional heating method followed for formation of protein carbohydrate complex is time consuming, so the application of the novel technologies like ultrasound, plasma in complex formation must be investigated and studied well. In this study the different ultrasound treatments in combination with heat treatment was studied in formation of soy protein rice starch complexes and their properties. Among the treatments applied; ultrasound treatment at 60% with heating proved to be efficient in formation of soy protein rice starch interaction complex which is significantly different compared to the raw soy protein isolate. The results obtained for rheological and textural profile data also confirmed that the higher consistency and viscosity of the samples treated at ultrasound 60%, compared to the other treatments. The attachment of rice starch to the soy proteins enhances the functionality of both the biomolecules which can be potentially used as fat replacers, thickeners or stabilizers in food industry.

Keywords: ultrasound, protein polysaccharide complex, rheology, consistency, structural modification

1. Introduction
Proteins are one of the important biomolecules present in the foods and they are made up of amino acids. They are widely used food biopolymer with high nutritional value and provide the desired colour, smell, state, texture, and taste of different foods. Proteins have diversified properties like emulsifying, foaming, gelling and solubility; these properties are generally enhanced due to the structural changes of the proteins. The structural modification of proteins provides a way for the development of numerous new products like gels and emulsions which can be extensively used in foods. If used in higher amounts the proteins can potentially replace the carbohydrate and fat content of the foods with reduced energy values. Numerous factors like pH, temperature, structural stability affects the usage and stability of proteins in foods which limits it commercial applications (de Oliveira, Coimbra, de Oliveira, Zuñiga, & Rojas, 2016) [2]. To overcome these limitations numerous methods are being applied and the glycation of the proteins with carbohydrates is one among them which increases the protein functionality and makes it more stable in nature (Kutzli, Weiss, & Gibis, 2021) [6].

Polysaccharides act as a major store house of energy and contribute to the structural integrity of the plant and animal cells. They are widely available in nature which makes them less expensive and have wide range of application as gelling agents, thickeners in agro processing and food processing industries. The polysaccharide can interact with the other biomolecules like protein, lipids to enhance its properties. The protein polysaccharide interaction can result due to the hydrophobic or electrostatic interactions or hydrogen bonds. The numerous factors like nature of polymer, molecular weight, ionic strength, pH and temperature affects the stability and formation of protein-polysaccharide complexes (Gosh & Bandyopadhyay, 2009) [5]. The protein polysaccharide complex formation has been in use since the olden days using the traditional wet heating method which requires a high amount of energy and time for interaction. To overcome these drawbacks, different novel processing methods like ultrasound, microwave or combination of these processing methods with thermal treatments are used.

Ultrasound is a non thermal processing method in which the sound waves with frequency more than 20 kHz is used for various food applications like processing, preservation, extraction, inactivation of enzyme activity and also as a combination method.

In addition to these applications structural modification of biomolecules using ultrasound technology has gained importance in recent days. The application of ultrasound to protein molecules modifies its secondary structure which enhances the properties and functionality of the proteins which can lead to its increased application in food industry. The acoustic cavitation produced by ultrasound breaks the disulphide linkages and bonds present in the proteins which potentially modifies the tertiary and secondary structure. Some previous studies have demonstrated that application of ultrasound treatment alters the protein structure and enhances the functional properties of the protein. The native structures of the protein are protected by formation of protein polysaccharide complexes using ultrasound which increases the thermal denaturation temperature and thermal stability of the proteins. The protein polysaccharide complex leads to formation of structural network due the different types of bonding that occurs between proteins and polysaccharides, which contribute to the better textural and rheological properties. The main objective of this study is to determine the rheological and textural properties of the soy protein isolate rice starch complexes produced at various ultrasound intensities and to analyze the structural changes during complex formation using FTIR.

2. Materials and Methods

2.1 Materials Required

The rice starch and the soy protein isolate used for the study are purchased from Natures Velvet, Hyderabad, India and Urban Platter, Mumbai, India. They are stored in air tight containers till the completion of the study.

2.2 Formation of soy protein rice starch complexes

The soy protein and rice starch were mixed in the ratio of 3:1.5 per 50 ml of deionised water. The mixture is kept in a magnetic stirrer for about 2 hours at room temperature which enables uniform distribution of the protein and carbohydrate in the solution. The solution is then stored in refrigerated condition overnight to enable complete dissolution of the biomolecules in the solution. The modified method of the Zhao *et al*., 2021 [10] is followed where the solution is subjected to ultrasound treatment at various intensities namely 20%, 40%, 60%, 80% and 100% at a pulsation of 3 seconds ON and 1 second OFF for duration of 5 minutes using a probe Ultrasound equipment (SONICS, USA) with a frequency of 20 kHz and maximum power output of 750 W. After ultrasound treatment the samples were heated at 90 ºC ± 2 ºC for 1 hour to enable interaction between the soy proteins isolate and rice starch. The temperature of the complexes is brought down to room temperature after heating and stored at refrigerated condition for further analysis.

2.3 Properties of Soy Protein Isolate – Rice Starch complexes

The properties of the soy protein isolate rice starch complexes like water activity, texture, colour, and rheology were determined. The stored samples are used in required quantities to determine rheological and textural behaviour. The samples after freeze drying is used to determine the changes in colour and water activity occurred during complex formation.

2.3.1 Colour

The effect of different ultrasound treatment on the colour of the soy protein rice starch conjugates was evaluated using bench top Hunter lab Colorflex EZ colorimeter (Hunter Associates Laboratory, Inc., Reston, VA, USA) for the freeze dried samples in a method adopted by (Cermeño *et al*., 2019) [1]. The values are determined in a 3 D colour space using the L*, a*, and b*, where L* determines the brightness range, a* determines the range from greenness to redness and b* determines the range from blueness to the yellowness respectively. The total colour difference (ΔE) value was determined using the formula (Equation (1)).

$$\Delta E = [(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2] / 2 \ldots\ldots\ldots\ldots\ldots (1)$$

Where
L_0^*, a_0^*, b_0^*- Values obtained for sample
L*, a*, b*- Values obtained for control

2.3.2 Rheology

The rheology of the soy protein rice starch complexes were analyzed using a MCR 52 modular compact Rheometer (Anton Paar, Austria). A 25 mm parallel plate type probe was used to determine the viscosity of the samples in a temperature controlled flat base maintained at 25ºC. The values were obtained and were fitted using the Rheoplus software in Herschel - Bulkley model (Equation (2)).

$$\sigma = \sigma_0 + K\dot{\gamma}^{\eta} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Where
σ_0 - Yield stress,
K - Consistency index and
η - Flow behavior index.

2.3.3 Texture Profile Analysis

The consistency, cohesiveness, index of viscosity and firmness of the soy protein – rice starch complexes were determined using a Texture Analyser – TA HD Plus (Stable micro system, UK). The samples were poured in an acrylic container and the value are obtained using a Back extrusion probe (A/BE) provided with a 35 mm disc. The penetration depth of 5 mm is provided to measure the desired parameters. The raw data obtained was processed using TA software to get the final values.

2.3.4 Water Activity

The water activity of the freeze dried soy protein rice starch complexes obtained using ultrasound treatment were determined using water activity meter (Aqua Lab, USA). One mg of sample is placed in a plastic container and kept in the water activity meter to determine the values.

2.4 Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR analysis is used to confirm the formation of the protein carbohydrate complexes (Zhang *et al*., 2020) [9]. The FTIR analysis is carried out using an ATR - FTIR (PerkinElmer, USA) spectrophotometer with a MIR TGS detector coated with ZnSe for the soy protein isolate rice starch complexes without freeze drying of samples. The samples are placed in the KBr plate and they were analyzed to determine the characteristics peak for the sample using the software NIOS2 Main. The total of 16 scans was performed for each sample at a scanning resolution of 4 cm^{-1} and in the wavelength region of 400 cm^{-1} to 4000 cm^{-1}.

2.5 Statistical Analysis

All the data were collected independently in triplicates. One – way analysis of variance (ANOVA) Tukeys test was carried out for the obtained data using Origin Pro version 8.5 with a statistical significance at $P<0.05$ for individual values. The L value is more for soy protein isolate rice starch complexes produced compared to the raw soy protein. The complex formed using ultrasound treatment at 20% intensity

3. Results and Discussion

3.1 Properties of Soy Protein Isolate – Rice Starch complexes

3.1.1 Colour

The L, a and b values of the samples treated with different ultrasound intensities were measured in triplicates and given in Table 1. The L, a and b values obtained differed significantly ($P<0.05$) compared to the raw soy protein isolate and soy protein isolate rice starch complexes formed. The results suggest that the addition of rice starch to the soy protein isolate increases the brightness (L) and decreased the redness (a) and yellowness (b) of complex formed. The complex formed using ultrasound at 20% intensity had the maximum brightness compared to all samples, where as it is found to be least for ultrasound 60% treatment. Among the different treatments the soy protein isolate rice starch complexes produced using 60% ultrasound treatment had higher a and b values and the 20% ultrasound treatment has lower values. All the samples had decreased yellowness and redness compared to the raw soy protein isolate.

Table 1: Colour values for the ultrasound treated Soy protein isolate rice starch complexes

Sample	L	a	b	ΔE
SOY	73.33 ± 0.01^{f}	3.41 ± 0.005^{a}	17.87 ± 0.005^{a}	-
US 20%	81.44 ± 0.08^{a}	1.33 ± 0.007^{f}	13.98 ± 0.012^{f}	42.61 ± 0.02^{a}
US 40%	79.13 ± 0.02^{b}	1.66 ± 0.004^{d}	14.79 ± 0.01^{d}	23.10 ± 0.03^{b}
US 60%	76.4 ± 0.01^{e}	1.94 ± 0.002^{b}	15.24 ± 0.005^{b}	9.24 ± 0.01^{c}
US 80%	76.90 ± 0.04^{d}	1.87 ± 0.006^{c}	15.09 ± 0.02^{c}	11.42 ± 0.02^{d}
US 100%	79.06 ± 0.03^{c}	1.43 ± 0.001^{e}	14.32 ± 0.005^{e}	24.7 ± 0.05^{e}

3.1.2 Rheology

The soy protein isolate rice starch complexes were analyzed for rheology and the data were fitted in Herschel - Bulkley model (Equation (2)) to determine the consistency index and flow behavior of the complexes formed. The presence of yield stress in all the samples indicates the samples are Non Newtonian in nature and the values of yield stress, consistency index and flow behavior index obtained for all the samples are given in the Table 2. All the samples had the flow behavior index (n) greater than 1 indicating that all the samples are shear thickening in nature. The statistical analysis of the results indicate that the soy protein isolate rice starch complexes produced with 60% ultrasound treatment is significantly different ($P<0.05$) compared to the soy protein and had the highest yield stress and consistency index which are similar to the results obtained by Olivas & Sepulveda, 2014 [7]. This suggests that this sample is highly viscous in nature and requires more initial stress for any deformation compared to the others. The highly viscous nature of the sample can be due to the increased interaction of the protein with rice starch. The ultrasound treatment at initial stages increases the yield stress but the application of high intensity ultrasound breaks down the integral structure and hydrogen bonds present. This leads to the low yield stress and consistency index of the samples produced using 80% and 100% ultrasound treatment. The consistency index of all the samples increased indicating that the interaction of soy protein with rice starch increased the thickening nature and viscosity of the samples and they can be potentially used as thickeners and stabilizers in food industries.

Table 2: Rheological characteristics of ultrasound treated Soy protein isolate rice starch complexes

Sample	Yield Stress	K	n
SOY	18.43 ± 1.77^{c}	0.002 ± 0.0004^{b}	4.13 ± 0.48^{a}
US 20%	$29.95 \pm 3.47^{a,b}$	$0.036 \pm 0.008^{a,b}$	$3.62 \pm 0.19^{a,b}$
US 40%	$31.10 \pm 5.47^{a,b}$	0.038 ± 0.003^{a}	$2.58 \pm 0.04^{c,d}$
US 60%	33.54 ± 5.11^{a}	0.057 ± 0.02^{a}	1.32 ± 0.25^{e}
US 80%	$29.97 \pm 1.88^{a,b}$	$0.027 \pm 0.02^{a,b}$	$1.83 \pm 0.45^{d,e}$
US 100%	$23.26 \pm 1.41^{b,c}$	$0.030 \pm 0.03^{a,b}$	$3.04 \pm 0.46^{b,c}$

3.1.3 Texture Profile Analysis (TPA)

The maximum positive force obtained gives the firmness and the positive area denotes consistency whereas the maximum negative peak and area denotes the cohesiveness and index of viscosity of the samples. Table 3 gives the values obtained for the samples using a single bite test. The statistical analyses of the samples indicate that the 60% ultrasound treatment had higher firmness, consistency, cohesiveness and index of viscosity compared to all the samples. The ultrasound treatment at intensities of 20%, 40% and 60% increases the consistency, firmness, cohesiveness and index of viscosity of the samples after that higher the ultrasound intensity the values decreases. Application of ultrasound treatment modifies the structure of protein and when high intensity treatment is given the structural deformation occurs which leads to irreversible change in the protein structure leading to the less firmness and consistency. Due to the binding of rice starch with soy protein the nature of the complex gets modified as the rice starch gives the desired consistency and cohesiveness, which can attribute to the designing of food products with desired properties.

Table 3: Texture Profile Analysis of ultrasound treated Soy protein isolate rice starch complexes

Sample	Firmness	Cohesiveness	Consistency	Index of Viscosity
SOY	12.88 ± 0.89[c]	-7.17 ± 0.82[a]	27.39 ± 2.13[d]	-6.87 ± 1.66[a]
US 20%	24.67 ± 2.15[b]	-12.01 ± 0.86[b]	44.07 ± 5.23[b]	-28.46 ± 3.90[b,c]
US 40%	26.87 ± 1.49[b]	-12.06 ± 0.61[b]	45.10 ± 3.60[b]	-29.90 ± 3.89[b,c]
US 60%	34.10 ± 3.48[a]	-12.19 ± 0.54[b]	67.18 ± 1.95[a]	-32.34 ± 1.67[c]
US 80%	16.05 ± 0.35[c]	-8.65 ± 1.42[a]	37.61 ± 0.71[b,c]	-16.58 ± 7.87[a,b]
US 100%	14.49 ± 0.59[c]	-8.55 ± 1.08[a]	35.91 ± 1.78[c]	-14.37 ± 7.47[a]

3.1.4 Water Activity

The water activity of the soy protein rice starch complexes formed using different ultrasound treatment is given in Table 4, and the statistical analysis shows that there is a significant difference between the raw soy protein and soy protein isolate rice starch complexes. The results indicate that the water activity of all the samples is 0.65 and so they possess no threat for microbial contamination and can be stored for a longer period of time. The water activity of the raw soy protein differs significantly ($P<0.05$) from the soy protein isolate rice starch complexes. The formation of soy protein isolate rice starch complexes increases the water activity as they were dissolved in water and treated with ultrasound. The ultrasound treatment intensity is found to be directly proportional to the water activity of the samples, as the treatment intensity increases the water activity increases.

Table 4: Water activity of ultrasound treated Soy protein isolate rice starch complexes

Sample	Water Activity
SOY	0.576 ± 0.002[d]
US 20%	0.639 ± 0.004[b]
US 40%	0.636 ± 0.003[c]
US 60%	0.6407 ± 0.006[a,b]
US 80%	0.6409 ± 0.007[a]
US 100%	0.6415 ± 0.001[a]

3.2 Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) was used to determine the structural changes occurring due to the interaction between the soy protein isolate rice starch complexes (Dong & Cui, 2021) [4]. The Fig 1 (a) – 1 (e) shows the characteristics FTIR spectra obtained for the soy protein isolate rice starch complexes obtained for different ultrasound treatment in comparison with raw soy protein and rice starch. The FTIR spectra obtained confirms the presence of water bonds in the region of 3200 cm^{-1}, the intensity of the peaks obtained for the complexes is high compared to the raw soy protein and rice starch. The complexes had the characteristics peak for the soy protein in the regions of 1200 cm^{-1} to 1700 cm^{-1} which includes the Amide I, Amide II and Amide III regions of the proteins and also the fingerprint regions of polysaccharides. The peaks obtained are broad and have high intensity compared to the raw soy protein indicating the interaction occurs due to the hydrogen formation between the biomolecules. Due to the formation of protein carbohydrate complex a new peak is found in the region of 1000 cm^{-1} to 1100 cm^{-1} resulting due the formation of glycosidic linkages between the soy proteins isolate and rice starch and shift in peaks occurs which is similar to the results obtained by Dev, Pandit, & Singhal, 2021 [3]. The FTIR spectra obtained for the samples suggest that the ultrasound treatment at 60% has higher intensity peaks compared to the other treatments indicating the higher interaction between the biomolecules.

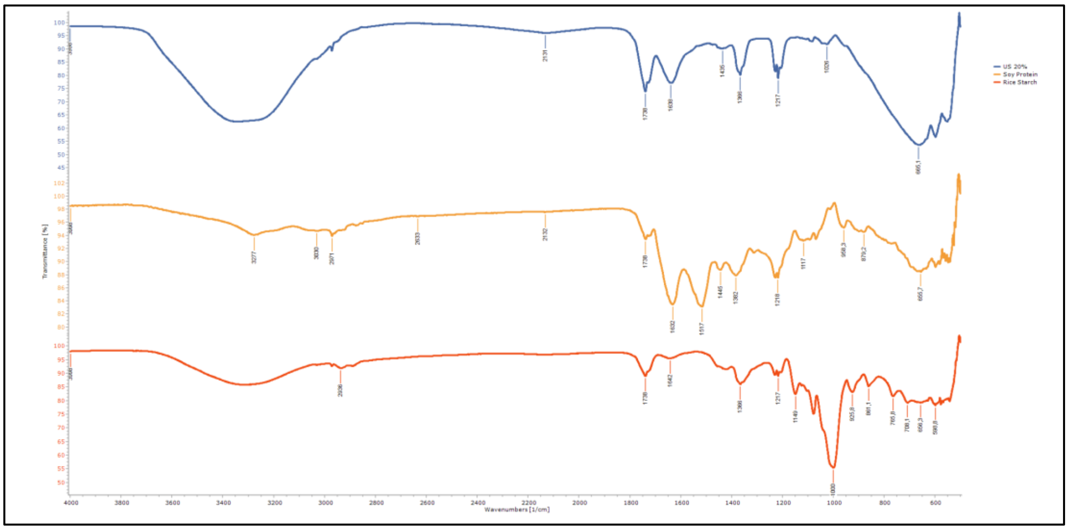

Fig 1(a): FTIR Spectra of soy protein isolate rice starch complex with ultrasound 20%

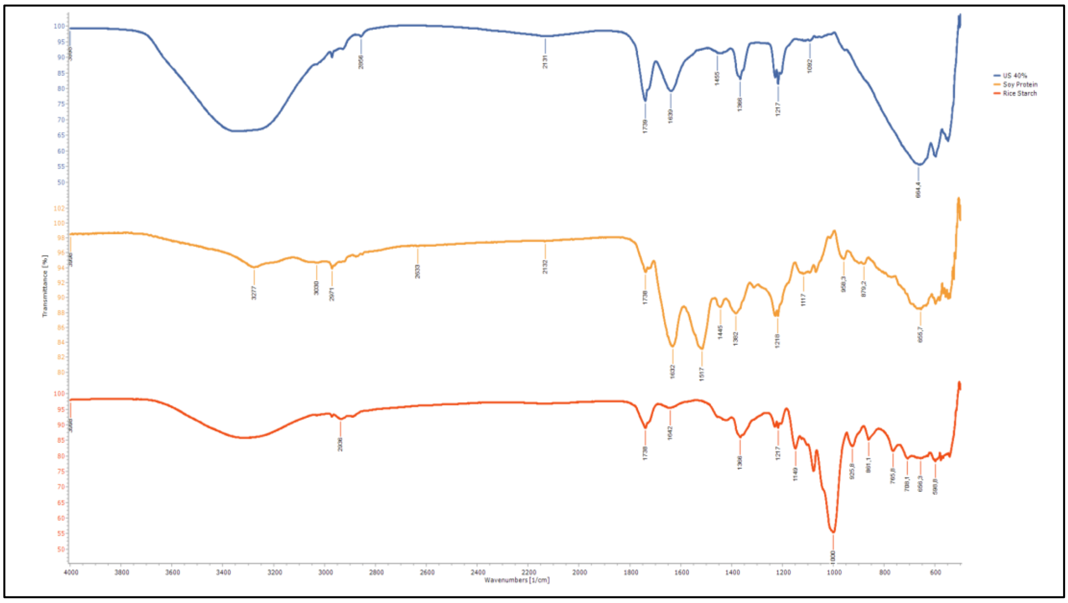

Fig 1(b): FTIR Spectra of soy protein isolate rice starch complex with ultrasound 40%

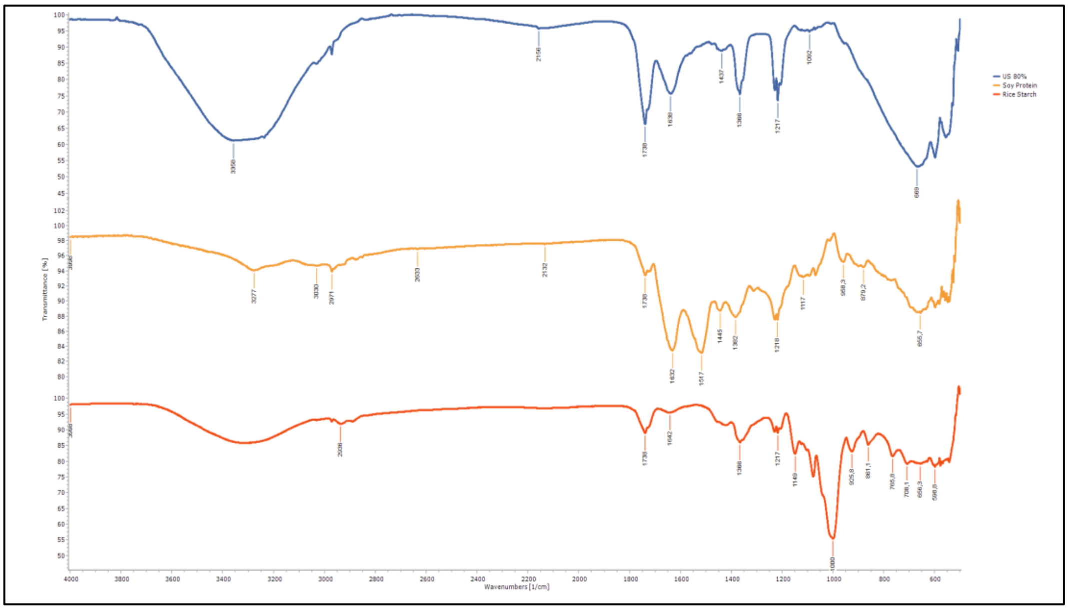

Fig 1(c): FTIR Spectra of soy protein isolate rice starch complex with ultrasound 60%

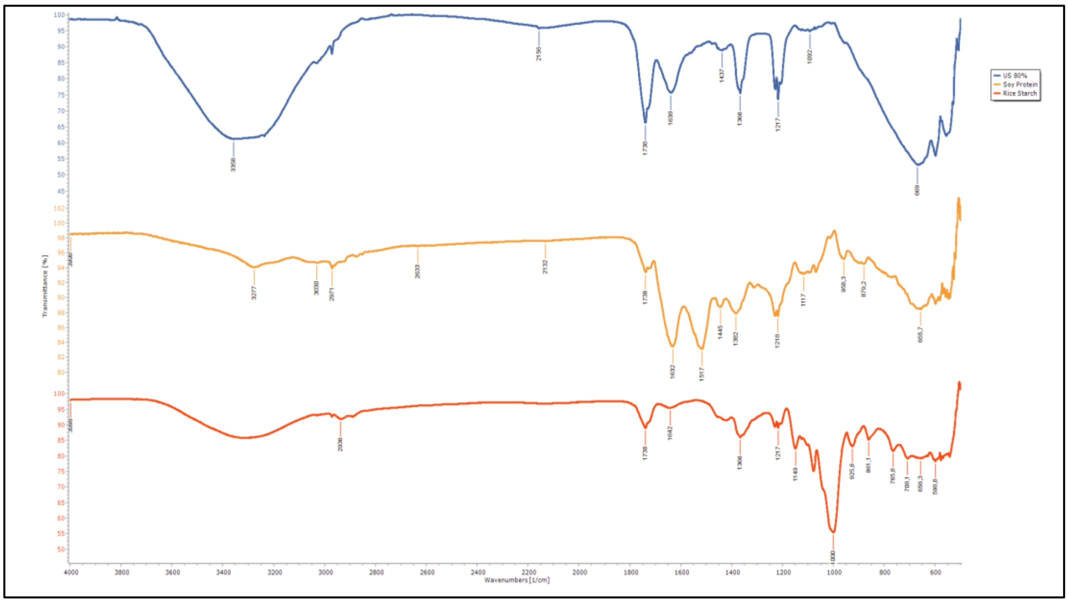

Fig 1(d): FTIR Spectra of soy protein isolate rice starch complex with ultrasound 80%

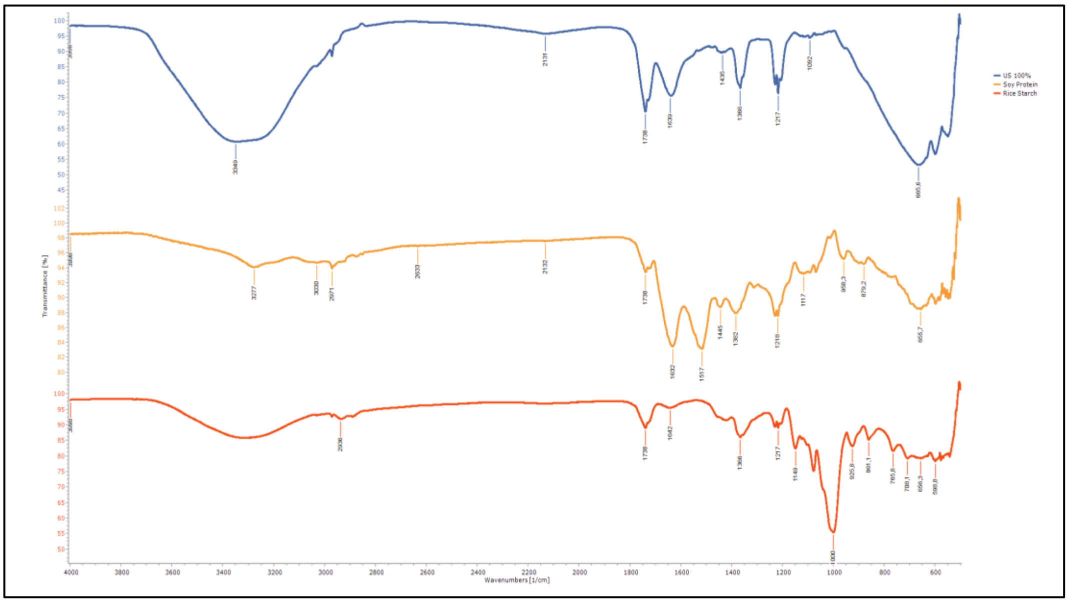

Fig 1(e): FTIR Spectra of soy protein isolate rice starch complex with ultrasound 100%

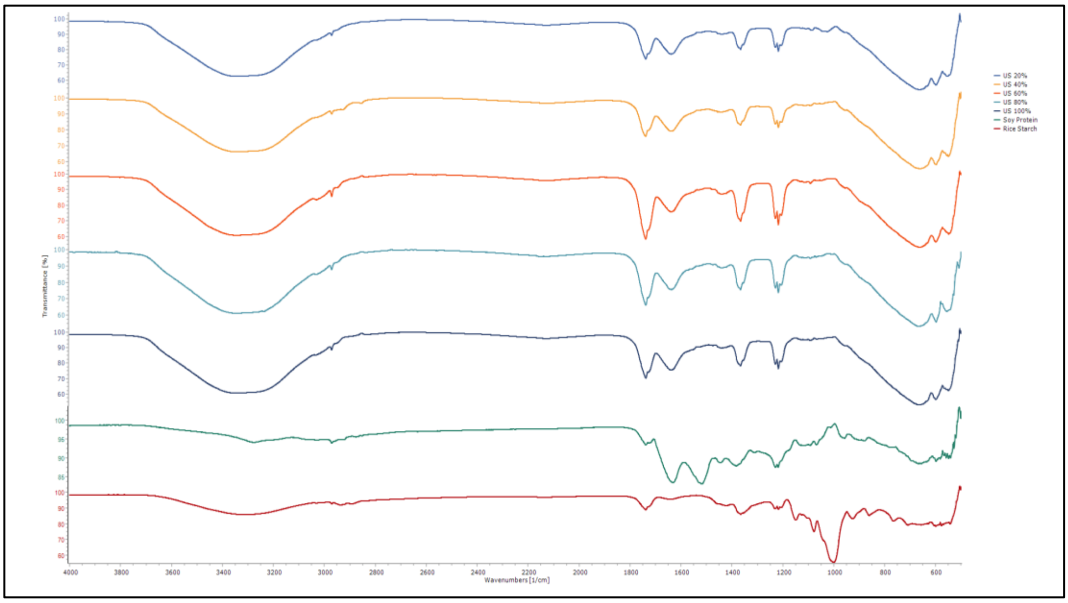

Fig 1(f): FTIR Spectra of soy protein isolate rice starch complex with ultrasound 20%

4. Conclusion

The ultrasound treatment modifies protein structure and enables binding of polysaccharide forming glycation reaction products. The rheological and textural characteristics of the soy protein isolate rice starch complexes increases due to the glycation reaction and they can have high functional and increased application in food industry. The protein carbohydrate complexes interaction have a wide range of application in diversified fields like biomedical and pharmaceuticals where they are used for targeted drug delivery and encapsulation of bioactive compounds. Thus the formation of protein-polysaccharide complexes using ultrasound as a pretreatment can lead to formation of a high acceptable product. Based on the results obtained for the consistency, firmness and viscosity the complex formed can be used as a potential replacer for synthetic thickeners and emulsifiers used in food industry.

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