Heliyon 10 (2024) e24140

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

The effect of high hydrostatic pressure on the structure of whey proteins-guar gum mixture

Saeed Mirarab Razi [a,**], Mohebbat Mohebbi [a,*], Seyyed Mahdi Mirzababaee [b], Mohammad Ali Hesarinejad [c], Mohammad Khalilian Movahed [a]

[a] Department of Food Science and Technology, Faculty of Agriculture, Ferdowsi University of Mashhad, PO Box: 91775-1163, Mashhad, Iran
[b] Department of Food Industry Machineries, Research Institute of Food Science and Technology, Mashhad, Iran
[c] Department of Food Processing, Research Institute of Food Science and Technology, Mashhad, Iran

ARTICLE INFO

Keywords:
Hydrocolloid
High hydrostatic pressure
Protein-polysaccharide
Particle size

ABSTRACT

The effect of high hydrostatic pressure (HHP) on the structural properties of whey protein concentrate (WPC) and guar gum mixture has been investigated at pH 5. WPC (6 % w/v) and guar gum (0.25 % w/v) mixture was freeze dried after adjusting pH and treated at different pressure levels (0–600 MPa) for 0–30 min. The solubility of treated powders decreased significantly ($p < 0.05$) as treatment time and pressure levels increased. Thermal analysis showed an increase in denaturation temperature after HHP treatment at 600 MPa. A more crystalline structure was observed in samples treated with 600 MPa for 20 and 30 min. With increasing pressure and time, particle size of the samples increased and the highest particle size was belonged to sample treated at 600 MPa for 30 min (759.66 nm). SEM results exhibited that by applying the pressure, irregularity of shapes and particle size increased while the apparent cracks decreased. FTIR results indicated that HHP treatment changed shift in bond and peak intensity. As reported in the current study, the application of HHP treatment as a green physical technology on protein-polysaccharide mixture could be used to improve interaction of protein and polysaccharide.

1. Introduction

High hydrostatic pressure (HHP) is a non-thermal technology that is regarded as an alternative to heat treatment. Not only it has the ability of providing microbiologically safe food products, but also it can preserve vitamins, proteins, and sensory quality of food products better than other techniques [1]. HHP is a physical process where food is pressurized evenly across all directions [2]. It has many applications in the production of various food ingredients such as starch and hydrocolloids, and food products such as sauces, jellies, fruit jams, juices, cooked ham as an alternative to the thermal process, but there are not commercial HHP-treated dairy products, yet [3–6]. Functional properties of proteins, especially milk proteins might be modified after exposing to HHP which causes a change in technological properties and the final quality of dairy products [7].

The sensitivity of various bonds in milk proteins to HHP is different, and hydrophobic interactions are the most sensitive bond to HHP. Electrostatic interactions have greater resistance to HHP compared to hydrophobic interactions and lower resistance compared to hydrogen bonds. However, covalent bonds are least affected by HHP. As covalent bonds have the highest resistance to HHP, the

* Corresponding author.
** Corresponding author.
E-mail addresses: saeed.mirarab86@yahoo.com (S. Mirarab Razi), m-mohebbi@um.ac.ir (M. Mohebbi).

https://doi.org/10.1016/j.heliyon.2024.e24140
Received 17 October 2023; Received in revised form 19 December 2023; Accepted 4 January 2024
Available online 7 January 2024

primary structure of protein remains unchanged while the secondary and tertiary structures alter [8]. In extreme environmental conditions, caseins are more resilient compared to whey proteins which are due to its molecular structure which can hold together by hydrogen bonds, hydrophobic bonds, and colloidal calcium phosphate interactions [9]. Whey proteins are subject to denaturing because hydrogen bonds and hydrophobic interactions primarily control their tertiary structure [10]. The stability of β-lactoglobulin and α-lactalbumin as the most abundant whey proteins differs when exposed to PHH. β-lactoglobulin is sensitive to HHP and denatured irreversibly by the formation of complexes between β-lactoglobulin and κ-casein from 100 MPa, while denaturation of α-lactalbumin starts at pressure more than 400 MPa [11]. Protein denaturation may increase as pressure, temperature and time increase [12]. The order of sensitivity of whey proteins to HHP is as lactoferrin<β-lactoglobulin < immunoglobulin<α-lactalbumin. The high sensitivity of α-lactalbumin to HHP is associated with many intra-molecular disulfide bonds and the absence of free sulphhydryl groups in the α-lactalbumin structure [11]. Consequently, whey proteins can be denatured or left intact after exposure to HHP, resulting in different functional properties in HHP-treated whey proteins. However, in order to develop the applicability of whey proteins, their functional properties required to be manipulated and the addition of polysaccharide is one method to achieve this [13].

Guar gum is a high molecular weight polysaccharide with hydrophilic properties. There are hydroxyl groups in the polymer structure of guar gum, which can help it produce various derivatives with several industrial applications. Guar gum is an insoluble polysaccharide in fats, hydrocarbons, alcohols, esters, and ketones but it has high solubility in water. Contrary to other gums, the total viscosity of guar gum may reach in cold water. The hydration rate and viscosity of guar gum depend on a number of parameters, including pH, concentration, temperature, presence of other substances, etc. [14,15].

Proteins and polysaccharides are widely used in different food colloids, foams and emulsions. The interactions between these biopolymers have a great role in the stability and structure of foams, emulsions, microencapsulation, delivery systems, etc. [16–21]. These biopolymers can associate with physical interactions. Non-covalent interactions (strict exclusion, hydrogen bonding, electrostatic and hydrophobic interactions, etc.) between protein and polysaccharide play a significant role in influencing the interfacial properties of adsorbed films and also the creation and stability of the dispersion [22]. Electrostatic interactions are predominant when polysaccharides are charged. When the pH falls below pI, strong, attractive electrostatic interactions occur between negatively charged polysaccharides and positively charged proteins. When pH is more than pI, proteins carry a negative charge and a weaker reversible complexes tend to form proteins and polysaccharides [22].

Although environmental conditions like temperature, pH, ionic strength, etc., have substantial effects on the interaction of protein and polysaccharide, non-thermal technologies like HHP can effect on this interaction [23]. The aim of the present investigation was to understand the impact of HHP (time and pressure) treatment parameters on the interaction of whey proteins and guar gum. Interaction of WPC and guar gum is important because it has various application in the food formulation. On the other hands, the mixture of WPC and guar gum can be used in the formulation of process cheese and also foam structure dessert. So many researches have been done on the structure of whey protein and guar gum, so we didn't study the effect of high pressure on guar gum and WPC, alone [24–29].

2. Materials and methods

2.1. Materials

Whey protein concentrate (WPC, containing 77 % protein, dry basis, produced using a cold-processed method) was obtained from Kalleh dairy Co. (Amol, Iran). Guar gum was provided from Sigma-Aldrich (Dorset, UK). All chemicals used were analytical grades.

2.2. Preparation of WPC–guar gum mixtures

WPC at a concentration of 10 wt% and guar gum at concentration of 1 wt% were prepared with dispersing specific amount of protein and polysaccharide in distilled water and the solutions were stirred overnight. Afterwards, specific amount of protein and polysaccharide were mixed to obtain a mixture solution containing 6 % WPC and 0.25 % guar gum. pH of solutions were adjusted to 5 using HCl 0.1 N. This mixture (6 % WPC and 0.25 % guar gum, pH 5) has a proper foaming properties that can be used in foam structure food formulations.

2.3. High pressure treatment

70 mL samples of the WPC-guar gum mixtures were sealed in polyethylene bags utilizing a vacuum packaging system. Subsequently, these samples treated by high hydrostatic pressure in the pressure of 0, 300, and 600 MPa, at 25 °C (initial temperate was variable according to increasing of temperature by 3 °C for every 100 MPa increase in pressure) for 0, 10, 20, and 30 min in a hydrostatic pressurization unit (RIFST, Mashhad, Iran). The treated samples, then were freeze-dried (FDU-8606, Operon, Gimpo-si, Korea) and obtained powders were analyzed. Also, the mixture of WPC and guar gum, which was not processed with HHP (0 MPa, 0 min) were considered as a control sample.

2.4. Solubility

The solubility of treated and untreated samples was determined with preparation a dispersion with concentration of 1 % w/v. For this aim, specific amount of powders were dispersed in deionized water and agitated in a water bath at 30 °C for 30 min. Afterwards, this dispersions were centrifuged (Orum Tadjhiz Digital Centrifuge Refrigerator System, HSC 10000) at 8000 g or 8500 RPM for 15

min, then, their supernatants were dried at 125 °C until constant weight. The solubility of powders were determined as follow:

$$\text{Solubility } (\%) = \frac{w_1}{w_2} \times 100$$

where, w_1 and w_2 are the weight of soluble powder in the supernatant, and the initial weigh of sample, respectively [30].

2.5. Fourier transform infrared (FTIR) spectroscopy

The interactions between the functional groups of WPC and guar gum were assessed by conducting FTIR spectroscopy with a Shimadzu FTIR-8400 S device from Japan. This involved analyzing the disappearing or displacements of shifts in various spectra. Subsequently, the freeze-dried powders were combined with KBr in a 1 to 100 ratio, followed by grinding. These powders were converted into tablets (2 cm) using a hydraulic press (60 kN) before analysis. An OPUS spectroscopy software (Version 4.2, Germany) was used for analyzing the spectra. The specified transmission curve data points were within the range of 4000 to 400 cm−1.

2.6. X-Ray Diffraction (XRD) analysis

The XRD profiles of different samples were investigated using a Philips X-ray diffractometer (PW1730 × 142, PANalytical, Netherlands). The 2θ angle was adjusted, ranging from 5° to 50°, while the scanning rate remained constant at 1°/s. Operating voltage was 40 KV.

2.7. Particle size

The specimens' particle size was analyzed by employing a laser light scattering type particle size analyzer (Mastersizer 2000, England).

2.8. Zeta-potential measurement

The zeta-potential value of specimens were identified with a dynamic light scattering (DLS) instrument (Brookhaven Instruments Corp., USA) apparatus. The specimens were diluted using distilled water before analysis to avoide of multiple scattering.

2.8.1. Scanning electron microscopy (SEM)

SEM analysis was performed using a scanning electron microscope (Leo,VP14, Germany). After fixing and sputtering, powders covered using 20 nm of a slim conductive gold layer. Then, the produced powders were scanned by AIS2300c software (voltage of 20 kV).

2.9. Thermal stability

A differential scanning calorimetry (DSC) analysis using a DSC-60 A instrument (Shimadzu Co., Ltd., Kyoto, Japan) was used to specify denaturation and enthalpy of powders. Temperature scans were recorded from −50 to 200 °C at a scan rate of 5 °C/min.

2.10. Data analysis

The data collected was evaluated using the SPSS statistical software (Version 16, 201 Armonk, USA). To determine significant differences, a one-way analysis of variance was conducted, and the means were compared using the Duncan test at a 95 % significance level ($p < 0.05$).

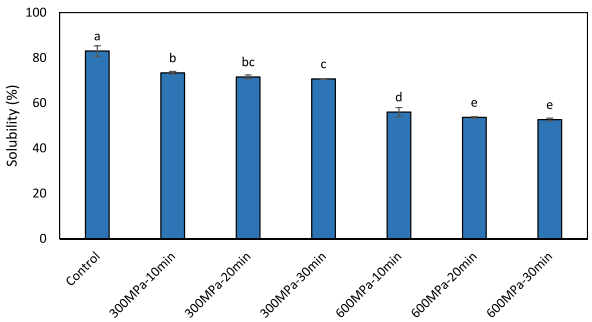

Fig. 1. The effect of HHP treatment on the solubility of WPC-guar gum powder.

3. Results and discussion

3.1. Solubility

The effect of HHP treatment on the solubility of guar gum powder WPC is depicted in Fig. 1. As shown in Fig. 1, the solubility of all samples decreased significantly ($p < 0.05$) after pressurization. Our findings showed that the intensity and duration of the applied pressure had an effect on the solubility of the powders. Powder solubility was significantly reduced when pressurization was performed at 600 MPa. Powder solubility of WPC-guar gum after processing at 300 MPa for 30 min decreased from 70.66 % to 52.66 % after 600 MPa at the same time (Fig. 1). The low solubility of powders after treatment at 600 MPa could be related to increased aggregation after pressurized release. Moreover, surface hydrophobicity of powders may be increased, leading to the exposure of hydrophobic groups that were previously hidden [31].

The high solubility was observed in control sample (83.00 %) and solubility decreased as the intensity and duration of applied pressure increased. Hydrophobic interactions, which are crucial for the formation of aggregated upon press release, can be happened in higher pressure, which reduces powder solubility [31]. Krešic, Lelas [31] reported that the solubility of pressured WPC and WPI was lower compared to non-pressured samples. They stated that higher solubility of non-pressurized samples was related to a high proportion of native whey protein.

3.2. FT-IR

FT-IR spectroscopy was applied to further illustrate the impact of HHP on the structure changes and interaction of whey proteins and guar gum. The amide I band, specifically the C═O stretching vibrations of the peptide backbone in the wavenumber range of 1700–1600 cm−1, provided valuable insights into the secondary structures of the protein. To be more exact, the bonds at 1610–1640 cm−1 (β-sheet), 1640–1650 cm−1 (random coil), 1650–1664 cm−1 (α-helix), and 1664–1695 cm−1 (β-turn) are important [32].

To specify the impact of HHP processing on the structure of the guar gum mixture WPC, FT-IR spectroscopy was performed at 4000-400 cm wavecount 1.

As can be seen in Fig. 2, different peaks were observed in the regions of around 661, 1074, 1154, 1240, 1315, 1399, 1450, 1537, 1656, 2782, 2927, 2966, 3076 and 3297 cm-1. The band at 1654 cm-1 is related to the α-helix structure of the protein sample [33]. After HHP treatment in all samples, especially treatment at 600 MPa, the band's intensity increased which indicate the secondary structure of protein and also the interaction of WPC-guar gum have changed [33]. The HHP treatment at 600 MPa may unfold WPC structure and expose more α-helix structures on the surface of the protein, which might increase its interaction with guar gum and increase in band intensities.

3.3. XRD

XRD diffraction was carried out to evaluate the amorphous structure of the WPC-guar gum powders after pressured at different pressure and time. Fig. 3 shows the X-ray diffractograms of WPC-guar gum powders. The XRD profile of the WPC-guar gum mixture powders after pressurization at 300 MPa did not change significantly from the control samples. However, a significant change took place after pressurized treatment at 600 MPa for 20 and 30 min.

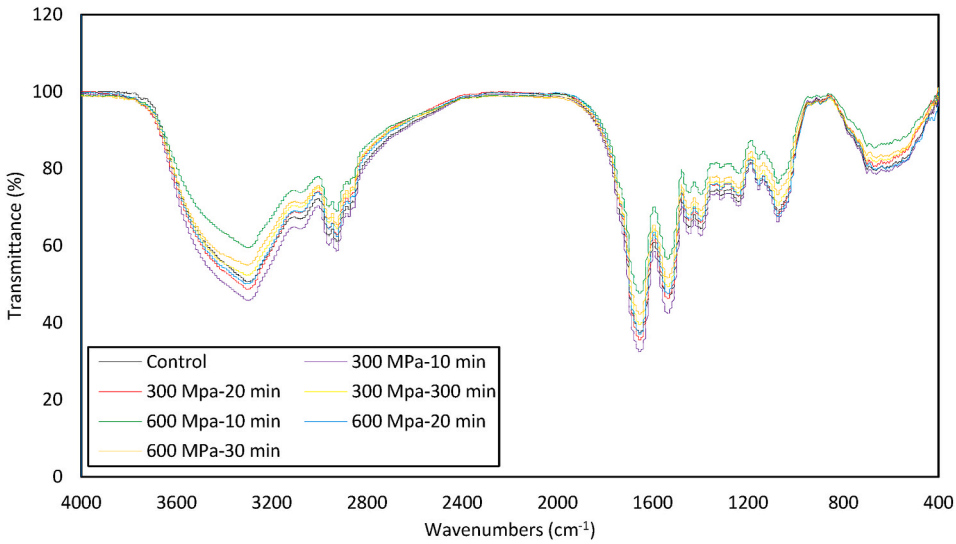

Fig. 2. The effect of HHP treatment on FTIR spectra of WPC-guar gum mixture.

HHP treatment increased denaturation of whey proteins. β-lactoglobulin is more sensitive to high pressure compared to α-lactalbumin. Actually, β-lactoglobulin can be denatured at a pressure of 100 MPa, whereas α-lactalbumin denaturation, due to lack of free sulfhydryl groups, starts at pressures higher than 400 MPa [11]. Cheftel [34] reported that at low protein concentration, when pressure is low (less than 300 MPa) protein denaturation is reversible, while at higher pressure (more than 300 MPa) an irreversible and extensive effects were observed. According to our findings, the increase in time and pressure increased the denaturing of proteins. In XRD spectra, sharp peaks show crystalline diffraction. Our results showed that a crystalline structure was observed after pressure treatment at 600 MPa; the peak at $2\theta = 18.45^{\circ}$ indicates that the crystallinity of these samples was relatively high.

3.4. Particle size

Fig. 4 shows the particle size of guar gum WPC samples without/with high pressure processing. Smallest particle size was belonged to control sample. The HHP treatment of the WPC–guar gum mixtures caused a great increase in the droplet size of the samples. Control sample had an average droplet size of 494.7 nm, while treatment at the pressure of 300 MPa increased the droplet size of samples from 545 nm (10 min) to 607.37 nm (30 min). As pressure reached to 600 MPa, the droplet size increased from 632.8 nm (10 min) to 759.66 nm (30 min). In general, particle size was significantly ($p < 0.05$) higher after processing at 600 MPa for 20 min and 30 min. Lee, Clark [35] and GALAZKA, LEDWARD [36] investigated the impact of HHP on whey protein concentrate (WPC) and β-LG and reported that HHP increased surface hydrophobicity of proteins and led to protein unfolding. Higher pressure (600 MPa) combined with long-term treatment (20 min and 30 min) can cause protein denaturation [37] which increases particle size in samples. Furthermore, after a pressure treatment at 600 MPa, the interaction between WPC and guar gum was higher, resulting in large particles.

3.5. Zeta-potential

Electrostatic interaction between WPC and guar gum was determined using the zeta potential of these samples at pH 5. Zepa potential values in all samples were negative (Fig. 5). Guar gum has a negative charge at pH 5 and also WPC is negatively charged. The results showed that net-negative charge increased in pressured samples at 600 MPa. The lowest zeta potential was observed for untreated and treated samples at a pressure of 300 MPa for 10 and 20 min. This finding suggests that electrostatic interactions between WPC and guar gum were weak in these samples [38]. Wang, Jiang [38] reported that high-pressure processing enhanced the zeta potential of oyster protein. They stated that high pressure can cause more ionizable acids on the protein surface.

3.5.1. Scanning Electron Microscopy

SEM was conducted to evaluate the impact of HHP treatment on the morphology of the WPC-guar gum mixture. Fig. 6 shows the optical microscopy pictures of guar gum-WPC samples without/with HHP processing. As shown, the freeze-dried samples exhibit a diverse range of irregular structures in terms of size and shape. Irregular surfaces of particles can be related to their shrinkage during the spray drying process [39]. The dimensions of the unprocessed sample were quite large. To a certain extent, the control particles were spherical (irregular in shape) with visible agglomeration points and cracks. The apparent cracks decreased at pressure samples. This feature is important for the encapsulation process due to greater protection of core [39]. In general, in all samples, interaction between whey proteins and guar gum was observed because the pH of the samples decreased to 5. As shown in Fig. 6a, most particles of the control sample are less than 1 μm in size (Mag 30 k). In all pressure-treated samples (Fig. 6 b-g), much larger particles were

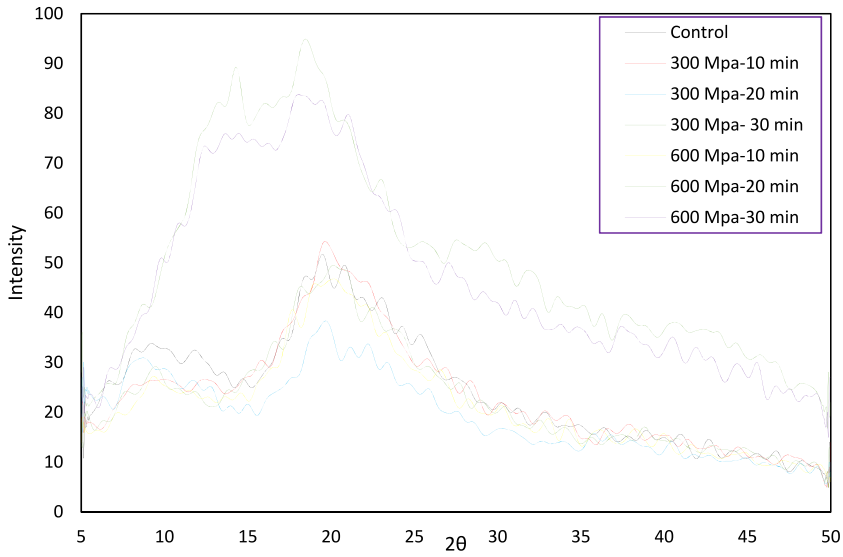

Fig. 3. X-Ray Diffraction of WPC-guar gum mixture after pressurization at different pressure (0–600 MPa) and time (0, 30 min).

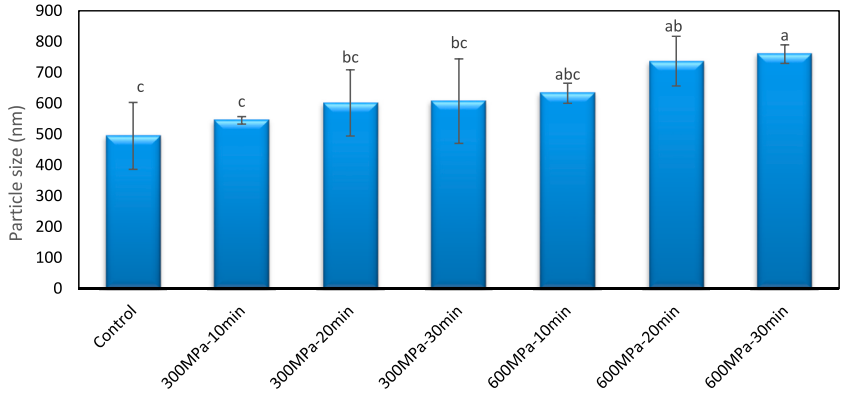

Fig. 4. The effect of HHP treatment on the particle size of WPC-guar gum powder.

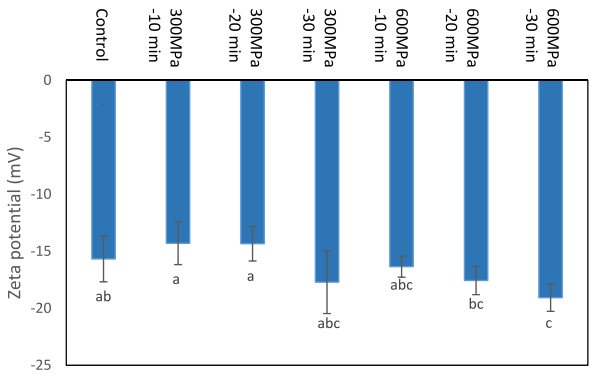

Fig. 5. The effect of HHP treatment on the zeta potential of WPC-guar gum powder.

observed which were consistent with the particle size results. In addition, by applying pressure, the irregularity of shapes grew.

The extent of the pressure can change compressibility and particle size of the protein. HHP can also change conformational rearrangement of proteins, with applying high pressure treatment globular proteins can unfold to a limited extent, so protein aggregation and protein-polysaccharide interaction via disrupted hydrophobic groups can form bigger particle size [40].

3.6. Differential scanning calorimetry

The behaviour of the WPC-guar gum mixture during heating is associated with a change in thermal properties. The correlation between the changes in enthalpy and the unfolding of WPC is directly proportional to the degree of denaturation, and this relationship can be observed through DSC [40]. As can be seen in Fig. 7, all samples exhibited one broad endothermic peak with a peak height corresponding to the denaturation temperature of the major whey proteins, β-LG, which was approximately 77 °C. Denaturation of WPC involves dissociation of intermolecular bonds. Dissanayake and Vasiljevic [40] reported the same results for whey proteins under the impacts of heat treatment and hydrodynamic high-pressure shearing. These researchers stated that during denaturation of whey proteins the endothermic total enthalpy is associated mainly to the disruption of internal hydrogen bonds in protein and water. Additionally, it is also associated, albeit to a lesser degree, with the creation of bonds between whey protein and water, the presence of excessive hydrogen bonds in water surrounding polar groups, and the disruption of van der Waals bonds between polar groups.

4. Conclusion

This study examined the effect of HHP on the structure of the WPC/guar gum mixture at pH 5. The HHP- treated samples showed larger particle size and higher zeta potential. HHP treatment significantly decreased powder solubility. FTIR results showed, after HHP treatment especially at 600 MPa, the secondary structure of protein and also the interaction of WPC-guar gum have changed. The XRD and DSC findings showed that the amorphous structure and denaturation temperature of WPC-guar gum mixtures changed after HHP treatment, respectively. Pressure at 600 MPa increased the denaturing temperature of the samples. Most particles of the control sample was less than 1 μm in size. SEM images confirmed the results of particle size. Various foodstuffs contain proteins and polysaccharides. Both biopolymers (individually or in combination) have the potential to alter the structure, texture, shelf life and stability of food products because of their thickening, gelling and surfactant properties. HHP could potentially have some significant impacts on the

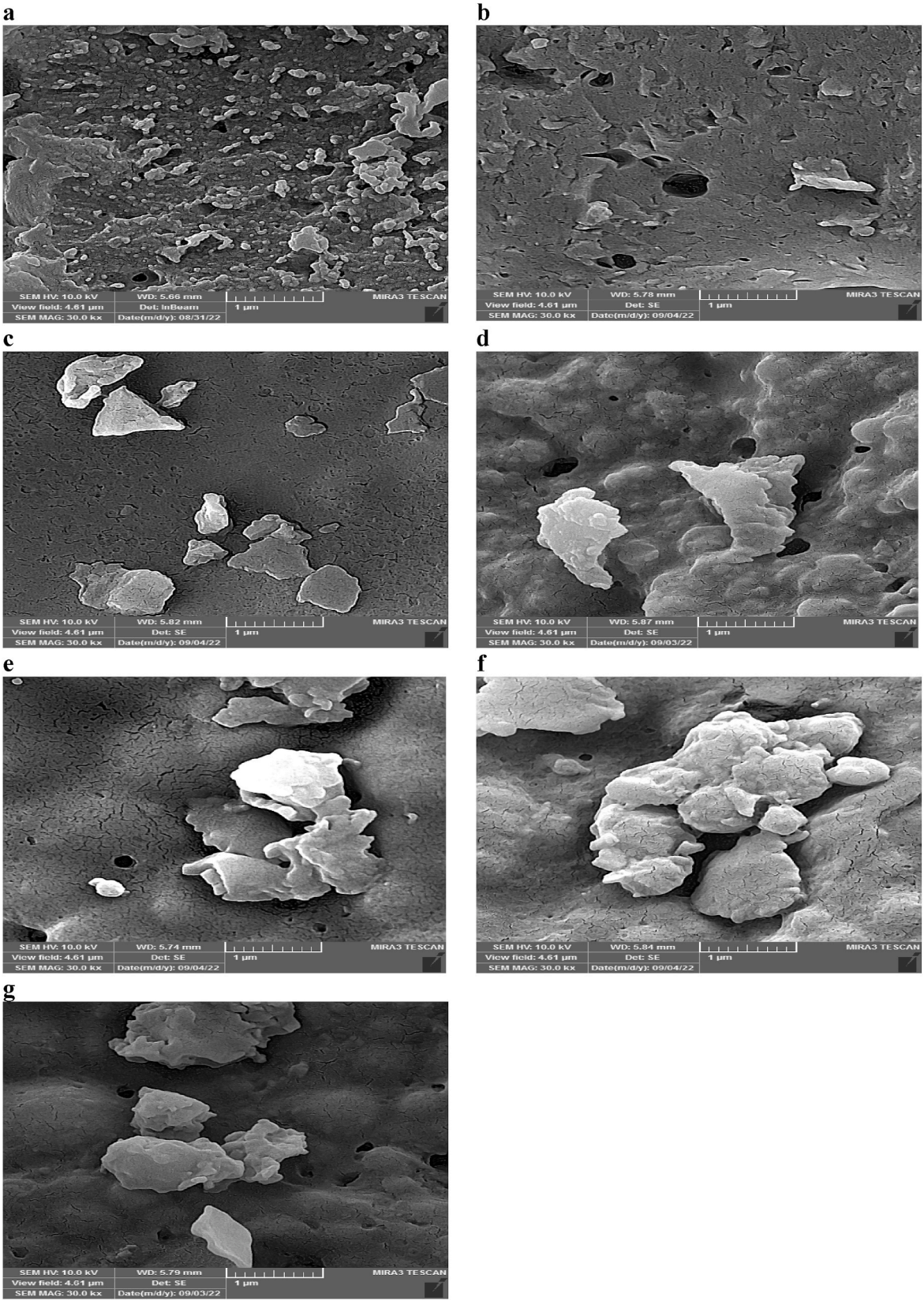

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Fig. 6. Scanning Electron Microscopy (30 k ×): (a) untreated sample; (b) 300 MPa-10 min; (c) 300 MPa-20 min; (d) 300 MPa-30 min; (e) 600 MPa-10 min; (f) 600 MPa-20 min; (g) 600 MPa-30 min.

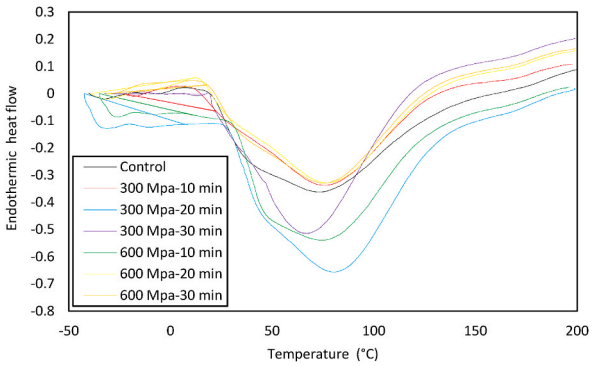

Fig. 7. Differential scanning calorimetry (DSC) of WPC-guar gum mixture after pressurization at different pressure (0–600 MPa) and time (0, 30 min).

electrostatic interactions and microstructure of these biopolymers such as proteins and polysaccharides. Consequently, this could also affect various other characteristics of the resulting food products. Hereupon, the findings of the present investigation can provide valuable insights into the interactions between WPC and guar gum at varying HHP levels. Additionally, this composite can be employed as a substitute for fat, a stabilizer, an enhancer for encapsulation, and a means to enhance texture in diverse food matrices.

Ethics approval and consent to participate

Not applicable.

Consent for publication

We, the authors, gladly declare our consent to publish the article in this journal.

Funding

Saeed Mirarab Razi was partially supported by a grant from the Ferdowsi University of Mashhad, Iran for the Postdoc research project. The financial support is gratefully acknowledged.

Consent for publication

All authors read and approved the final manuscript.

Availability of data and materials

The data will be available from the authors upon request.

CRediT authorship contribution statement

Saeed Mirarab Razi: Writing – original draft, Software, Formal analysis, Data curation. **Mohebbat Mohebbi:** Writing – review & editing, Supervision. **Seyyed Mahdi Mirzababaee:** Writing – original draft, Formal analysis. **Mohammad Ali Hesarinejad:** Software, Methodology, Data curation. **Mohammad Khalilian Movahed:** Writing – review & editing, Project administration, Investigation, Formal analysis, Data curation.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Mohebbat Mohebbi reports was provided by Ferdowsi University of Mashhad. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We are grateful to the Research Institute of Food Science and Technology for the use of its facilities for this study.

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