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Effects of polyhydroxy compounds on the properties of starch-protein composites modified by high-pressure processing

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Abstract

Biopolymer composites based on starch-protein blends are responsible for a variety of food properties. The aim of this research was to study the effects of high pressure processing (HPP) and polyhydroxy compounds on the properties of starch-protein composites as determined by the degree of starch gelatinization (DG), surface hydrophobicity (H_0), exposed and total free sulfhydryl (SH) groups, and morphology as they relate to their pasting properties. Tapioca starch (TS) and whey protein isolate (WPI) were chosen to form composites. The impacts of pressure ranging from 300-600 MPa on tapioca starch-whey protein isolate composites (mTS-WPI) with and without polyhydroxy compounds were investigated. Increased pressure up to 500 MPa significantly increased the DG, H_0, and exposed free SH groups of the composites, with dramatic increases observed at 600 MPa. HPP also altered the microstructure of the composites, especially at 500 and 600 MPa of HPP. Addition of polyhydroxy compounds decreased the DG, H_0, exposed free SH group content of the composites in a concentration and compound type dependent manner. Addition of glucose had more effect on the mTS-WPI than that of fructose and glycerol at the same concentration. Polyhydroxy compounds also had an impact on the pasting properties of the composites, exhibiting higher pasting viscosity and temperature than composites to which no polyhydroxy compounds were added. This research indicates that different types and concentrations of polyhydroxy compounds could be used to stabilize and prevent starch gelatinization and protein denaturation in starch-protein based foods that are treated by HPP.

Keywords: High-pressure processing, HPP, Starch-protein composite, Polyhydroxy compound

1. Introduction

The use of gels from natural protein and polysaccharide biopolymers has increased in the food industry, but applications of these gels can be limited due to certain intrinsic and extrinsic factors. The properties of protein gels are modified by commonly encountered environmental factors in food such as the presence of ions, changes in pH, and extreme temperature [1]. Polysaccharide gels tend to have a more rigid structure that accounts for their tendency towards low water holding capacity (WHC) and weak flexibility [2,3]. However, gels from protein/ polysaccharide composites have been shown to have a more stable structure with improved physicochemical and mechanical properties [4,5] that can mitigate their limitations. Chemical, enzymatic or physical modifications of these gels can enhance their physicochemical properties. However, only enzymatic and physical modifications are considered environment-friendly [6].

High-pressure processing (HPP) can extend the shelf-life of certain food products by reduction or inactivation of enzyme activity and destruction of microorganisms that in most cases causes minimum effect on nutritional value and flavor [7]. HPP often is referred to as a physical, non-thermal processing method. The high hydrostatic pressures used in HPP can disrupt non-covalent bonds in biopolymers, resulting altered starch gelatinization and protein denaturation [8,9]. HPP has been applied to a variety of food products, including beverages, meat, seafood,

and ready-to-eat products [10]. However, food products may be composed of ingredients such as sugar that can alter the properties of starch and protein during HPP.

Glycerol, glucose, and fructose are considered polyhydroxy compounds because they are molecules that are composed of more than 2 hydroxyl groups. Glucose and fructose are monosaccharide isomers (M_w=180 g/mole) and are sweeteners widely used in the food industry. Glycerol is a smaller polyhydroxy compound (M_w=92 g/mole) and is used in the food industry as an additive, humectant and preservative. Polyhydroxy compounds such as these are known to enhance the stability of proteins [11] and can inhibit starch gelatinization [12]. Research studies have shown the effects of sugar or sugar mixtures on the pasting, gelatinization, and rheological properties of starches [12-16] or the characteristics of protein gels [17]. However, the literature is void of studies on the effect of polyhydroxy compounds on starch-protein composite gels induced by HPP. Therefore, the objective of this study was to investigate the effect of HPP on the physicochemical properties of starch-protein composite gels as affected by the type and concentration of polyhydroxy compounds. This knowledge could help to elucidate the interactions of starch, protein and polyhydroxy compounds as affected by HPP and could be applied to the quality and safety improvement of starch-protein based food products containing polyhydroxy compounds.

2. Materials and methods

2.1 Materials

Tapioca starch (TS) was purchased from Thai Wah Co., Ltd., Bangkok, Thailand. Whey protein isolate (WPI; 93.30% protein dry basis) was purchased from Matell Intertrade Co., Ltd., Khonkaen, Thailand. Analytical grade glycerol was purchased from Qchemical Co., Ltd., Bangkok, Thailand. D(+)-glucose anhydrous was purchased form Elago Enterprises Pty Ltd., New South Wales, Australia and D(-)-fructose was purchased from Loba Chemie Pty Ltd., Mumbai, India.

2.2 Sample preparation for high-pressure processing treatment

Modified tapioca starch-whey protein isolate composites (mTS-WPI) were prepared with 10% TS and 5% WPI but varied with the addition of either 0, 5, 10, 20, or 30% glycerol, fructose, or glucose (w/w). TS, WPI, and either glycerol, fructose, or glucose were mixed in distilled water for 2 h with moderate stirring at room temperature (25±5 ˚C), after which 10 g of the mixtures were transferred to 7 cm x 10 cm polyethylene bags and hermetically sealed. The suspensions immediately were stored at 4 ˚C for 24 h to ensure full hydration. The suspensions were subjected to either 300, 400, 500, or 600 MPa pressure treatment for 20 min using a high-pressure unit (HPP-600 MPa/5.0L, Kefa High Pressure Food Processing Co., Ltd., Baotou, China).

For the composites that contained glycerol, glucose, or fructose, all assays described below except morphology analysis by scanning electron microscope (SEM) were performed on freshly processed composites within 48 h after the HPP treatment was completed. For the composites that contained no glycerol, glucose, or fructose, only the degree of gelatinization was determined on the freshly processed composites within 48 h after the HPP treatment was completed. All of the remaining fractions, both with and without glycerol, glucose, and fructose, were freeze-dried using a DL-0.5 Freeze Dryer (Kinetic Engineering Co., Ltd., Thailand), ground and sieved (100 mesh or 149 μm screen), then packed in aluminum foil bags and stored at -18 ˚C until analyzed using the assays described below.

2.3 Degree of gelatinization

The degree of gelatinization (DG) of starch granules was determined according to the method described by Larrea-Wachtendorff et al. [18]. The freshly prepared mTS-WPI samples were diluted 100-fold and placed into a cell counting chamber (Hemocytometer), then observed under a microscope equipped with a polarization filter at 400X of magnification. The DG was calculated according to the equation (1) where *NB* is the number of starch granules with birefringence and *N* is the number of total starch granules.

$$DG=\left(1-\frac{NB}{N}\right)\times 100 \quad (1)$$

2.4 Scanning electron microscopy

The morphology of the mTS-WPI samples were imaged at 500X magnification using a field-emission scanning electron microscope (FE-SEM) model JSM 7800F (JEOL, Japan) according to the method of Zhang et al. [19]. The freeze-dried samples were mounted on a carbon tape and coated with gold in a vacuum evaporator. An acceleration voltage of 2 kV was used during micrography.

2.5 Surface hydrophobicity

The surface hydrophobicity (H_0) of the mTS-WPI samples was measured using 1,8- anilinonapthalenesulfonate (ANS^-) as described by Liang and Tang [20]. ANS^- stock solution (8 mM) was prepared by dissolving the ANS^- in 10 mM of phosphate buffer (pH 7.0). The composite samples were diluted with 10 mM of phosphate buffer (pH 7.0) to obtain a final protein suspension concentration of 0.001-0.05 % (w/v). The protein suspension (4 mL) was mixed with 20 µL of ANS^- stock solution and vortexed for 5 s. The fluorescence intensity (FI) of the mixtures was measured at an excitation wavelength of 370 and emission wavelengths of 470 nm using a microplate reader (Infinite M200 Pro, Tecan Austria, GmbH, Austria). The FI of each sample was subtracted by the FI of the buffer. The H_0 was calculated by linear regression analysis of the initial slope of the subtracted FI versus the protein concentration.

2.6 Exposed and total free sulfhydryl groups

The exposed and total free sulfhydryl (SH) groups of the mTS-WPI samples were determined using Ellman's reagent [5,5' dithiobis (2-nitrobenzoic acid); (DTNB)] as described by Du et al. [21]. The mTS-WPI samples were dissolved in buffer (86 mM TRIS, 90 mM glycine, 4 mM EDTA, pH 8.0) to obtain a final protein concentration of 0.05 % (w/v). Diluted sample (1 mL) was mixed with 10 µL of Ellman's reagent (4 mg of DTNB/mL of standard buffer) and allowed to incubate at room temperature for 1 h. The mixture was centrifuged at 12,000xg at 4 ˚C for 10 min. The supernatant was collected and the absorbance measured at 412 nm using a UV-Vis spectrophotometer (Helios Alpha, Thermo Electron Corporation, England) to determine exposed SH groups. The total SH group content was determined using the same technique with a denaturing agent (8 M urea and 0.5 % (w/v) sodium dodecyl sulfate) in the buffer. Free sulfhydryl groups (µmole SH/g protein) of each sample were calculated using a molar extinction coefficient of 1.36×10^4 $M^{-1}cm^{-1}$.

2.7 Pasting properties

The influence of polyhydroxy compounds on the pasting properties of mTS-WPI was analyzed according to the method of Zhang et al. [19] using Rapid Visco Analyzer (RVA, Tecmaster, Perten Instruments, Sweden). The mTS-WPI samples were diluted with 50% (w/w) of distilled water to reduce the concentrations of starch and protein to the practical range of evaluation. The mixture (25 g) was transferred into RVA canister and the analysis was performed by stirring at 960 rpm for 10 s and then slowing to 160 rpm. The temperature was set at 50˚C for 1 min, then increased from 50 to 95 ˚C at the rate of 12 ˚C/min and held at 95 ˚C for 2.5 min, cooled back to 50 ˚C at the rate of 12 ˚C/min and finally held at 50 ˚C for 2 min. The peak viscosity (PV), breakdown viscosity (BD), final viscosity (FV), setback viscosity (SB) and pasting temperature (PT) were recorded.

2.8 Statistical analysis

Experiments were performed triplicate. Data were analyzed by analysis of variance (ANOVA) using statistical package for the social science (SPSS). Duncan's multiple range tests were used to compare means with significance determined at $p \leq 0.05$.

3. Results and discussion

3.1 Effect of HPP on mTS-WPI composites without polyhydroxy compounds

The influence of HPP at different pressures on the DG, H_0, SH groups, and morphology using SEM of mTS-WPI composites without the addition of polyhydroxy compounds is shown in Table 1 and Figure 1. Although considered a non-thermal processing treatment, it has long been known that the thermodynamic effect of pressure-induced adiabatic heating can cause the temperature in the HPP chamber to increase during HPP [22]. However, it is unlikely that the slight increase in temperature during HPP processing is responsible for any of the effects reported herein.

Table 1 Influence of pressure on the degree of gelatinization (DG), surface hydrophobicity (H_0), exposed and total free sulfhydryl (SH) groups of modified tapioca starch-whey protein isolate composites.

Pressure (MPa)	Degree of gelatinization (%)	Surface hydrophobicity (x 10^4)	Sulfhydryl group (µmole SH/g protein)	
			Exposed	Total
0	$0^{d} \pm 0$	$47.61^{e} \pm 0.54$	$14.70^{c} \pm 0.56$	$21.51^{b} \pm 0.39$
300	$0^{d} \pm 0$	$63.64^{d} \pm 0.12$	$15.54^{bc} \pm 0.09$	$21.90^{b} \pm 0.19$
400	$1.36^{c} \pm 0.03$	$76.17^{b} \pm 0.13$	$16.54^{a} \pm 0.02$	$22.51^{a} \pm 0.18$
500	$15.12^{b} \pm 0.03$	$82.16^{a} \pm 1.01$	$16.42^{ab} \pm 0.49$	$22.11^{ab} \pm 0.14$
600	$67.83^{a} \pm 0.66$	$71.93^{c} \pm 1.40$	$15.14^{c} \pm 0.21$	$22.10^{ab} \pm 0.10$

Means within a column with different superscripts are significantly different ($p \leq 0.05$).

3.1.1 Effect of HPP on degree of gelatinization (DG) of mTS-WPI composites

Table 1 shows the influence of HPP at different pressures on the degree of gelatinization (DG) without the addition of polyhydroxy compounds. The mTS-WPI that was not subjected to HPP exhibited zero DG. The mTS-WPI composites subjected to higher pressures of HPP caused a significant stepwise increase ($p \leq 0.05$) on the DG of the order of 0 MPa = 300 MPa < 400 MPa < 500 MPa < 600 MPa. The increase in DG from 300 and 600 MPa is second order ($R^2 = 0.98$), suggesting that higher pressure of HPP corresponds to much greater the impact on DG.

3.1.2 Effect of HPP on surface hydrophobicity (H_0) of mTS-WPI composites

Surface hydrophobicity (H_0) measurements were used to evaluate the impact of HPP on the properties of protein molecules in the mTS-WPI composites without the addition of polyhydroxy compounds. Shown in Table 1, the pressure of HPP had a significant effect on the H_0 ($p \leq 0.05$) of all treatments, with a significant and stepwise increase in H_0 in composites subjected to increasing pressure up to 500 MPa, after which a significant decline occurred. The increased H_0 caused by increasing pressure could be attributed to the pressure-induced unfolding of the protein molecules in the mTS-WPI, which exposed buried hydrophobic residues that usually form the hydrophobic cores that maintain the structural integrity of proteins. The results reported here agree with previous research that showed that HPP can affect the secondary, tertiary and quaternary structures of protein molecules [23]. The exposed hydrophobic residues of proteins, as indicated by increased H_0, can alter their molecular function by affecting protein–protein interactions. Therefore, this information could facilitate our understanding of the functional properties of proteins in complex food systems under HPP. It should be noted that the significant decrease in H_0 ($p \leq 0.05$) from 500 to 600 MPa could be explained as a result of the entropy-driven reformation of intermolecular and intramolecular interactions caused by excess molecular unfolding of proteins at 600 MPa during and after HPP, the result of which could be the decrease of surface hydrophobicity as a more stable structure is formed [24]. This effect was observed in soy protein isolate, but the decrease was observed after 400 MPa [25].

3.1.3 Effect of HPP on free and total sulfhydryl (SH) groups of mTS-WPI composites

Measurements of SH groups also were used to evaluate the impact of HPP on the properties of protein molecules in the mTS-WPI composites (Table 1). The effect of HPP on the free SH groups of the mTS-WPI composites increased with increasing pressure until 400 MPa, then decreased when pressure reached 600 MPa, in the order of 0 MPa = 300 MPa < 400 MPa = 500 MPa > 600 MPa. This agrees with previous research that showed that high pressure can induce unfolding of β-conglycinin, a protein from soy protein isolate, which exposed the free SH groups previously buried or bound and increased the free SH group content [25]. However, total SH groups did increase with increasing pressure up to 400 MPa but did not significantly decline thereafter.

3.1.4 Effect of HPP on morphology of mTS-WPI composites and comparison to previous results

Figure 1 shows the impact of HPP at 0, 400, 500 and 600 MPa for 20 min on the scanning electron microscope (SEM) images of the freeze-dried mTS-WPI composites without the addition of polyhydroxy compounds. As shown in Figure 1A, intact TS granules of different sizes were separate from WPI, and the TS granules were mostly spherical and smooth surfaced. After being subjected to HPP at 400 MPa (Figure 1B), the SEM images show some changes to the TS and WPI. Although most of TS granules remained spherical and were similar in appearance to those that were not subjected to HPP, some TS granules exhibited irregular shapes with less smooth surfaces, perhaps due to the small but significant increase in DG observed in the mTS-WPI composites at 400 MPa (Table 1). The WPI began to aggregate and form irregular shapes with uneven surfaces, which seems to visually confirm the protein denaturation suggested by significantly higher H_0 and free SH reported previously in Table 1.

At higher pressure (500, 600 MPa), TS granules were more deformed and merged with WPI molecules (Figure 1C and 1D). These phenomena also can be explained by the results reported previously in Table 1. Increased pressure induced the gelatinization of starch, i.e. increased DG, and increased protein unfolding and exposure of more hydrophobic regions of the protein molecules, i.e. increased H_0. These results are seen in the dramatic loss of structure shown in Figures 1C and 1D, which indicates that pressures of 500-600 MPa caused the formation of a more complex composite of gelatinized TS and denatured WPI. This result was consistent with the result in Proso millet (*Panicum miliaceum*) starch granules that began to lose structure at pressure of 450 MPa and formed a gel-like structure at 600 MPa [26]. Taken together, these results indicate that the structural characteristics of proteins and starches are modified by HPP treatment. What is not known is how these structural modifications affect the functional properties of the composites.

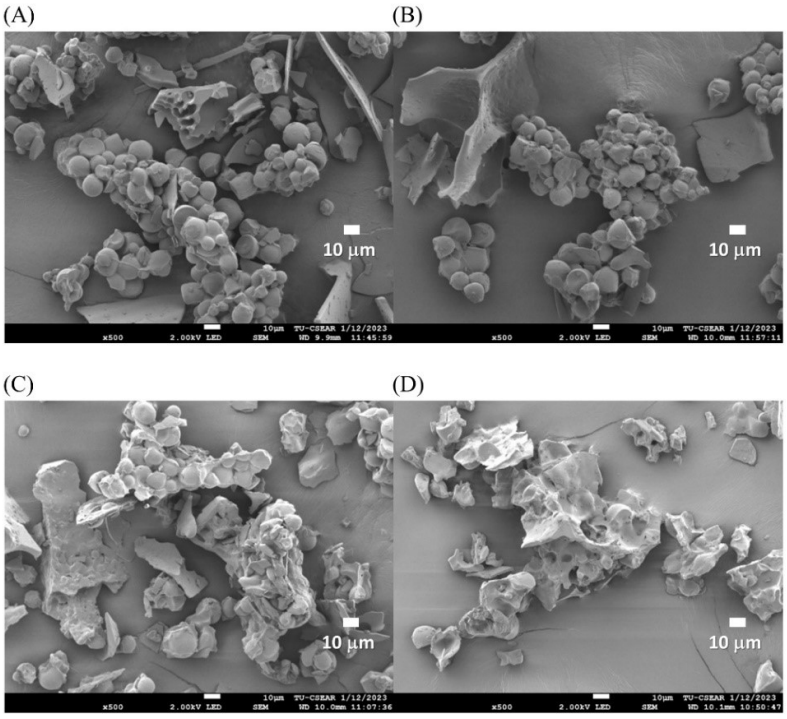

Figure 1 Influence of (A) 0 MPa, (B) 400 MPa, (C) 500 MPa, and (D) 600 MPa on the SEM images of HPP-treated modified tapioca starch-whey protein isolate composites.

3.2. Effect of HPP on mTS-WPI composites with the addition of polyhydroxy compounds

The next step in this research was to determine if mTS-WPI composites with the addition of glucose, fructose, and glycerol can mitigate the alteration of the structural characteristics caused by HPP as reported in Table 1 and visualized in Figure 1 because polyhydroxy compounds can alter the pasting, gelatinization, and rheological properties of starches [12-16] or the characteristics of protein gels [17]. Because mTS-WPI composites subjected to 500 MPa for 20 min exhibited significant gelatinization (DG) and significantly higher hydrophobicity (H_0) and free SH groups than composites exposed to 600 MPa, the mTS-WPI composites subjected to 500 MPa were chosen to study the impact of polyhydroxy compounds on the properties of composites. The influence of HPP at different pressures on DG, H_0, and free and SH groups of mTS-WPI composites with the addition of different types (glycerol, fructose, and glucose) and concentrations (0, 5, 10, 20, and 30%) of polyhydroxy compounds is shown in Table 2.

Table 2 Influence of HPP at 500 MPa for 20 min on the degree of gelatinization (DG), surface hydrophobicity (H_0), exposed and total free sulfhydryl (SH) groups of modified tapioca starch-whey protein isolate composites with the addition of glucose, fructose, and glycerol.

Type and percent of polyhydroxy compound (%)	Degree of gelatinization (%)	Surface hydrophobicity (x 10^4)	Sulfhydryl group (μmole SH/g protein)	
			Exposed	Total
Glycerol				
0	$15.12^{a} \pm 0.03$	$154.86^{a} \pm 0.85$	$15.40^{a} \pm 0.36$	20.87 ± 0.30
5	$3.41^{b} \pm 0.06$	$124.94^{c} \pm 0.93$	$14.08^{b} \pm 0.10$	20.81 ± 0.21
10	$1.60^{e} \pm 0.12$	$116.07^{f} \pm 0.20$	$12.91^{c} \pm 0.26$	21.03 ± 0.42
20	$1.03^{f} \pm 0.04$	$112.70^{g} \pm 0.10$	$12.04^{d} \pm 0.49$	20.97 ± 0.19
30	$0.69^{g} \pm 0.09$	$108.25^{h} \pm 1.14$	$11.93^{d} \pm 0.13$	20.75 ± 0.13
Fructose				
0	$15.12^{a} \pm 0.03$	$154.86^{a} \pm 0.85$	$15.40^{a} \pm 0.36$	20.87 ± 0.30
5	$3.07^{c} \pm 0.28$	$133.42^{b} \pm 0.92$	$14.06^{b} \pm 0.13$	20.79 ± 0.39
10	$1.31^{f} \pm 0.25$	$123.58^{c} \pm 0.30$	$13.20^{c} \pm 0.10$	20.74 ± 0.52
20	$0.70^{g} \pm 0.02$	$121.73^{d} \pm 0.25$	$11.97^{d} \pm 0.08$	20.77 ± 0.42
30	$0.35^{h} \pm 0.02$	$112.54^{g} \pm 0.41$	$8.99^{f} \pm 0.34$	20.88 ± 0.47
Glucose				
0	$15.12^{a} \pm 0.03$	$154.86^{a} \pm 0.85$	$15.40^{a} \pm 0.36$	20.87 ± 0.30
5	$2.24^{d} \pm 0.18$	$124.73^{c} \pm 0.90$	$14.17^{b} \pm 0.55$	20.88 ± 0.21
10	$1.07^{f} \pm 0.15$	$119.05^{e} \pm 0.80$	$13.31^{c} \pm 0.10$	20.97 ± 0.34
20	$0.67^{g} \pm 0.01$	$116.36^{f} \pm 1.54$	$10.79^{e} \pm 0.13$	21.02 ± 0.40
30	$0.15^{h} \pm 0.03$	$98.48^{i} \pm 0.44$	$6.93^{g} \pm 0.44$	21.01 ± 0.03

Means within a column with different superscripts are significantly different ($p \leq 0.05$).

3.2.1 Effect of polyhydroxy compounds on degree of gelatinization (DG) of HPP-treated mTS-WPI composites

Table 2 shows the influence on the DG of HPP-treated mTS-WPI composites with the addition of different types (glycerol, fructose, and glucose) and concentrations (0, 5, 10, 20, and 30%) of polyhydroxy compounds. All three polyhydroxy compounds significantly decreased the degree of gelatinization compared to the HPP-treated composite to which no polyhydroxy compound was added. Although the addition of glycerol to the mTS-WPI composites significantly reduced DG at each concentration, it was not as effective at reducing DG as either fructose or glucose. A concentration effect was observed, as increasing polyhydroxy concentrations of each compound significantly reduced DG to the point where 30% glycerol, fructose and glucose reduced DG by more than 98% compared to the composites to which no polyhydroxy compound was added.

We hypothesize that the mitigating effect of polyhydroxy compounds on DG is related to the effective number of hydroxyl groups per molecule (n_{OH}^{eff}/n), which for glucose, fructose and glycerol are 4.00, 3.44 and 2.36, respectively [27,28]. Thus, more effective hydroxyl groups per molecule promotes stronger intermolecular hydrogen bonding with water leading to decrease free water in the system, resulting in reduced swelling and gelatinization of starch granules in accordance with their type and concentration.

3.2.2 Effect of polyhydroxy compounds on surface hydrophobicity (H_0) of HPP-treated mTS-WPI composites

Table 2 shows the influence on the surface hydrophobicity (H_0) of HPP-treated mTS-WPI composites with the addition of different types (glycerol, fructose, and glucose) and concentrations (0, 5, 10, 20, and 30%) of polyhydroxy compounds. The addition of all three polyhydroxy compounds to the mTS-WPI composites significantly decreased H_0 compared to the HPP-treated composite to which no polyhydroxy compound was added. A concentration effect was observed, as increasing polyhydroxy concentrations of each compound significantly reduced H_0 in the range of 19-30% for glycerol, 14-27% for fructose, and 19-36% for glucose compared to the HPP-treated composite to which no polyhydroxy compound was added.

Polyhydroxy compounds have been shown to increase the hydrophobic interactions between non-polar amino acids [29], resulting in the inhibition of protein denaturation. Sugars also have been shown to protect heated proteins from denaturation, as was shown in heated whey protein wherein added sugar increased the midpoint transition temperature (T_m) which protected the protein from denaturation [30]. However, considering that the H_0 of the mTS-WPI composites was doubled by HPP treatment at 500 MPa (Table 1), it can be concluded that the addition of polyhydroxy compounds to HPP-treated composites provided some protection but did not completely mitigate against protein denaturation caused by HPP. This result may explain why similar enzymes are fully denatured by HPP in some systems and only partially denatured by the same HPP conditions in others. For example, enzyme inactivation of polyphenol oxidase in HPP-treated strawberry puree at 600 MPa was reported to increase from 40% in puree at 6.5 ºBrix to 100% (complete inactivation) in puree at 9.3 ºBrix [31].

3.2.3 Effect of polyhydroxy compounds on total and free sulfhydryl (SH) groups of HPP-treated mTS-WPI composites.

Table 2 shows the influence on the total and SH groups of HPP-treated mTS-WPI composites with the addition of different types (glycerol, fructose, and glucose) and concentrations (0, 5, 10, 20, and 30%) of polyhydroxy compounds. The results showed that polyhydroxy type and concentration had no significant impact on the total free SH groups ($p>0.05$) but did significantly decrease exposed SH groups. These results confirmed that polyhydroxy compounds could be used to stabilize protein structure in HPP by preventing protein unfolding. The effect of polyhydroxy compound type on the decrease of exposed free SH groups was in the following order: glucose > fructose > glycerol, which could be due to the higher effective number of hydroxyl groups per molecule of glucose than that of fructose and glycerol [27,28], as described earlier in section 3.2.1. Higher effective number of hydroxyl groups per molecule can promote stronger H-bonding with water, decreasing the mobility of water molecules in the system and decreasing protein denaturation of HPP.

3.2.4 Effect of polyhydroxy compounds on morphology of HPP-treated mTS-WPI composites.

Figure 2 visualizes the impact of type (glycerol, fructose, and glucose) and concentration (5 and 10%) of polyhydroxy compound addition to freeze-dried mTS-WPI composites. Addition of 5 and 10% polyhydroxy compounds did not seem to alter the granules of TS after HPP when compared to the granules from Figure 2A-1, which visualizes the TS granules without either polyhydroxy addition or HPP treatment. The granules of TS in Figure 2 were composed of various sizes and shapes, including spherical shapes with smooth surface that remained intact.

As noted earlier in section 3.1.4, pressure of 500 MPa deformed the TS granules which merged with WPI molecules and caused a dramatic loss of structure (Figure 2A-2), which is attributed to the pressure-induced

gelatinization of starch, exhibited as increased DG, and increased protein unfolding and exposure of more hydrophobic regions of the protein molecules, i.e. increased H_0. The morphology of starch granules in Figure 2 suggests that the low DG observed with the addition of polyhydroxy compounds and decreased protein denaturation as determined by decreased H_0 and exposed SH group contents (Table 2) in the mTS-WPI indicate that the structural characteristics of proteins and starches that are modified by HPP treatment are mitigated in the presence of polyhydroxy compounds.

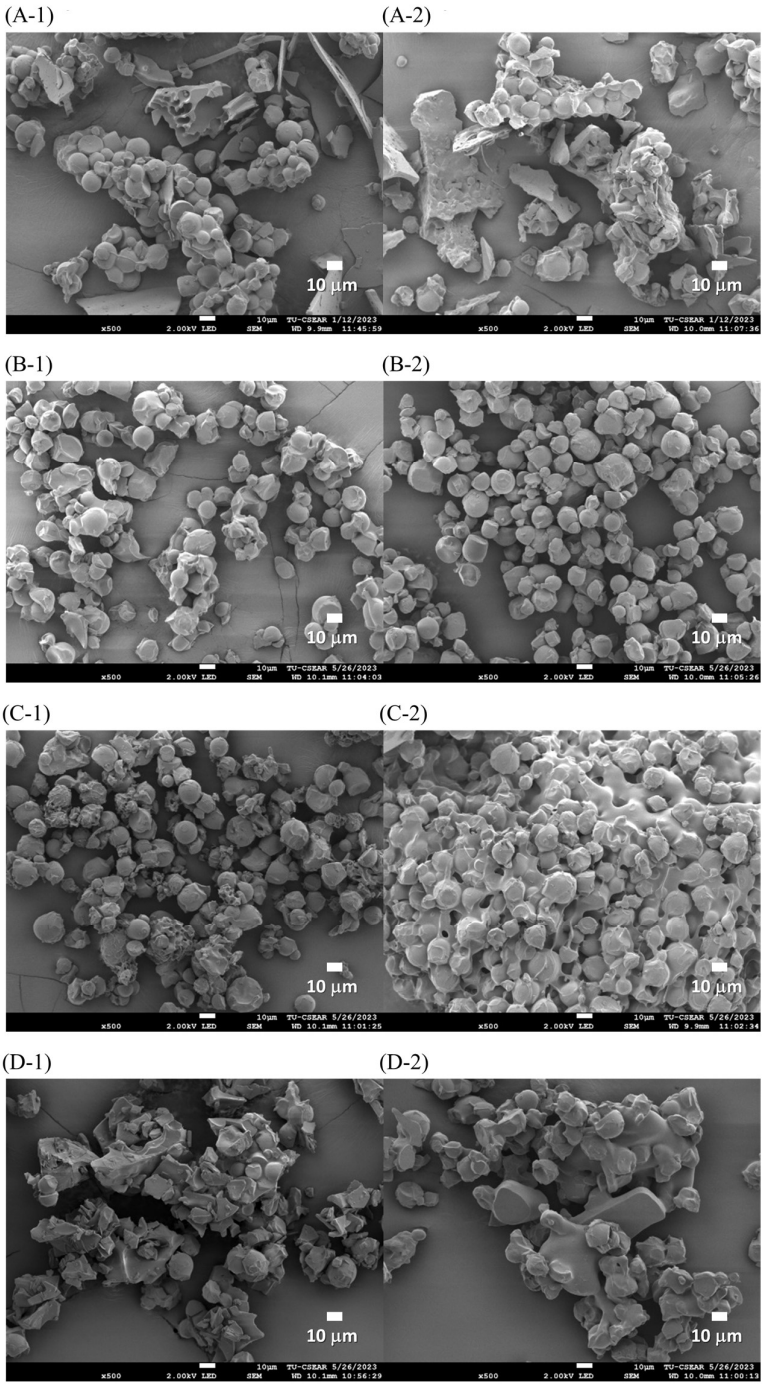

Figure 2 Influence of polyhydroxy compound type and concentration on the SEM images of the modified tapioca starch-whey protein isolate composites treated with HPP at 500 MPa for 20 min: (A-1) without HPP, (A-2) without polyhydroxy compound (B-1) 5% glycerol, (B-2) 10% glycerol, (C-1) 5% fructose, (C-2) 10% fructose, (D-1) 5% glucose and (D-2) 10% glucose.

3.2.5 Effect of polyhydroxy compounds on pasting properties of HPP-treated mTS-WPI composites

Table 3 shows the influence on pasting properties of HPP-treated mTS-WPI composites with the addition of different types (glycerol, fructose, and glucose) and concentrations (0, 5, 10, 20, and 30%) of polyhydroxy compounds. All of the viscosity measurements exhibited significant increases in the presence of polyhydroxy compounds, especially at 20% and 30%. The pasting temperature also was significantly higher in the presence of 20% and 30% polyhydroxy compounds. As shown earlier in Table 2, the addition of the polyhydroxy compounds altered the structural properties (DG, H_0 and exposed SH groups) of the composites during HPP treatment, so it is likely that the differences in pasting properties were caused by differences in the relationship between starch, protein and polyhydroxy compounds during heating from 50-95 ˚C under high shear. In other words, the HPP-treated composites were structurally different before they were heated into a starch paste than the composites that were not treated with HPP, so they exhibited different properties.

Pasting viscosity (PV) is the viscosity measured after the starch paste reaches the pasting temperature, which in our study ranged from 75-84 ˚C. The increase in pasting viscosity and temperature were affected by the polyhydroxy compounds in the following order: glucose > fructose > glycerol, likely due to the difference in the effective number of hydroxyl groups per molecule. This effect on pasting viscosity and temperature was dramatic at 20% and 30% polyhydroxy addition, which can be explained by the higher capability of glucose to interact with water molecules than that of fructose and glycerol, perhaps indicating that hydroxyl groups of the polyhydroxy compounds not only decreased starch gelatinization before heating but also interacted with water in the system during heating, affecting the degree of swelling of the starch granules. SEM imaging (Figure 2) indicates differences between the composites composed of different polyhydroxy compounds.

Breakdown viscosity measures the decrease in viscosity caused by breakdown of the swollen starch granules. When the composites were held at 95 ˚C for 2.5 min, the presence and concentration of polyhydroxy compounds caused an increase in breakdown viscosity, however, composites to which polyhydroxy compounds were added exhibited less breakdown (57-61%) compared to the composite that did not contain a polyhydroxy compound (71%). This suggests more stability to breakdown of the starch granules, perhaps related to protection from the altered protein in the system.

The setback viscosity and final viscosity are related measures and the higher final viscosity, i.e. positive setback viscosity, observed in all composites compared to the pasting viscosity probably indicates some degree of retrogradation. When compared to its breakdown viscosity, the composite that did not contain a polyhydroxy compound exhibited a 3.4 fold increase in viscosity, however, the addition of polyhydroxy compounds exhibited increases in the range of 1.7 to 2.3 fold. This indicates that less retrogradation is occurring, which could be due to the impact of the polyhydroxy compounds on molecular movement and rearrangement.

Table 3 Influence of polyhydroxy compound type and concentration on the pasting properties of the modified tapioca starch-whey protein isolate composites treated with HPP at 500 MPa for 20 min.

Polyhydroxy compound	Concentration % (w/w)	Peak viscosity (PV in cP)	Breakdown viscosity (BD in cP)	Final viscosity (FV in cP)	Setback viscosity (SB in cP)	Pasting temperature (PT in ˚C)
none	-	$1713^{j} \pm 40$	$489^{j} \pm 5$	$1654^{g} \pm 47$	$430^{gh} \pm 2$	$77.03^{f} \pm 0.04$
Glycerol	5	$2041^{Bi} \pm 11$	$800^{Ci} \pm 7$	$1655^{Bg} \pm 78$	$414^{Ah} \pm 10$	$76.60^{Ag} \pm 0.14$
	10	$2182^{Bg} \pm 35$	$928^{Cg} \pm 11$	$1702^{Bg} \pm 24$	$449^{Bfgh} \pm 1$	$76.60^{Ag} \pm 0.07$
	20	$2432^{Be} \pm 16$	$1038^{Be} \pm 8$	$1905^{Ce} \pm 12$	$511^{Ce} \pm 4$	$79.00^{Ad} \pm 0.28$
	30	$2850^{Cc} \pm 17$	$1093^{Bd} \pm 16$	$2274^{Cc} \pm 4$	$517^{Ce} \pm 5$	$82.80^{Bb} \pm 0.07$
Fructose	5	$2143^{Agh} \pm 20$	$863^{Ah} \pm 4$	$1697^{ABg} \pm 22$	$417^{Ah} \pm 6$	$75.93^{Bi} \pm 0.18$
	10	$2387^{Ae} \pm 42$	$1049^{Ae} \pm 19$	$1798^{Af} \pm 30$	$459^{Bfg} \pm 7$	$76.43^{Agh} \pm 0.32$
	20	$2732^{Ad} \pm 33$	$1206^{Ab} \pm 20$	$2147^{Bd} \pm 18$	$621^{Bd} \pm 5$	$78.40^{Be} \pm 0.07$
	30	$3282^{Bb} \pm 20$	$1345^{Aa} \pm 2$	$2726^{Bb} \pm 67$	$789^{Bb} \pm 49$	$82.75^{Bb} \pm 0.07$
Glucose	5	$2101^{Ah} \pm 18$	$831^{Bi} \pm 7$	$1699^{Ag} \pm 5$	$429^{Agh} \pm 6$	$76.13^{Bhi} \pm 0.11$
	10	$2319^{Af} \pm 2$	$992^{Bf} \pm 8$	$1814^{Af} \pm 4$	$487^{Aef} \pm 3$	$76.75^{Afg} \pm 0.00$
	20	$2749^{Ad} \pm 35$	$1159^{Ac} \pm 27$	$2249^{Ac} \pm 11$	$660^{Ac} \pm 4$	$79.33^{Ac} \pm 0.04$
	30	$3545^{Aa} \pm 20$	$1370^{Aa} \pm 24$	$3142^{Aa} \pm 29$	$967^{Aa} \pm 33$	$84.20^{Aa} \pm 0.07$

Uppercase letters indicate significant difference ($p \leq 0.05$) between mean of each polyhydroxy compound at the same concentration.
Lowercase letters indicate significant difference between means within a column ($p \leq 0.05$).

4. Conclusion

The results from this study showed that HPP altered the structural characteristics mTS-WPI composites which were further affected by the presence of polyhydroxy compounds in the order of glucose > fructose > glycerol, most likely due to the difference in the effective number of hydroxyl groups per molecule. Different types and concentrations of polyhydroxy compound could be used to stabilize and prevent starch gelatinization and protein denaturation and applied in starch-protein based foods that are treated by HPP. The practical implications of this research lie in the impact of HPP on food properties as the changes of ingredient interactions and functionalities by creating the composite model system composed of polysaccharides and proteins which are two major food biopolymers.

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