Physico-chemical properties of camelina protein altered by sodium
bisulfite and guanidine-HCl

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ABSTRACT

The objective of this research was to investigate the unfolding behaviors of camelina protein isolate (CPI) induced by sodium bisulfite (NaHSO_3) and guanidine-HCl (Gdm.Cl). Protein was extracted from defatted camelina meal through alkali solubilization and acid precipitation and modified with varying amount of NaHSO_3 (0-12% of the protein dry basis) and Gdm.Cl (0-250% of the protein, dry basis). NaHSO_3 treatment broke the disulfide bonds of the CPI and increased free sulfhydryl content and surface hydrophobicity. As NaHSO_3 concentration increased, the viscosity, elastic modulus (G') and water resistant of NaHSO_3 -modified camelina protein (SMCP) dispersion decreased, and the protein became aggregated due to high surface hydrophobicity. Gdm.Cl treatment broke the CPI's hydrogen bonds but decreased their surface hydrophobicity. Similarly, viscosity, G' , and water resistant of Gdm.Cl-modified camelina protein (GMCP) dispersions decreased as Gdm.Cl increased and protein aggregation formed. The reducing effect of NaHSO_3 was greater than Gdm.Cl for CPI's protein-protein interaction and less obvious for the viscosity and water resistant. Gdm.Cl reduced CPI's decomposing temperature at high concentration, but no remarkable effect was observed for NaHSO_3 treatment. These findings on physicochemical changes of SMCPs and GMCPs may contribute to the application of camelina proteins in the biodegradable materials products.

Keywords: Camelina protein isolate, Sodium bisulfite, Guanidine-HCl, Unfolding, Protein interaction

1. Introduction

To date, the application of environment-friendly biopolymers from the natural sources has drawn much attentions to replace petroleum-based synthetic polymers such as phenol–formaldehyde (PF), urea–formaldehyde (UF), and melamine–formaldehyde (MF)(Sun, 2011). Soy protein, one of the most commonly investigated bio-based material, has exhibited its processability and biocompatibility in fabricating the biodegradable polymers with bulk and surface modification(Song et al., 2011). However, soy protein comprises a portion of humans' diets, thereby creating competition between utilization of soy protein for protein-based products or human food. Therefore, alternative bio-resources must be discovered.

Camelina sativais, an oilseed crop of easy availability, has been primarily used for biodiesel (Kasetaite et al., 2014). Recently, the crop has drawn increasing attention due to its abundance in high quality oil, namely the omega-3 and polyunsaturated fatty acids, which is potential sources for animal feedings and film manufacturing. Accordingly, the demand for biofuels and unsaturated camelina oil resulted in increased production of camelina meal (CM) as protein resources for value-added products (Reddy et al., 2012). CM, a by-product of camelina seed's oil extraction process, typically contains 10-15% residual oil, 40% crude protein, 5% minerals, and 10-12% crude fiber. Camelina protein (CP) is a mixture of 2S (albumin storage protein) and 12S fraction (cruciferin), which are linked by disulfide bonds (Nguyen et al., 2013). Li et al. (2014) isolated four fractions of CP from the meal: albumins (water-soluble), globulins (5% NaCl-soluble), prolamins (70% ethanol-soluble), and glutelins (0.1 N NaOH-soluble). Acid-sedimentation could also be used to extract glutelin, the primary fractions, as soy protein for further processing. Similar to soy protein isolate (SPI), ongoing studies have investigated application of CM and its protein isolate for the biodegradable materials. Zhao et al.(Zhao et al., 2014)developed CP-based water-stable films with potential for tissue engineering in a cytocompatibility study. Reddy (Reddy et al., 2012) used vinyl-grafted CM for thermoplastic application. However, information about the extraction and modification of CP is still limited.

Kim et al. studied the relationship between viscosity and soy protein adhesion (Kim, 2015). They found that the NaHSO₃ unfolded soy protein had an optimum viscosity range for an improved adhesion. Either higher or lower than the optimum viscosity range, adhesion was

1 reduced. Studies also showed that controlling protein's unfolding degree was the most widely
2 used method to modify protein's functional properties (Abtahi and Aminlari, 1997; Jiang et al.,
3 2010; Kishore et al., 2012). We used the similar NaHSO_3 loading level used for soy proteins to
4 treat camelina proteins. However, we did not receive the similar results as to soy protein
5 (unpublished data). We speculated that camelina protein is different from soy protein in
6 configurations. This triggers us to study unfolding kinetics of camelina protein symmetrically.

7 Sodium bisulfite (NaHSO_3) and guanidine-HCl (Gdm.Cl) are two effective unfolding
8 reagents. NaHSO_3 , a strong reducing agent, unfolds protein's higher structures by breaking their
9 intermolecular S-S to expose the buried hydrophobic groups of the globulin. In Sun and Qi's
10 studies (Qi et al., 2011), NaHSO_3 cleaved the S-S and decreased the viscosity of SPI
11 suspension during in situ modification. NaHSO_3 also has function to increase the hydrophobicity
12 of glycinin of SPI dispersion. Gdm.Cl, a chaperon agent, that destroys protein's hydrogen bond
13 to unfold structures (Kiefhaber and Baldwin, 1995; Zhong et al., 2001). Huang and Sun (Huang
14 and Sun, 2000a) used Gdm.Cl to unfold SPI, and they observed that SPI structure was stabilized
15 at low Gdm.Cl concentrations (0.5M and 1M) and was disrupted at higher concentrations (3M)
16 by comparing their denaturing temperatures. Based on the previous studies on SPI, we
17 speculated that, due to their different unfolding mechanisms, NaHSO_3 modified camelina protein
18 (SMCP) and Gdm.Cl modified camelina protein (GMCP) would demonstrate unique behaviors.
19 To the best of our knowledge, in spite of many studies on the modification of SPI-based
20 biodegradable materials, little was known for the physiochemical properties and structures of
21 unfolded camelina protein isolate (CPI).

22 The unfolded protein structure resulted in 1) increased free functional groups such as free
23 SH to increase reactivity, 2) enhanced surface hydrophobicity to increase water resistance, or 3)
24 decreased viscosity of protein solution to facilitate further processing. Thus, the objective of this
25 study was to unfold CPIs by disrupting their H bond or S-S bond using NaHSO_3 or Gdm.Cl and
26 comparing their unfolding behaviors. We confirmed the unfolding behaviors of camelina protein
27 by free sulfhydryl group, surface hydrophobicity, and viscosity. We also characterized the
28 physicochemical properties such as particle size, rheological properties, water resistance, and

thermal properties.

2. Materials and Methods

2.1 Materials

Camelina meal (CM) with 15% lipids (db), 32.4% crude protein (db), and 11.0% moisture content (db) was provided by Field Brothers Inc. (Pendroy, MT, US). CM with particle size <0.5 mm was obtained by milling the CM using a cyclone sample mill (Udy Corp., Fort Collins, CO, USA). CM was then defatted with hexane at a solid/liquid ratio of 1:10 (w/v) for 2 h at room temperature in three cycles. The defatted camelina meal (DCM) was placed in a fume hood in a very thin layer (~2 mm) for 24 h to evaporate residual hexane. NaHSO₃, Gdm.Cl, Modified Lowry Protein Assay, hydrochloric acid (HCl), and sodium hydroxide (NaOH) were purchased from Fisher Scientific (Fair Lawn, N.J.).

2.2 Isolation of camelina protein

CPI was separated from DCM using the method described by Li et al. (2014) with some modifications. DCM samples were mixed with distilled water at a solid/liquid ratio of 1:30 (w/v), stirred for 2 h, and then centrifuged to wash the soluble carbohydrate. Residues were collected and re-suspended in water at a solid/liquid ratio of 1:30 (w/v), adjusted to pH 12 using 2 N NaOH with continuous stirring for 2 h, and centrifuged. Supernatants were adjusted to pH 4.5 and centrifuged to precipitate protein fractions. Isolated CP was washed twice with distilled water, dissolved in distilled water with pH adjusted to 7.0, and lyophilized. The CPI contained approximately 83% protein, (Nitrogen was converted to protein using a factor of 6.25) as evaluated by Elemental Analyzer (PerkinElmer 2400 Series II CHNS/O).

2.3 Preparation of SMCP and GMCP dispersions

CPI was resolved in deionized water at a solid/liquid ratio of 1:10 (w/v). For SMCP, NaHSO₃ (0, 1, 3, 6, 9, 12% of CPI, dry basis) was added in to CPI solution and the resulting solutions were named SMCP-0, 1, 3, 6, 9, 12, respectively. For GMCP, Gdm.Cl (2, 5, 10, 50, 100, 250% of the CPI, dry basis) was added the resulting solutions were named GMCP-0, 2, 5, 10, 50, 250, respectively. The CPI dispersion was adjusted to pH 8.0 by adding 2 N NaOH, and the

1 reaction was implemented with mild stirring at room temperature for 2 h. The reaction was
2 terminated by adjusting pH to 4.5 for further characterization.

3 ***2.4 Determination of free sulfhydryl groups***

4 SH groups of CPI, SMCP, and GMCP were determined using Ellman's reagent DTNB
5 (5,5'-dithiobis-(2-nitrobenzoic acid)) according to the method described by Kazuko Shimada
6 (Shimada and Cheftel, 1988), with some modification. Samples were dissolved in reaction buffer
7 (0.1M sodium phosphate, pH 8.0, containing 1mM EDTA at pH 8) at a concentration of 0.25%
8 (50 mg of protein/20 mL of buffer). Mixtures were gently stirred for 24 h at room temperature
9 and then centrifuged at 20,000g for 15 min at 4 °C. Supernatant fractions were analyzed for SH
10 group content, and 50 μ L of Ellman's Reagent Solution (4 mg of DTNB/mL of reaction buffer)
11 was added to a 250 μ L aliquot of the protein supernatant with 2.5mL of reaction buffer. After the
12 solution was rapidly mixed and allowed to stand at 20 °C for 15 min, absorbance was read at 412
13 nm. Buffers were used instead of protein solutions as a reagent blank. A molar extinction
14 coefficient of 14,150 M⁻¹cm⁻¹ was used to calculate micromoles of SH/ g of protein.

15 ***2.5 Surface hydrophobicity***

16 Surface hydrophobicity was determined using ANS (8-Anilinonaphthalene-1-sulfonic acid)
17 as a fluorescence probe as described by Hu (Hu et al., 2013) with modification. Lyophilized CPI,
18 SMCP, and GMCP samples in buffer (1 mg/ml in 0.1 M phosphate buffer at pH 7.4) were
19 centrifuged at 20,000g for 15 min at 4 °C. After determining the protein content of supernatant
20 fraction using Lowry's method, each supernatant was diluted to 0.1mg/ml, and then 60 μ L ANS
21 solution (8.0 mM in 0.1 M phosphate buffer, pH 7.4) was added to 3 mL sample solution. Sample
22 solutions were excited at 365 nm, and the relative fluorescence intensity (RFI) of emission
23 spectra was recorded from 400 nm to 540 nm using a Hitachi F-7000 fluorescence
24 spectrophotometer (Hitachi, Ltd., Tokyo, Japan) with a slit width of 10 nm.

25 ***2.6 Particle size***

26 The SMCP and GMCP dispersion were prepared and stored at 4 °C overnight to ensure that
27 the SMCP and GMCP samples were completely dissolved. Particle sizes of samples were

measured by light scattering using Horiba Laser Scattering Particle Size Distribution Analyzer LA-910. Particle size was reported as volume distribution and volume-mean diameter.

2.7 Rheological properties

A Bohlin CVOR 150 rheometer (Malvern Instruments, Southborough, MA) was used to characterize viscoelastic properties of SMCP and GMCP dispersions. A parallel plate head was used with 20-mm plate diameter and a 500-um gap. A thin layer of silicon oil was spread over the circumference of the sample to prevent sample dehydration during testing. All experiments were performed in triplicate at 1% strain (within its linear viscoelastic region). Apparent viscosity was sweep at 25 s⁻¹ shear rate for 150s, mean values were calculated and reported. Frequency sweep analyses were performed for a frequency range of 0.1rad s⁻¹ to 100 rad s⁻¹.

2.8 Water soaking test

SMCP and GMCP dispersion were prepared for the water soaking test. In a typical experiment, dispersion was uniformly brushed onto a glass panel and cured at 110 °C for 120 min. After cooling down to room temperature, the protein-covered glass panel was soaked in distilled water for 120 min, followed by 100 °C drying for 30 min. Protein lost was calculated as

$$Protein\ loss = \frac{W_i - W_f}{W_i - W_g} \times 100\% \quad (1)$$

where W_g is the weight of the glass panel, W_i is the weight of the glass panel and protein after 110 °C curing, and W_f is the weight of the glass panel and protein after 100 °C drying.

2.9 Thermal gravimetric analysis

TGA of CPs was conducted with a Perkin-Elmer TGA 7 (Perkin-Elmer, Norwalk, CT, USA) in a nitrogen atmosphere. Approximately 5 mg of ground powder was weighed into a platinum cup and scanned from 45 to 700 °C at a heating rate of 10 °C /min. Derivative thermogravimetry curves. Maximum degradation temperature was calculated at the peak temperature of the derivative thermogravimetry curves.

3. Results and Discussion

3.1 Free sulfhydryl groups

SH groups that are on the surface of protein molecule and readily accessible to reaction with Ellman's reagent are referred as "free SH" groups (Kalapathy and Hettiarachchy, 1996). NaHSO_3 is an agent known to unfold protein's structure by disrupting the intermolecular S-S bond; therefore, increasing SH concentration of SMCP denotes increasing unfolding degree. In Fig.1 (A), it was observed that free SH content of SMCP increased significantly with NaHSO_3 content. Interestingly, free SH content reached 17.20 $\mu\text{mol/g}$ at SMCP-3, and remained stable around 18 $\mu\text{mol/g}$ for SMCP-6, 9, 12, indicating that 3% NaHSO_3 content was able to reduce the available S-S bonds of CPI. Kalapathy and Hettiarachchy (Kalapathy and Hettiarachchy, 1996) reported similar results when they modified SPI using NaHSO_3 . They also observed increasing SH group content of SPI in the presence of NaHSO_3 , and the break of S-S also resulted in reduced viscosity of SPI dispersion. This phenomenon was in agreement with our studies for viscosity, as discussed in Section 3.5. However, compared to the free -SH content of SMCP-9, that of SMCP-12 dropped, possibly due to the shielding effect of excessive HSO_3^- to form the sulfonate group (RS-SO_3^-) (Qi et al., 2012). For GMCP, the content of free SH group also increased with the content of Gdm.Cl, indicating the unfolding had significant effect on camelina protein structure. Unlike the thiol-exchange effect of NaHSO_3 to break protein's disulfide bond, Gdm.Cl was a reagent destroying the hydrogen bond of protein matrix, and then embedded free SH groups became accessible. Thus, Gdm.Cl's reducing effect to disulfide bond was limited compared to NaHSO_3 , and the maximum amount of free SH group of GMCP was obviously less than the unfolded SMCP.

3.2 Surface hydrophobicity

Protein's hydrophobic groups at the side chain of amino acid are mostly buried at folded state, forming a compact hydrophobic core, while the surface was more hydrophilic. Therefore, we used the protein's surface hydrophobicity to describe protein's unfolding degree by detecting ANS binding capacity because ANS is accessible to surface hydrophobic groups and exhibited fluorescence properties. The intensity of ANS binding capacity increased as unfolding hydrophobic groups increased. RFI of SMCP -0, 3, 6, 9 and GMCP -0, 10, 50, 250 were reported.

As expected, the RFI of SMCPs increased as NaHSO_3 content increased. No significant

1 difference was observed between SMCP-3 and CPI, but RFI increased significantly for SMCP -6
2 and SMCP-9. As NaHSO_3 broke more S-S at high concentrations, CPI's structures were
3 destroyed, and buried hydrophobic groups were exposed. This was in agreement with the
4 findings discussed in Section 3.1 regarding free SH groups. Zhang and Sun (Zhang and Sun,
5 2008) obtained similar surface hydrophobicity trend of NaHSO_3 -modified SPI by determining
6 SDS-binding capacity, indicating that NaHSO_3 could unfold SPI and cause the increase of
7 surface hydrophobicity.

8 However, a decrease of the RFI of GMCPs was observed. For GMCPs, no significant
9 difference was observed between CPI and GMCP-10, but RFI decreased at GMCP-50, and
10 increased again at GMCP-250. Many studies have described the “molten globule” state of
11 proteins, thereby explaining the RFI quenching phenomenon for GMCP unfolding progress.
12 Gdm.Cl unfolded the proteins in two ways. With low Gdm.Cl content, the protein turned into a
13 molten globule state with destroyed tertiary structures but stabilized secondary structure with
14 lower hydrophobicity than the folded protein. Proteins under the molten state presented
15 pronounced secondary structure and considerable conformational mobility compared to native
16 and fully unfolding state (Ohgushi and Wada, 1983). With high Gdm.Cl content, the secondary
17 structure unfolded and the hydrophobic groups were exposed (Huang and Sun, 2000; Povarova et
18 al., 2010). Similar denaturing phenomenon on the cotton protein was observed when Gdm.Cl
19 was used as reducing agent (He et al., 2014). Rashid et al. (2005) also found that the quenching
20 phenomenon of Gdm.Cl denatured human placental cystatin (HPC) from the native protein up to
21 1.5M Gdm.Cl for emission fluorescence spectroscopy; proteins in the presence of more than 2M
22 Gdm.Cl exhibited significant increase in surface hydrophobicity, suggesting the breakdown of
23 secondary structures. These findings are consistent with our results suggesting that with low
24 concentration of Gdm.Cl, CPI was unfolded into the molten state with reduced surface
25 hydrophobicity.

27 **3.4 Particle size**

28 The effect of NaHSO_3 and Gdm.Cl on volume-mean diameter of CPI dispersions is shown in
29 Fig.3. (A) (B), with increasing NaHSO_3 , SMCPs decreased from 10.21 μm to 5.97 μm (from CPI

to SMCP-3) and then increased to 21.48um (SMCP-12). The initial decrease of particle volume of SMCP was attributed to the disappearance of S-S. Similar dissociation phenomenon of protein particles was also reported for SPI as S-S-induced conformational rearrangement(Hu et al., 2013). With the accumulation of hydrophobic groups, particle size increase from SMCP-3 to SMCP-6 and SMCP-12 due to hydrophobic interaction (Section 3.2) that connected the protein. For GMCPs, the volume median of GMCPs increased gradually at low Gdm.Cl content (0-10%). However, from GMCP-50 to GMCP-250, the volume median increased significantly from 42.91 um to 159.93 um due to the Gdm.Cl-induced CPI aggregation. This aggregation phenomenon was also 0

As shown in Fig 3. (C) (D), NaHSO₃ and Gdm.Cl had significant effects on particle size distribution of CPI dispersion corresponding to the SMCP and GMCP's volume medium. Non-treated CPI exhibited unimodal particle distribution with a peak appeared around 10 um. Compared to the untreated CPI, single peak of GMCP-5 was right shifting. Then, bimodal curves appeared at 10um and 100um for GMCP-10. Further increase of Gdm.Cl content resulted in unimodal distribution and right-shifting of the peak distribution for GMCP-50, and GMCP-250, indicating their aggregation behaviors. For SMCP, a left-shifting peak was observed for SMCP-3, and then protein particles grew larger at SMCP-6, 9, 12.

3.5 Rheological properties

3.5.1 Viscosity

Viscosity, a significant rheological property, governs flow-ability behavior of protein dispersions and influences their handling, processing, and efficiency for further modification (Sun, 2011). Apparent viscosities of SMCP-0, 1, 3, 6, 9, 12 and GMCP-0, 5, 10, 50, 100, 250 are shown in Fig 4. In general, both SMCPs and GMCPs exhibited lower viscosity than the native CPI. Because S-S and hydrogen bond both help maintain the protein structures, unfolding protein exhibited decreasing with increasing NaHSO₃ content for SMCPs. For GMCPs, the lowest viscosity was 130.53 cp for GMCP-5, and slowly increased to 151.59cp for GMCP-250. Unlike the continuous decreasing viscosity of SMCPs, protein aggregation (Section 3.4) caused the increase of GMCP's viscosities at high Gdm.Cl concentrations (GMCP-50, 100, 250). Furthermore, we observed that GMCPs were less viscous than SMCPs, indicating that hydrogen

bond was more influential than disulfide bond on shearing behaviors in this hydro-protein system.

3.5.2 Frequency sweep

Chemical bonds between CPI molecules may be disrupted during the increasing shearing force. Thus, protein's rheological property varies due to their individual bonding. This variation could be used to evaluate the degree of intermolecular interaction. Fig. 5 shows the effect of Gdm.Cl and NaHSO₃ treatments on the variation of storage modulus (G') for CPI dispersions (10%, w/v). As shown in Fig 8, G' increased linearly at a certain frequency region (0.5-5 rad/s) for all samples, followed by a sharp increase. Similar frequency-modulus files for SPI were reported (Hu et al., 2013; Tunick, 2011).

Tunick (Tunick, 2011) classified protein interaction into three major types: entangled networks (of biopolymers), cross-linked bonding, or physical (non-covalent linkages) bonding. Entangled networks are highly frequency dependent, suggesting weakest bonding. Cross-linking is a strong covalent bond with minimal frequency dependence. Physical bonding refers to non-covalent interactions that are intermediate between strong and weak bonds, including hydrogen bond and hydrophobic interaction. In order to better describe the G' variation of CPI dispersion, we used the following equation to characterize the frequency sweep.

$$\log G' = n \log \omega + K \quad (2)$$

where n and K are constants with n being the degree of frequency dependence (Tunick, 2011).

Parameters of equation (2) were shown in Table 1 by fitting linear curves. The n values for GMCP and SMCP increased at higher NaHSO₃ or Gdm.Cl concentration, denoting the effectiveness of Gdm.Cl and NaHSO₃ in unfolding CPI structures and breaking the protein interaction. However, GMCP-250 was an exception, in which its n value decreased significantly, meaning the protein was less frequency dependent. This may be due to the strong protein aggregation at GMCP-250 during the unfolding process. By comparing n values of SMCPs and GMCPs, we found that SMCPs were generally more frequency dependent than GMCPs, suggesting that NaHSO₃ may be more effective in breaking the protein-protein interaction. Because the covalent S-S bond is stronger than the non-covalent hydrogen bond; therefore,

SMCP's intermolecular interaction were more reduced.

3.6 Water soaking test

Fig. 6 shows the weight loss of SMCP -1, 3, 6, 9, 12 and GMCP-5, 10, 20, 50, 250. Weight loss during the water soaking test for cured SMCPs and GMCPs was primarily governed by the degree of protein interaction (Section 3.5.2). A high conjugation degree caused water-resistant protein, while loose structures stimulated increased protein loss (Qi et al., 2012; Wang et al., 2007). The weight loss of SMCPs and GMCPs increased as NaHSO₃ and Gdm.Cl content increased. At low salt concentration (<10%), a comparison of SMCP and GMCP weight loss revealed that, GMCPs were less dissolved in water than the SMCPs. This result was in agreement with previous protein interaction evaluation by frequency sweep: NaHSO₃ was more effective in unfolding protein structure and breaking their interaction, which resulted in high protein loss at high salt concentration. For SMCPs, excessive additions of NaHSO₃ into the protein system are detrimental to the water resistance of CPI because SH resulting from deoxidization were blocked as a sulfonate group (RS-SO₃⁻) in the presence of excessive HSO₃⁻ during the NaHSO₃ reducing reaction, so the protein became more hydrophilic. Similar results were also reported by Li et al. and Zhang and Sun (Li et al., 2011; Zhang and Sun, 2008) for the investigation of water resistance of NaHSO₃-modified SPI. For GMCPs, the protein loss increased significantly due to electrostatic force and salt-in effect at 50% Gdm.Cl or higher.

3.7 Thermal gravimetric analysis

Fig. 7 shows derivative thermogravimetry (DTG) curves of SMCP-0, 3, 6, 9 and GMCP-0, 10, 50, 250 as a function of temperature from 50 °C to 600 °C. CPI, SMCPs, and GMCPs exhibited their maximum degradation rate in the temperature region from 325 °C to 345 °C. SMCP peaks were overlapping before 340°C, suggesting that NaHSO₃ did not influence CPI's maximum degradation temperature, but it induced more weight loss after 340 °C. Comparatively, high Gdm-HCl concentrations decreased CPI's maximum degradation temperature; GMCP-50 and GMCP-250 peaks were left-shifting. DTG curves of SMCP and GMCP were in agreement with the studies for glutelin fraction of CPI by Li et al. (Li et al., 2014).

3.8 Proposed mechanism to the SMCP and GMCP

The proposed mechanism of NaHSO₃ and Gdm.Cl induced unfolding to CPI were depicted in Fig. 8. NaHSO₃ was a strong reducing agent to cleave the S-S bond between molecules by thiol-disulfide exchange reaction (Gilbert, 1995). Intermolecular S-S bonds of CPI were destroyed and peptide chains became more independent and less compact due to decreasing CPI's secondary and tertiary structures. Hydrophobic groups became exposed and induced CPI aggregation due to hydrophobic interaction; therefore, protein interactions became weaker.

Gdm.Cl unfolded the CPI into a molten state. CPI's tertiary structures were destroyed by formation of a hydrogen bond with -NH₂ from Gdm.Cl instead of an amine group from the peptide chain (Pace and Grimsley, 2005). Then the Gdm.Cl penetrated into the protein matrix, and its globular shape began to inflate. Therefore, particle size of the CPI increased, and CPI's surface hydrophobic decreased. During this progress, CPI's secondary structure was present, but the tertiary and quaternary structures exhibited reduced stability.

4. Conclusions

Both NaHSO₃ and Gdm.Cl treatment had significant effects on CPI's structural and physicochemical properties. NaHSO₃ cleaved the S-S and increased surface hydrophobicity, which was demonstrated by increasing free SH group content and ANS binding capacity. NaHSO₃ resulted in reduced viscosity and protein-protein interaction as well as an increase of particle size (10.21-21.48mm). Gdm.Cl broke the hydrogen bond among molecules but decreased CPI's surface hydrophobicity, leading to reduced viscosity and protein interaction as well as a significant increase of particle size (10.21-159.53 mm). The decreasing in protein interaction resulted in increased protein loss of SMCPs and GMCPs during water soaking. The reducing effect of NaHSO₃ was greater than Gdm.Cl for CPI's intermolecular interaction and less obvious for the viscosity and water resistant. Gdm.Cl reduced the decomposition temperature of CPI, but NaHSO₃ demonstrated no obvious reducing effect on the thermal properties of CPI. This information shed light on the structural conformation and bio-processing of camelina protein.

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Figure captions

- Fig. 1. Effect of NaHSO_3 and Gdm.Cl treatment on the free SH group of CPI dispersions.
- Fig. 2. Effect of NaHSO_3 (A) and Gdm.Cl (B) treatment on the surface hydrophobicity of CPI dispersions.
- Fig. 3. Effect of NaHSO_3 and Gdm.Cl treatment on the volume medium and particle size distributions of CPI dispersions.
- Fig. 4. Effect of NaHSO_3 and Gdm.Cl treatment on the viscosity of CPI dispersions.
- Fig. 5. Effect of NaHSO_3 (A) and Gdm.Cl (B) treatment on the variation of storage modulus of CPI dispersions for the frequency sweep.
- Fig. 6. Effect of NaHSO_3 (A) and Gdm.Cl (B) treatment on the weight loss of 10% cured CPI after water soaking.
- Fig. 7. Derivative thermogravimetry curves of SMCP (A) and GMCP (B).
- Fig. 8. Proposed mechanism for the unfolding process of CPI by NaHSO_3 and Gdm.Cl

1 **Table captions**

2 Table 1.

3 Rheological properties of NaHSO₃ and Gdm.Cl treated CPI dispersions for frequency sweep at 0.1 to 100 rad/s.

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5

6

Fig. 1. Effect of NaHSO₃ (A) and Gdm.Cl (B) treatment on the free SH group of CPI dispersions. Content of NaHSO₃ and Gdm.Cl was on the dry base of CPI.

(A)

(B)

Fig. 2. Effect of NaHSO_3 (A) and Gdm.Cl (B) treatment on the surface hydrophobicity of CPI dispersions. Content of NaHSO_3 and Gdm.Cl was on the dry base of CPI.

(A)

(B)

(C)

(D)

Fig. 3. Effect of NaHSO₃ and Gdm.Cl treatment on the volume medium and particle size distributions of CPI dispersions. (A) Volume medium of SMCP (B) Volume medium of GMCP (C) Particle size distribution of SMCP (D) Particle size distribution of GMCP. Content of NaHSO₃ and Gdm.Cl was on the dry base of CPI.

Fig. 4. Effect of NaHSO_3 and Gdm.Cl treatment on the viscosity of CPI dispersions. (A) Content of NaHSO_3 and Gdm.Cl was on the dry base of CPI

(A)

(B)

Fig. 5. Effect of NaHSO_3 (A) and Gdm.Cl (B) treatment on the variation of storage modulus of CPI dispersions for the frequency sweep. Content of NaHSO_3 and Gdm.Cl was on the dry base of CPI.

(A)

(B)

Fig. 6. Effect of NaHSO₃ (A) and Gdm.Cl (B) treatment on the weight loss of 10% cured CPI after water soaking. Content of NaHSO₃ and Gdm.Cl was on the dry base of CPI.

(A)

(B)

Fig. 7. Derivative thermogravimetry curves of SMCP (A) and GMCP (B). Content of NaHSO_3 and Gdm.Cl was on the dry base of CPI.

○: hydrophobic groups; ●: hydrophilic groups

Fig. 8. Proposed mechanism for the unfolding process of CPI by NaHSO₃ and Gdm.Cl

Table. 1
Rheological properties of NaHSO₃ and Gdm.Cl treated CPI dispersions for frequency sweep at 0.1 to 100 rad/s.

NaHSO ₃ content (%)	Fitting slope	R ²
0	0.15	0.997
1	0.17	0.990
3	0.19	0.973
6	0.26	0.973
9	0.31	0.995
12	0.33	0.987

Guanidine-HCl content (%)	Fitting slope	R ²
0	0.15	0.997
2	0.18	0.994
5	0.22	0.978
10	0.24	0.989
50	0.29	0.980
250	0.14	0.963

Abbreviations:

- DCM: Defatted camelina meal
- CPI: Camelina protein isolate
- NaHSO₃: Sodium bisulfite
- Gdm.Cl: Guanidine- hydrochloride
- SMCP: NaHSO₃-modified camelina protein
- GMCP: Gdm- HCl-modified camelina protein
- DTNB (5,5'-dithiobis-(2-nitrobenzoic acid))
- ANS: 8-Anilinonaphthalene-1-sulfonic acid
- RFI: Relative fluorescence intensity
- S-S: Disulfide bond
- SH: free sulfhydryl group
- DTG: Derivative thermogravimetry curves

Highlight:

We unfolded camelina protein isolate by two reagents: NaHSO_3 (cleave S-S) and guanidine-HCl (destroy hydrogen bond).

Both treatment decreased camelina protein isolate dispersion's viscosity and protein-protein interaction.

NaHSO_3 modified camelina protein isolate was more hydrophobic than guanidine-HCl modified camelina protein.

NaHSO_3 was more efficient in destroying the structure of camelina protein isolate, while Gdm.Cl treatment reduced more viscosity and water resistant.