Effect of High-Hydrostatic Pressure and Temperature on Rheological Characteristics of Glycomacropeptide

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ABSTRACT

The influences of high pressure and temperature on the rheological characteristics of glycomacropeptide (GMP) were studied using a controlled rate rheometer. GMP dispersions at a concentration of 12.5% (w/w) were subjected to high pressure from 100 to 400 MPa for 30 min and temperature from 20 to 80°C for 15 min followed by rheological measurements at a shear rate ranged between 0 and 200 s⁻¹. Shear stress-shear rate data of both pressure and heat induced GMP samples fitted Herschel-Bulkley model well with yield stress. It exhibited shear-thinning behavior with flow behavior index ranged between 0.882 and 0.996. Consistency coefficient and apparent viscosity increased with pressure up to 300 MPa while those parameters decreased at 400 MPa. The rheology of GMP was influenced by temperature. The consistency coefficient and apparent viscosity at 100 s⁻¹ obeyed the Arrhenius relationship with activation energies ranged between 8.17 to 12.38 kJ/mol. Lower activation energy signified lesser molecular aggregation or unfolding of protein molecules during thermal treatment of GMP.

(Key words: consistency coefficient, flow behavior index, glycomacropeptide, high pressure and temperature)

Abbreviation key: GMP = glycomacropeptides, HPP = high hydrostatic pressure processing, K = consistency coefficient, n = flow behavior index.

INTRODUCTION

High-hydrostatic pressure processing (HPP) has been reported a promising area for various foods applications and will now deliver the ultimate convenience for consumers (Meyer et al., 2000; Sizer et al., 2002).

Various researchers have reported the applications of HPP in different areas of food processing like inactivation of microorganisms and enzymes (Alderton et al., 1976; Oxen and Knorr, 1993; Arroyo et al., 1997; Berlin et al., 1999; Mussa et al., 1999; Denys et al., 2000), denaturation and functionality of proteins (Hayakawa et al., 1992; Knorr, 1995; Lee et al., 1999) structure change of food materials (Van Camp and Huyghebaert, 1995; Hosseini-nia et al., 2002). The major researches on HPP have been focused on food protein and its functional properties, modification and gel rigidity and water holding capacity (Johnston et al., 1993). Such studies have involved in milk proteins, polysaccharides and combinations of them.

Knowledge of the rheological properties of food products is essential for the product development, quality control, sensory evaluation and design and evaluation of the process equipment. Rheological measurements have also been considered as an analytical tool to provide fundamental insights on the structural organization of food and play an important role in heat transfer to fluid foods. The flow behavior of a fluid can range from Newtonian to time dependent non-Newtonian in nature depending on its origin, composition and structure behavior and previous history (Rao, 1986). The role of protein structure on rheology of emulsion and gel is a complex one and fundamental rheological tests provide critical information on time dependent viscoelastic behavior and the molecular mechanisms surrounding the changes in structure when a protein undergoes gelation (Phillips et al. 1994). Food proteins and milk products are compositionally and structurally complex materials and can exhibit a wide range of rheological properties at different conditions. The rheological properties of these products are strongly influenced by temperature, concentration and physical state of dispersion (van Vliet and Walstra, 1980). Therefore, it is interesting to study the rheological properties of proteinous and dairy products as function of temperature and high pressure.

Glycomacropeptide (GMP) is a highly biologically active whey protein with superior purity and is considered by some to be a designer protein. It is one of the im-

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portant minor proteins present in whey along with lactoperoxidase, lactoferrin and protease peptones (Belem et al., 1999). GMP present in cheese whey is a C-terminal glycopeptide released from κ -casein by the action of chymosin at Phe-105 and Met-106 (Eigel et al., 1984). It is a unique peptide composed of a chain of 64 amino acids with a molecular weight of 6700 daltons. Researchers have identified five different heterogeneous sugar chains related to GMP derived from mature bovine milk. The most prominent of these is N-acetylneuraminic acid, commonly known as sialic acid. GMP has a purity level of over 90% and is also highly glycosylated with 7 to 8% sialic acid. The peptide has no aromatic amino acids (phenyl alanine, tyrosine, tryptophan) while rich in branched-chain amino acids (leucine, isoleucine, and valine) (Eigel et al., 1984).

The GMP is available in a light colored, mild tasting, free flowing powder ideal for usage in both functional foods and dietary supplements. It has several health benefits like dental caries and plaque reduction; immuno-modulatory effects and affords a passive defense mechanism to newborns (Broody, 2000). GMP is useful in the treatment of phenylketonuria and considered as an ideal ingredient in nutritional formulations for people suffering from hepatic diseases (Eryck et al., 2002). Keeping in view of all these health benefits, presently the health food manufacturers have extensively used GMP as an ingredient for their products however; no report is available on the rheological characteristics of GMP.

Therefore the objective of this work was to provide a study of rheological changes of GMP upon exposure to high pressures from 100 to 400 MPa for 30 min and temperature from 20 to 80°C for 15 min.

MATERIALS AND METHODS

Materials

The GMP samples were provided by Davisco Food International (MN, USA). As per manufacturer's analysis, glycomacropeptides contains $6 \pm 0.2\%$ moisture, $12.2 \pm 0.2\%$ total nitrogen, $85 \pm 1.5\%$ GMP ($N \times 6.94$), fat 0.6 ± 0.2 , ash $6.3 \pm 0.2\%$ and lactose $<1\%$. The purity of the GMP has been reported as 97 ± 1 and contains $8 \pm 0.4\%$ sialic acid.

Sample Preparation

Samples of 12.5% GMP solutions were prepared (w/w) by addition of required amount GMP and deionized water (conductance: 18 Ω , Milli-Q, Millipore, Bedford, USA) under stirring and kept for 6 h at refrigerated temperature (4 to 6°C) for hydration. The concentration was selected to avoid gel formation. Each sample was

prepared in duplicate. The pH of the GMP solution was 7.01.

High Hydrostatic Pressure Treatment

An isostatic high-pressure machine unit (Model# CIP 42260, ABB Autoclave System, Columbus, OH, USA) with chamber dimension of 0.55 m height and 0.1 m diameter was used to generate high-pressure levels. Distilled water containing 2% water soluble oil (Part No. 5019, Autoclave Engineers, Columbus, OH, USA) was used as the pressure medium for pressurization. A smooth pressure rise of 2.4 MPa s^{-1} after an initial delay of 15 s occurred during pressurization. The come-up time for pressurization ranged from 33 s to 2.8 min depending upon the pressure level, and the depressurization time was ~ 10 s. The temperature of the sample was controlled by circulating cold water. The medium temperature was recorded by a thermocouple (K-type) and a data logger during the experimentation.

The test pouches were submerged in water for the pressurization. The pressure applied to the test sample ranged between 100 and 400 MPa with an increase of 100 MPa for a residence time of 30 min at $20 (\pm 1^\circ\text{C})$. Pressure treatment time mentioned in the study did not consider the pressure build up or releasing time. Duplicate samples were used for each pressure treatment. The pressure treated pouches were immediately transferred to refrigerator (4 to 6°C) and subsequently studied the rheology.

Rheological Measurement

Rheological measurements were carried out in a controlled stress rheometer (AR 2000, TA Instruments, New Castle, DE, USA) with attached computer software (Rheology Advantage Data Analysis Program, TA). A double concentric cylinder (stator outer radius 20 mm; rotor inner radius 20.38 mm; rotor outer radius 21.96 mm; immersed height 59.5 mm and gap 500 microns) geometry was used for rheological measurement. The AR 2000 Concentric Cylinder System is based on efficient peltier temperature control and temperature was efficiently monitored during the experiments.

For each test, the measured volume of sample (approximately 6.48 ml) was transferred to sample compartments. The instrument was programmed for set temperature and equilibration for 10 min followed by two-cycle shear changes from 0 to 200 s^{-1} in 5 min and by back to 0 s^{-1} in next 5 min. All the rheological parameters were obtained from the software (Rheology Advantage, TA version 2.3). In order to perform a quantitative comparison of temperature and pressure treated samples various rheological flow models based

Table 1. Fitting of various flow models for GMP at 100 MPa for 30 min.

No.	Model	Yield (Pa)	Consistency coefficient (Pa·s ⁿ)	Flow behavior index (–)	Standard error
1	Newtonian model	–	0.0025 ¹	–	22.17
2	Power law model	–	0.0042	0.903	8.21
3	Casson model	0.0004	8.6 E-6	–	59.16
4	Bingham model	0.0208	0.0024	–	8.81
5	Herschel Bulkley model	0.0055	0.0031	0.927	7.11

¹Apparent viscosity, Pa·s.

on shear stress-shear rate were tested (Newton, Bingham, Casson, power law, Herschel Bulkley) and the best fit model was selected on the basis of standard error, which is defined as:

$$[\Sigma (X_m - X_c)^2 / (n - 2)]^{0.5} / \text{Range} \times 1000 \quad (1)$$

Where X_m is the measured value; X_c is the calculated value; n is the number of data points and range is the maximum value of X_m – the minimum value.

Statistical Analysis

Statistical analysis was carried out as per method described by Gacula and Singh (1994). Trends were considered significant while means of compared sets differed at $P < 0.05$ (Student's *t*-test).

RESULTS AND DISCUSSION

Flow Models

Shear stress-shear rate data of the heat and pressurized GMP samples were tested for various rheological models (Newtonian, Casson, Bingham, power and Herschel-Bulkley), and it was found that Herschel Bulkley model was fitted adequately (Table 1). The Herschel Bulkley model is represented as:

$$\tau = \tau_0 + K(\dot{\gamma})^n \quad (2)$$

Where τ is the shear stress (Pa), τ_0 is the yield stress, $\dot{\gamma}$ is the shear rate (s^{–1}), K is the consistency coefficient (Pa·s ^{n}), and n is the flow behavior index (dimensionless). The GMP samples exhibited definite yield stress. No significant ($P < 0.05$) variations in the up and downward curves of GMP samples were observed during heat or pressure treatment and therefore, the fluid were nonthixotropic. The average rheological parameter values of up and down curves are reported in the present paper.

Effect of High Pressure on Rheological Characteristics of GMP

The rheogram for pressure treated samples of GMP are presented in Figure 1. The control sample was considered at atmospheric pressure and 20°C. Shear stress increased with pressure at a constant shear rate up to 300 MPa and decreased at 400 MPa (Figure 1). The rheological parameters of pressure treated GMP samples are reported in Table 2. The yield stress increased with the increase of the pressure from 200 to 300 MPa and followed by decreasing at 400 MPa. The magnitude of the consistency coefficient (K) of control sample was found to be 0.0027 Pa·s ^{n} that consistently increased to 0.0033 Pa·s ^{n} during pressurization except at 400 MPa (Table 2). The flow behavior index (n) ranged between 0.927 and 0.966. The observed n values indicated that the GMP solution exhibited pseudoplasticity while no systematic trend was noticed for the same. The trend of apparent viscosity was similar to consistency coefficient, which increased linearly with pressure level. The magnitudes of consistency coefficient, apparent viscosity and flow behavior index decreased at 400 MPa. However, the changes in the magnitude of rheological parameters during pressurization were insignificant ($P > 0.05$). The decrease in rheological parameters at 400 MPa might be attributed by slight deformation of κ -CN bonds and change of orientation. The energy generation

Figure 1. Effect of pressure on rheology of glycomacropeptide.

Table 2. Effect of pressure on rheological parameters of GMP.

Pressure (MPa)	Yield stress (Pa)	Consistency coefficient (K), Pa·s ⁿ	Flow behavior index (n)	Standard error
0	0.0109	0.0027	0.966	6.043
100	0.0055	0.0031	0.927	7.105
200	0.0018	0.0032	0.955	5.919
300	0.0132	0.0033	0.961	7.668
400	0.0084	0.0031	0.940	5.851

from high-pressure is only 9.6 kJ/g mol per 10⁴ MPa and it could not completely disrupt those bonds (Haya-kawa et al. 1992). It supports the lower texture degradation during high pressure. The coagulation of protein observed at pressure level of 400 MPa, which was reflected through decrease in magnitudes of shear stress, consistency coefficient and apparent viscosity. Lopez et al. (1996) reported that denaturation of protein was expected at above 300 MPa while the pressure level above 200 MPa was sufficient for β -lactoglobulin. In the present study, the protein denaturation initiated at 300 MPa.

Effect of Temperature on Rheological Characteristics of GMP

Temperature has an important role on rheological characteristics of any food products. The effect of temperature on rheological characteristics of GMP is shown in Figure 2. It is evident from the Figure that an increase in temperature reduced the shear stress at a constant shear rate. The rheological parameters of GMP during heat treatment are reported in Table 3. The yield stress, consistency coefficient and flow behavior index decreased significantly ($P < 0.05$) during thermal treatment. However, the slight increase in magnitude of K at 80°C indicated phase transformation of GMP solution to gel. A unique trend was noticed for

flow behavior index. The magnitude of n decreased from 0.966 to 0.882 during heat treatment from 20 to 80°C. Van Camp and Huyghebaert (1995) advocated that the heat induced protein structure (gel) was strong with significant loss of water and dry in appearance. They also observed higher levels of cross-links in heat-induced gels through electron microscopy. These could be the possible reasons for the decrease in the values of n and K at higher temperatures.

Temperature dependency of consistency coefficient and apparent viscosity at constant shear rate (100 s⁻¹) is expressed by Arrhenius relationship (Equations 3 and 4):

$$K = A_K \exp(E_K/RT) \quad (3)$$

$$\eta = A_\eta \exp(E_\eta/RT) \quad (4)$$

Where K is the consistency coefficient, η is the apparent viscosity at shear rate of 100 s⁻¹, A_K , A_η are the pre-exponential constants; E is the activation energy; R is the universal gas constant and T is absolute temperature.

Figure 3 shows the temperature dependence of consistency coefficient and apparent viscosity of GMP. The coefficients of Equations 3 and 4 were computed using the least square technique. Magnitudes of the energy of activation relating to consistency coefficient and apparent viscosity ranged between 8.17 and 12.38 kJ/g mol respectively while the corresponding magnitudes of constants (A_K and A_η) were 9.57×10^{-5} Pa and 1.52×10^{-5} Pa·s respectively. The R^2 for both cases were greater than 0.976 while standard error were less than 0.052. These values indicated that both K and η follow Arrhenius model. There was significant difference in activation energies. This difference is due to a single value obtained from the instrument (η at 100 s⁻¹) and another from the entire experimental data (K value). The obtained activation energy for GMP was too low compared to other whey protein components. The E values were found to be very high for α -lactalbumin, β -lactoglobulin and lactoferrin (136-280 kJ/g mol) where cleavage and denaturation of proteins have been re-

Figure 2. Effect of temperature on rheology of glycomacropeptides.

Table 3. Effect of temperature on rheological parameters of GMP at atmospheric pressure.

Temperature (°C)	Yield stress (Pa)	Consistency coefficient (K), Pa·s ⁿ	Flow behavior index (n)	Standard error
20	0.0109	0.0027	0.966	6.043
40	0.0047	0.0020	0.950	8.041
60	0.0018	0.0015	0.933	8.016
80	0.0012	0.0016	0.882	12.27

ported at higher temperature (Sanchez et al. 1992). The predicted E value of GMP indicated that a minimum number of bonds were unfolded and denaturation of protein had not been completely occurred at a temperature range of 20–80°C.

CONCLUSIONS

The rheological characteristics of GMP samples studied without additional heat supply after high pressure treatment at 100 to 400 MPa for 30 min at 20°C. The rheological characteristics were also investigated for temperature range between 20 and 80°C for 15 min at atmospheric pressure. Both pressure and heat treated samples followed Herschel-Bulkley model and exhibited shear-thinning behavior. Samples pressurized at 400 MPa and thermally treated at 80°C behaved differently from those corresponding pressure and temperature treated samples. The observations indicated that it could be due to cleavage of κ -CN bonds during high pressure (400 MPa) and high temperature (80°C). However, heat induced protein structure was strong with significant loss of water while high pressure formed a weak network without much alteration on κ -CN bonds. In order to obtain specific gel characteristics and their relative contribution to the rheological characteristics, it might be of interest to study the infrared and electron microscopy of GMP during pressure and heat treatment.

Figure 3. Arrhenius plot depicting the temperature dependence of consistency coefficient and apparent viscosity of GMP.

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