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Flow behaviour and temperature stability of chicken muscle proteins – modified waxy corn starch blends

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Abstract: Chicken breast muscle powder (CBMP) was treated as a function of heating temperature, heating time and amount of alkali added. The pre-treated CBMP was then blended with modified waxy corn starch (MWCS) and characterized by flow analysis and temperature sweep. Flow analysis revealed that the blend behaved as a shear thickening and time dependent fluid with a yield stress. Statistical analysis showed that only linear and quadratic effects of heating temperature and heating time caused significant effects on flow behaviour index, consistency index and yield stress ($p<0.05$). Sample flow behaviour was found to change from rheopectic to thixotropy with increasing amount of alkali used. On the other hand, sample prepared by blending mildly treated CBMP and MWCS was thermally stable, where the G' was recorded to be independent of heating and cooling cycle from 5-95^{o}C and 95-5^{o}C.

Keywords: Chicken protein, modified waxy cornstarch, flow behaviour, temperature sweep

Introduction

Proteins and polysaccharides are widely used in foods to modify food texture or functional properties. By understanding the structure-functionality relationship of a blend system, one could develop fabricated foods based on consideration of the underlying science rather than on trial-and-error manipulation of food ingredients (Hirose, 1993).

A comprehensive review on protein-polysaccharide interactions have been covered in many review papers such as those reported in Ledward (1993), Doublier *et al.* (2000), Nishinari *et al*. (2000) and Turgeon et al. (2003). The behaviour and structure of the mixture are affected by many factors, for example mixing ratio, solvent quality, ionic strength, total concentration, molecular weight, charge density, pH, molecular conformation, process treatments and others.

In a mixed biopolymer system of protein and polysaccharide, it is reported that two phase separation phenomena can be observed (Doublier *et al*., 2000), namely segregative phase separation and associative phase separation. The former is evident when a net repulsion between the biopolymers occurs due to thermodynamic incompatibility whilst the latter happens when both polymers carry an opposite charge. Phase separation may lead to different desired or non-desired properties that directly modify the quality and stability of the food containing thereof. There are research works (Syrbe *et al*., 1998; Bryant and McClement, 2000; Turgeon and Beaulieu, 2001) reported that whey proteins and several polysaccharides, such as dextran, methylcellulose, xanthan or k-carrageenan, were incompatible thermodynamically in aqueous solutions, which resulted in phase separation under certain conditions of pH, temperature and ionic strength.

With increasing demands for detailed knowledge of the role of protein-polysaccharide interactions, in relation to their functionality, more studies on mixed biopolymers should be encouraged. In this study, a pre-treated chicken breast muscle protein (CBMP) was blended with modified waxy corn starch (MWCS) and characterized by flow analysis and temperature sweep. The MWCS used was crosslinked-hydroxypropylated waxy corn starch. It was used for it provides a paste that resist to thinning at low pH and high shear. Characteristics of the CBMP and MWCS used have been reported in our previous publication (Zhang *et al*., 2008).

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Materials and Methods

Materials

Commercial breast muscle was boiled at 80°C and oven dried at 90°C for 2 hours. The sample were ground and sieved through a 150 µm sieve. The chicken breast meat powder (CBMP) was stored in screw-capped glass bottle at 4°C. Modified waxy cornstarch (MWCS) and sodium hydroxide (NaOH) were obtained from Sim Company (Penang, Malaysia).

CBMP morphology

The surface morphology of chicken breast muscle aggregates (CBMP) was observed using a scanning electron microscope (Leo Supra 50 VP Field Emission SEM) after treatment. An amount of 3 g CBMP was heated at different temperatures (80, 90 and 100°C for 10 min) in 40 ml 0.75% (w/v) NaOH , after which the pH of the dispersion was adjusted to 7.0 with 3% citric acid. The dispersion was then centrifuged at 15000 x *g* for 30 min and an aliquot of the supernatant was dialyzed in distilled water overnight. Proteins in the dialyzed sample were extracted with 3 parts acetone and lyophilized.

Sample preparation

A response surface methodology of a three-level factorial design was adopted. Where required, 3 g of CBMP was treated with different volume of 0.75% (w/v) NaOH (40, 50 or 60 ml) at 80, 90 or 100°C and hold for 1, 5, or 10 min. After cooling to room temperature, samples were adjusted to pH 7 with 3% (w/v) citric acid before 4% MWCS was added and made up to 100.0 g with water. The suspension was subsequently heated to 90°C for 20 min and kept at 4°C overnight before analysis.

Rheological measurements

The rheological behavior of CBMP-MWCS paste prepared was characterized using continuous flow ramp and oscillatory temperature sweep. All measurements were carried out with a controlled stress AR 1000 Rheometer (TA Instruments, New Castle, Del., U.S.A.) equipped with a Peltier Temperature Controller.

Flow analysis

Continuous flow curves were obtained by recording shear stress values when samples were subjected to a programmed shear rate increased linearly from 0 to 90 per second and decreased linearly from 900 to 0 s^{-1} in 3 min, respectively. All measurements were carried out at 25°C using a parallel plate geometry (60 mm diameter and 0.5 mm gap).

Temperature sweep

Temperature sweeps were run over the range from 5 to 95°C at a heating rate of 5°C/min with constant strain amplitude of 4% determined from the linear viscoelastic region of the samples at 1 Hz. Samples were equilibrated at 95°C for 5 min before being cooled to 5°C at 5°C/min. Silicon oil was applied to avoid dehydration during heating.

Experimetnal design and statistical analysis

A three-level factorial response surface design was constructed using Design-Expert Version 5.0.7 (Minneapolis, U.S.A.). The independent variables studied were heating temperature (X_1), heating time (X_2) and amount of alkali used (X_3). The heating temperature level studied varied between 80 and 100°C, the heating time between 1 and 10 min, and the amount of alkali used between 40 and 60 ml of 0.75% NaOH. The experimental data was fitted to a second-order polynomial model as follows:

$$Y = \beta_o + \sum \beta_i X_i + \sum \beta_i X_i^2 + \sum \beta_j X_i X_j$$

Where Y is the response calculated, β_o is the constant coefficient, β_i is the linear coefficient, β_{ii} is the quadratic coefficient, β_{ij} is the cross product coefficient, and X_i and X_j are the independent variables studied. The model adequacy check was performed at a 5% level of significance.

Results

Morphology of CBMP

Figure 1 shows the scanning electron micrographs of chicken breast muscle protein (CBMP) aggregates recovered with acetone after being treated with 0.75 % (w/v) NaOH at 80, 90, and 100°C for 10 minutes. At a relatively mild treatment condition (80°C and 10 min), it is noted that the denatured CBMP tends to aggregate into a globular shape. However, as the denaturing temperature was increased to 90°C, CBMP failed to aggregate into globular shape but take on a paste-like morphology. When CBMP was heated to 100°C, the paste formed was much more compact.

Flow analysis of CBMP-MWCS blend

Experimental design matrix performed is shown in Table 1. To perform a quantitative comparison of the samples, the flow data of the upward curve were fitted to some mathematical equations or models. The best fitted model reported with an R^2 greater than 0.96

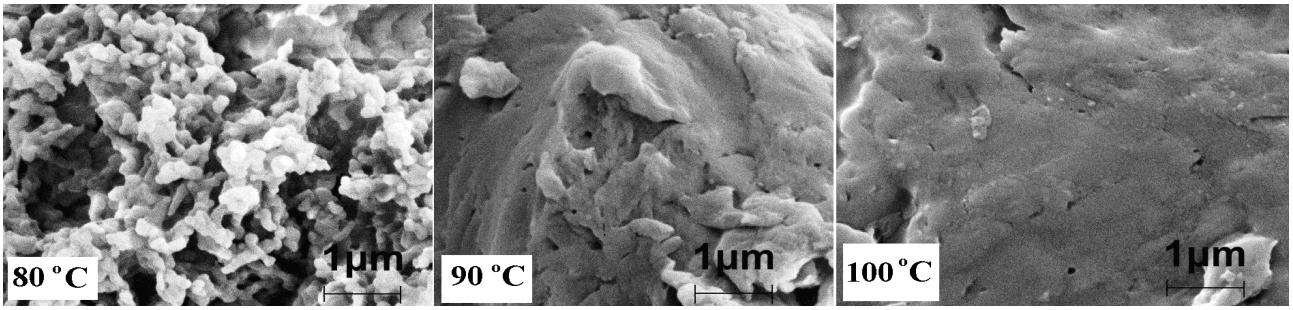

Figure 1. Scanning electron micrographs of lyophilized CBMP after being treated with 40 ml of 0.75% NaOH at different temperature for 10 minutes and extracted with acetone

Table 1. Experimental design matrix

Run Order	Independent variables		
	Temperature (°C)	Time (min)	Amount of 0.75% NaOH (ml)
	X_1	X_2	X_3
1	80	1	40
2	100	10	60
3	100	5	50
4	100	10	40
5	100	5	40
6	90	10	60
7	80	1	50
8	90	5	60
9	90	1	60
10	90	1	50
11	80	5	40
12	80	10	60
13	80	10	40
14	90	5	50
15	90	1	40
16	80	5	60
17	90	5	50
18	90	10	40
19	90	5	50
20	90	5	40
21	100	5	60
22	90	10	50
23	90	5	50
24	80	5	50
25	90	5	50
26	100	1	50
27	80	1	60
28	80	10	50
29	100	1	60
30	90	5	50
31	100	10	50
32	100	1	40

was Herschel-Bulkely model:

$$\sigma = \sigma_o + K\dot{\gamma}^n$$

where σ is the shear stress in (Pa), σ_o is yield stress (Pa), K is consistency coefficient (Pa s), $\dot{\gamma}$ is the shear strain in (1/s). and n is flow behaviour index (dimensionless). Thixotropy (Pa/s) values were calculated from the difference in area between upward and downward curves, i.e. the hysteresis surface area. Thixotropy is referred to a material flow that is shear thinning and time dependent (Mewis and Wagner, 2009).

Figure 2 through Figure 5 show that the flow behaviour index, consistency index, yield stress and thixotropy values of the blends ranged between 0.55 and 1.10; 0.15 and 1.65 Pa.s; 1.77 and 43.44 Pa; and -38130 and 5415 Pa/s, respectively.

Table 2 shows the analysis of variance of the regression parameters of the response surface quadratic models for various independent variables. The model presented showed R^2 values varied between 0.84 and 0.91 and coefficient of variance (CV) between 10.66 and 15.00.

The statistical analysis reveals that only the linear and quadratic effects of treatment temperature and the interaction between treatment temperature and treatment time caused significant effects on flow behaviour index, consistency index and yield stress ($p<0.05$) of the CBMP-MWCS blends. It is noted that when CBMP was treated with increasing treatment temperature and treatment time, the treated CBMP will produce a blend with lower flow behaviour index and yield stress, but higher consistency index when blended with MWCS.

However, as shown in Figure 2 and Figure 4, as treatment temperature and treatment time was increased from 80°C and 1 min to 90°C and 5 min respectively, the flow behaviour index and yield

Table 2. Regression coefficient and ANOVA of regression parameters of the response surface quadratic models

Regression coefficient	Flow behaviour index	Consistency index (Pa S)	Yield stress (Pa)	Thixotropy x 10^{-3} (Pa/s)
β_o	0.870	0.520	25.010	-12.656
β_1	-0.110***	0.220**	-6.800**	-2.567ns
β_2	-0.022ns	-0.004ns	0.570ns	0.586ns
β_3	-0.027ns	0.042ns	-1.420ns	3.269*
β_{11}	-0.110*	0.340**	-8.070*	-3.775ns
β_{22}	0.002ns	-0.015ns	0.340ns	-0.597ns
β_{33}	0.020ns	-0.015ns	0.660ns	5.629*
β_{12}	-0.077*	0.23*	-7.870**	5.438*
β_{13}	-0.048ns	0.12ns	-5.060ns	8.724**
β_{23}	-0.013ns	-0.019ns	-1.900ns	3.101ns
Lack of fit (p)	0.0388a	0.0769a	0.0124a	0.0482a
R^2	0.91	0.87	0.89	0.84
CV	10.66	12.0	15.0	11.14

βi : The regression coefficient for the main effects; βii : The regression coefficient for the quadratic effects; βij : The regression coefficient for the interaction effects. 1: Heating temperature; 2: Heating time; 3: amount of alkali used.

* Significant at p< 0.05; ** Significant at p< 0.01; *** Significant at p< 0.001;ns Not significant.
a Not significant (p>0.01)

stress observed increased to a maximum, after which the values decreased as treatment temperature and treatment time were further increased. The reverse was true for consistency index. Nevertheless, before the maximum or minimum was reached, the effect of treatment temperature and treatment time was marginal.

As for thixotropy values (Table 2), only the linear (β_3 = 3.269) and quadratic (β_{33} = 5.629) effects of amount of alkali added, and the interactive effects between treatment temperature and treatment time (β_{12} = 5.438), and treatment temperature and amount of alkali (β_{13} = 8.724) used were showing significant (p<0.05) positive effect. As displayed in Figure 5, when little amount (40ml) of 0.75% (w/v) NaOH was used, the blends showed antithixotropy properties. The anitithixotropy-ness is defined when flow causes a reversible, time dependent increase in viscosity (Mewis and Wagner, 2009). It was found to increase with higher heating temperature and lower heating time. However, when the amount of alkali increased from 40 ml to 60 ml, a drastic change in flow behaviour from antithixotropy to thixotropy was evident as treatment temperature and treatment time was increased.

The effect of treatment alkali concentration on the CBMP-MWCS blend flow behaviour is exemplified in Figure 6. It is clearly seen that at low alkali concentration treatment, CBMP-MWCS blend exhibits shear thickening properties, where apparent viscosity increased with increasing shear rate. In addition, we can see the time-dependent behaviour from the hysteresis loop, where the upward-curve and downward-curve are not super imposable. For this sample, viscosity increases with time when shear is applied and a better network will form after resting from shearing. However, such time dependence in recovering as indicated by the loop area was found diminished as the alkali concentration was increased to 50 ml NaOH. It is interesting to note that at extreme condition (60 ml NaOH), a different flow behaviour was registered. CBMP-MWCS blend was found exhibiting time dependent shear thinning properties with the apparent viscosity decreased with increasing shear rate.

Temperature sweep of CBMP-MWCS blends

Figure 7 shows the logarithm of storage modulus (G') of alkali- and heat-treated CBMP-MWCS samples as a function of heating temperature (5 -95°C). This test is sensitive to physical structure and chemical composition changes of a sample. As displayed in Figure 7(a), CBMP-MWCS samples were relatively more stable towards processing as G' was found to be independent of temperature. These samples were prepared by blending MWCS with CBMP pre-treated with 40 ml NaOH at a treatment temperature ranged from 80 to 100°C for 5 min. The sample thermal stability remained in the other two groups of sample (50ml an 60 ml NaOH) except those prepared by blending MWCS with CBMP pre-treated at 100°C. At this extreme heating temperature, CBMP molecules would have been degraded to a lower molecular weight fractions, hence forming

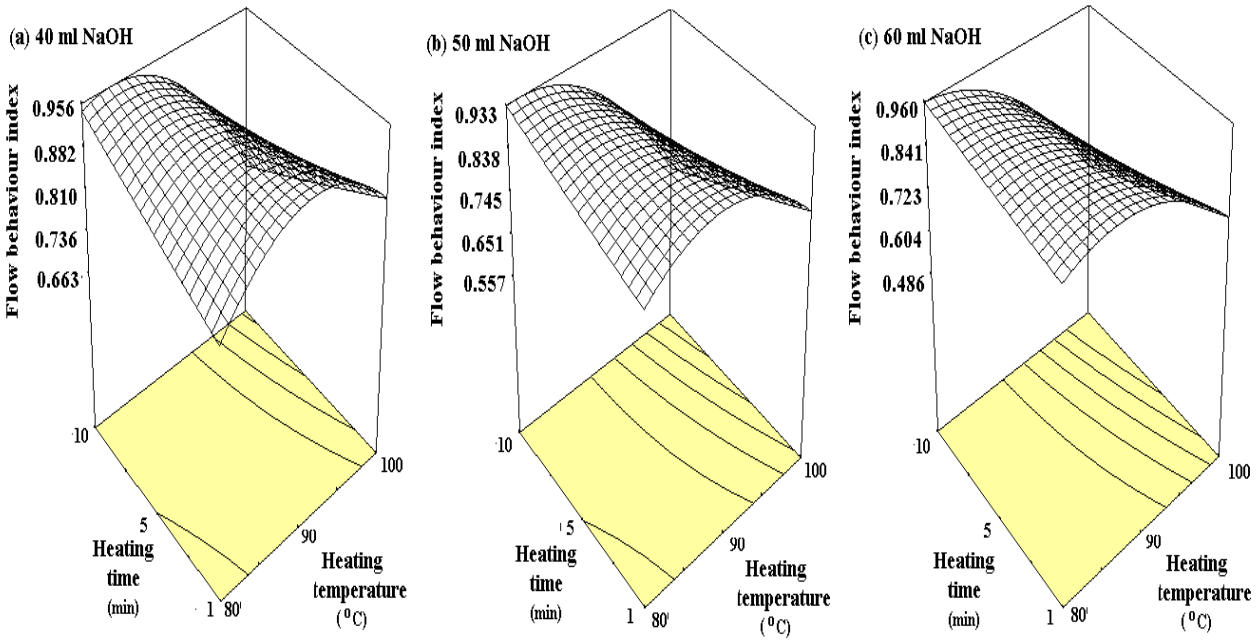

Figure 2. Flow behaviour index of CBMP-MWCS blends as a function of heating time, heating temperature and level of alkali modification

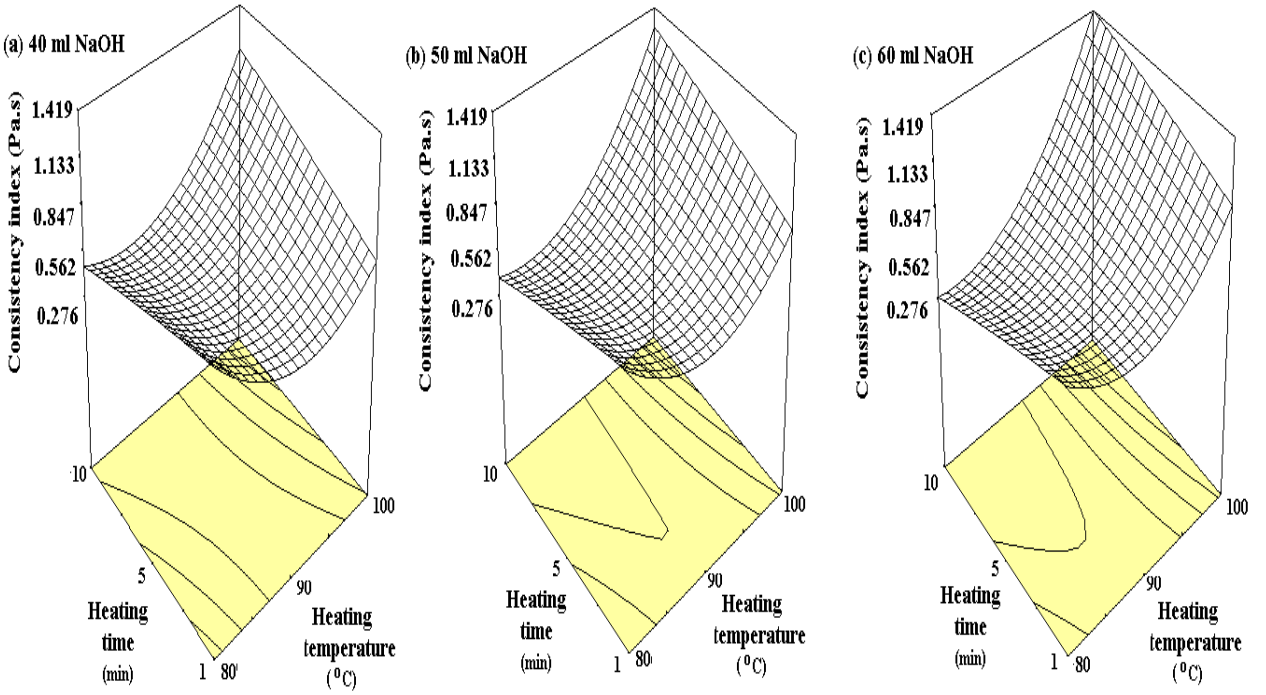

Figure 3. Consistency index of CBMP-MWCS blends as a function of heating time, heating temperature and level of alkali modification

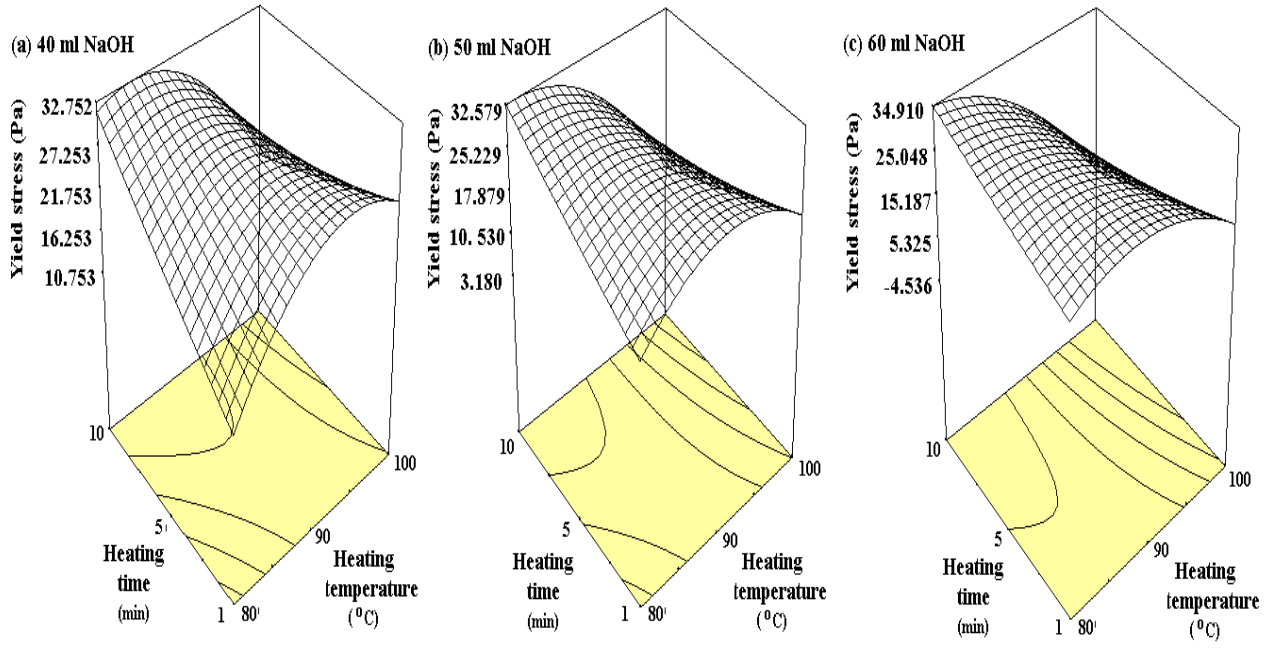

Figure 4. Yield stress of CBMP-MWCS blends as a function of heating time, heating temperature and level of alkali modification

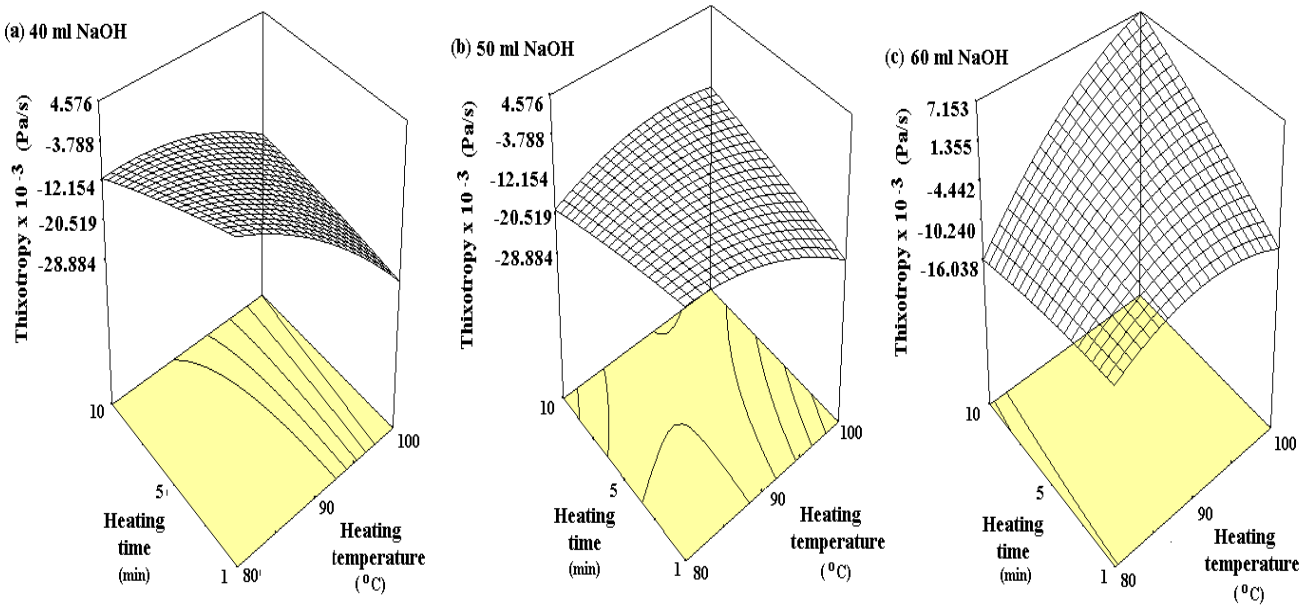

Figure 5. Thixotropy of CBMP-MWCS blends as a function of heating time, heating temperature and level of alkali modification

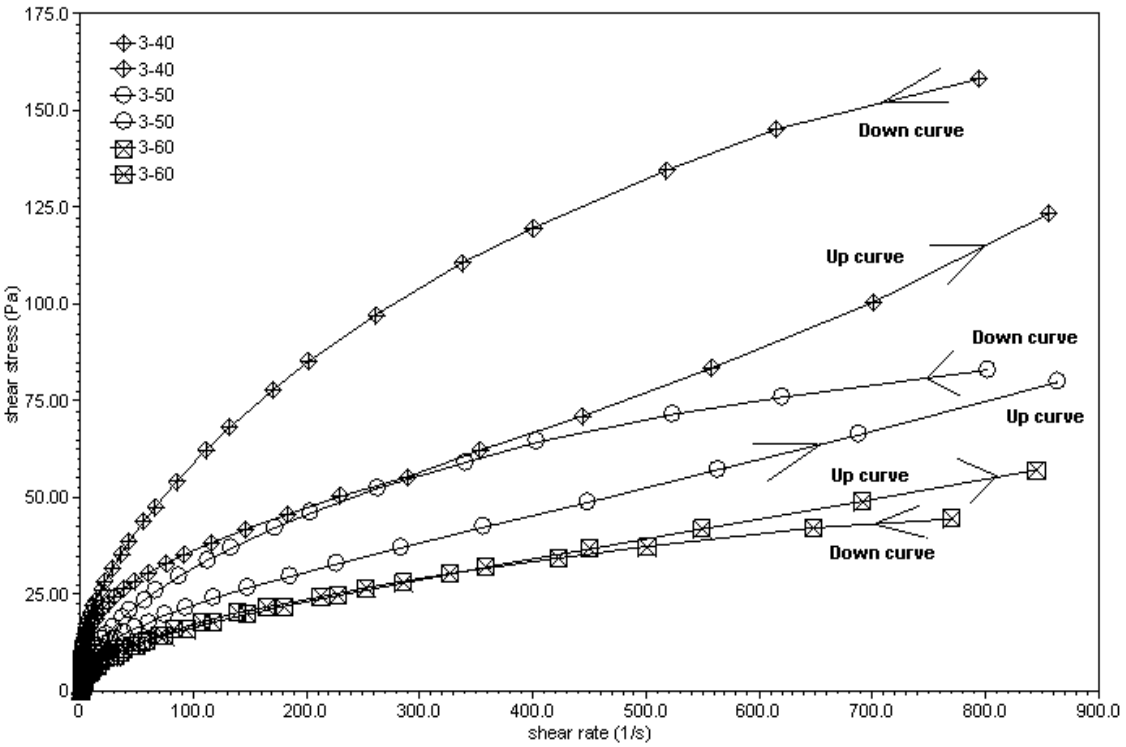

Figure 6. Typical flow curves of CBMP-MWCS samples treated with different amount of alkali (40 ml, 50 ml, and 60 ml) at 100°C for 10 min

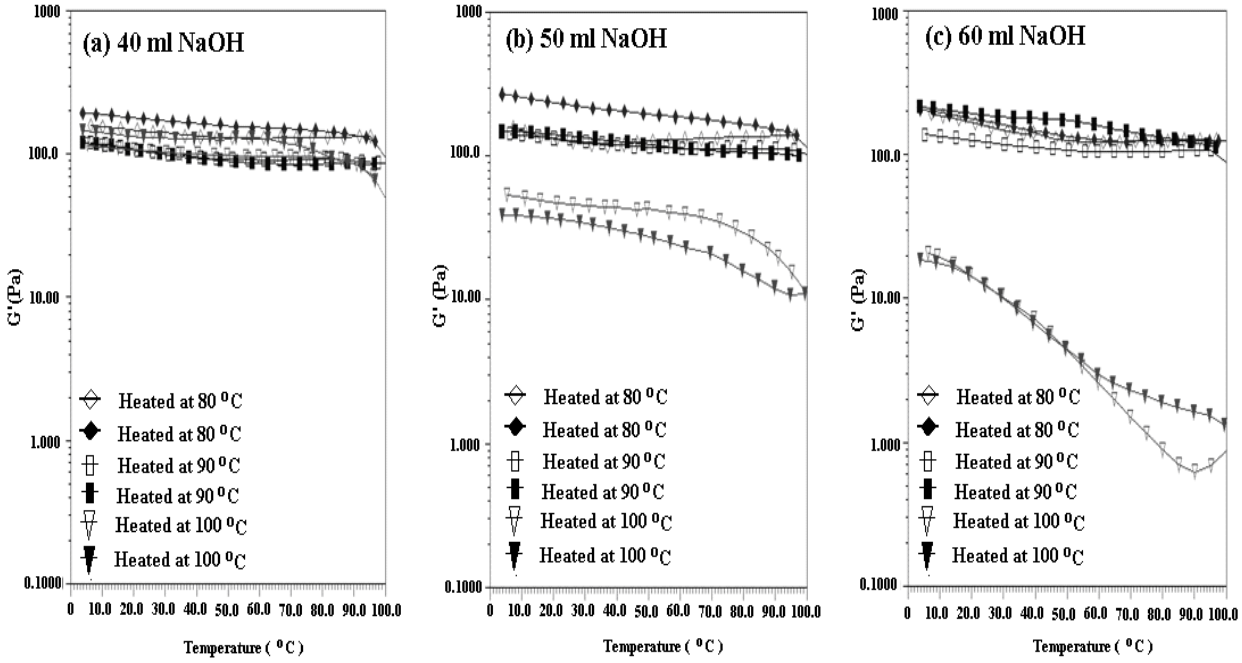

Figure 7. Temperature dependence of G'(open symbols: heating; close symbols: cooling) for CBMP-MWCS blends treated at different alkali modification levels and heating temperatures for 5 min

a weaker gel network with MWCS, denoted by a temperature sensitive G' response registered.

Discussion

From the morphology of the CBMP aggregates extracted, it is interesting to note that CBMP aggregates differently upon alkali and heat treatment. When treated at 80°C in an alkaline medium, CBMP was able to aggregate into a globular shape upon neutralization. But, when treated at higher temperatures (90°C and 100°C) it may have lost the capability to form globular aggregates, instead a paste was formed. It is believed that CBMP would have denatured and degraded to various degrees upon alkali and heat treatment. It might have transformed from a fully unfolded state through a molten globule like state (Hirose,1993) to a fully folded state and subsequently degraded into a smaller molecular weight fraction as the denaturing temperature was increased from 80 to 100°C. This might have directly modified the capability and transition path for CBMP to refold from the fully denatured state into a partially folded conformation. This suggests that protein aggregates of various characteristics could be obtained by manipulating the denaturing agents, such as heat and alkali which may cause marked changes in the protein structure. Walkenstrom *et al*. (1999) had reported the same on whey protein by varying process parameters like flow behaviour and temperature. Nevertheless, further analysis is needed to support this discussion.

When the pre-treated CBMP aggregates was blended with MWCS, and heated to 90°C for 20 min, the composite gels formed showed vastly different rheological properties most probably due to different interaction that was manifested between MWCS and CBMP of different conformation. As shown in Figure 2 through Figure 6, samples prepared can be characterized into two distinct groups: (i) shear thinning with time dependency (thixotropic), and (ii) shear thickening with time dependency (rheopectic). The former group is mainly shown in samples prepared by blending MWCS with CBMP that had undergone treatment with 60 ml NaOH, whilst the latter was shown by samples prepared with CBMP pre-treated with 40 or 50 ml NaOH.

According to Bagley and Dintzis (1999), shear thickening in dispersion system consisting rigid spherical particles is often considered a reflection of volume increase under shear, or dilatancy. This was reported to happen in cross linked waxy maize starch solution upon aseptic processing (Dail and Steffe 1990a, 1990b). In the work of Dintzis and Bagley (1995), crosslinked waxy maize starch was dissolved in potassium hydroxide and subjected to thermo-mechanical processing. They reported that the dilatancy was evident in samples that were not severely treated. To further elucidate the science behind the shear-thickening, the work of Kim *et al*. (2002) revealed that gently prepared starch solutions are macroscopically heterogeneous with regions of highly concentrated gel-like structures dispersed in dilute starch solution. Upon shearing, heterogeneous regions were broken up, resulting in an increase in the dissolved starch concentration, which account for the shear-thickening observed.

Similar trend was observed in this study, therefore the same explanation probably holds. It is noted that CBMP pre-treated with low alkali treatment may probably aggregate into globular shape upon pH adjustment. So, when blended with MWCS, CBMP aggregates may act as dispersed particles interspersed throughout the primary gel network of MWCS. These CBMP fillers may be broken up upon shearing and subsequently enhance the viscosity of the blends. However, CBMP that was pre-treated with 60 ml NaOH, did not aggregate the same but may have present in the form of a dissolved open chain that easily shear thin together with MWCS.

From the statistical analysis, it is found that progressive increase in pre-treatment temperature and pre-treatment time reduce the values of flow behaviour index and yield stress but enhance consistency index of the CBMP-MWCS blend. However, as shown in Figure 2- 4 , an optimum rheological properties with maximum flow behaviour index and yield stress, and minimum consistency, was shown at a pre-treatment condition of 90°C for 5 min, irrespective of the amount of alkali added.

Conclusion

Pre-treat CBMP with alkali and heat may render the protein molecules to denature. Upon pH adjustment, CBMP may aggregate differently depending on the level of pre-treatment carried out. The blend produced by mixing the pre-treated CBMP with MWCS was found to be thermally stable except those prepared with severely pre-treated CBMP. On the other hand, the shear-thickening effect observed in CBMP-MWCS blends may be attributed to higher concentration of dissolved protein molecules in the mixture due to breaking up of CBMP aggregates under shear.

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