CHAPTER 4

PHYSICOCHEMICAL PROPERTIES OF HEAT AND PREHEATED SOYMILK GELS SET USING HIGH PRESSURE

4.1 Materials

Soybean used in this experiment was Chiang Mai 60 variety purchased from Chiang Mai seed centre, Chiang Mai, Thailand. The dried beans (9.75% moisture content) were stored at 4°C until required.

4.2 Methods

4.2.1 Preparation of tofu samples

Tofu samples used in these experiments were of the filled (packed) tofu type (see earlier), prepared from soymilk by adding coagulant as described in section 3.2.1. After adding coagulant, the mixture was subsequently uniformly mixed for 15 sec using a magnetic stirrer (Hot plate/Stirrer, Heidolph MR3001, Germany) with a Teflon-coated magnetic stirring bar (8 mm diameter and 49 mm length). Then, the soymilk samples were divided into two separated portions. The first portion was subjected to heat treatment at 70°C for 60 min then cooled and stored at 4°C until used. The second portion was subjected to high pressure treatment using the following conditions; high pressure at 400 or 600 MPa with temperature 30 or 50°C for 20 min.

4.2.2 Chemical analysis of soybean, soymilk and tofu

Soybean, soymilk and tofu were analysed using the methods detailed in section 3.2.2.

4.2.3 Determination of the activities of trypsin inhibitors present

Activity of trypsin inhibitors of soybean, raw soymilk, heat treated (97-100°C for 7 min) soymilk and heat treated followed by high pressure treated (600 MPa and 50°C for 20 min) were determined using the methods described in section 3.2.3.

4.2.4 Rheological measurements on tofu materials

A controlled stress rheometer (TA instruments AR 2000, TA Instruments-Waters LLC, USA) was used to measure the dynamic viscoelastic properties of the samples. Measuring geometry used was a 25 mm diameter parallel plate with a gap width of 2.5 mm. Samples were loaded onto the rheometer and allowed to equilibrate to the measuring temperature (25°C for about 3 min). Excess sample was trimmed off carefully with a razor blade. A thin layer of low viscosity oil was applied to the exposed free edges to prevent evaporation of water. Subsequent examination of the samples after testing showed no evidence of any ingress of this oil into the sample.

The linear viscoelastic region of the heat and high pressure treated samples were determined by means of a series of oscillation strain sweeps (0.01 to 10% strain at angular frequency of 1 rad/sec) as shown in Figure 5.1a and b. Based on these results, a strain of 0.1% was chosen for the subsequent measuring of the oscillation frequency sweep. The frequency sweep was determined against a frequency range of 0.01 to 1 Hz for each set of treated samples. Each analysis was a replication of six measurements (n = 6).

This measurement was difference from those performed in chapter 3 where a control stress of 1 Pa was used. Since a different rheometer was used for the work presented in this chapter, a 0.1% control strain was simply applied. However, the measurement systems in both chapters were determined within a linear viscoelastic region. Therefore, the results of both measurements were relatively reliable.

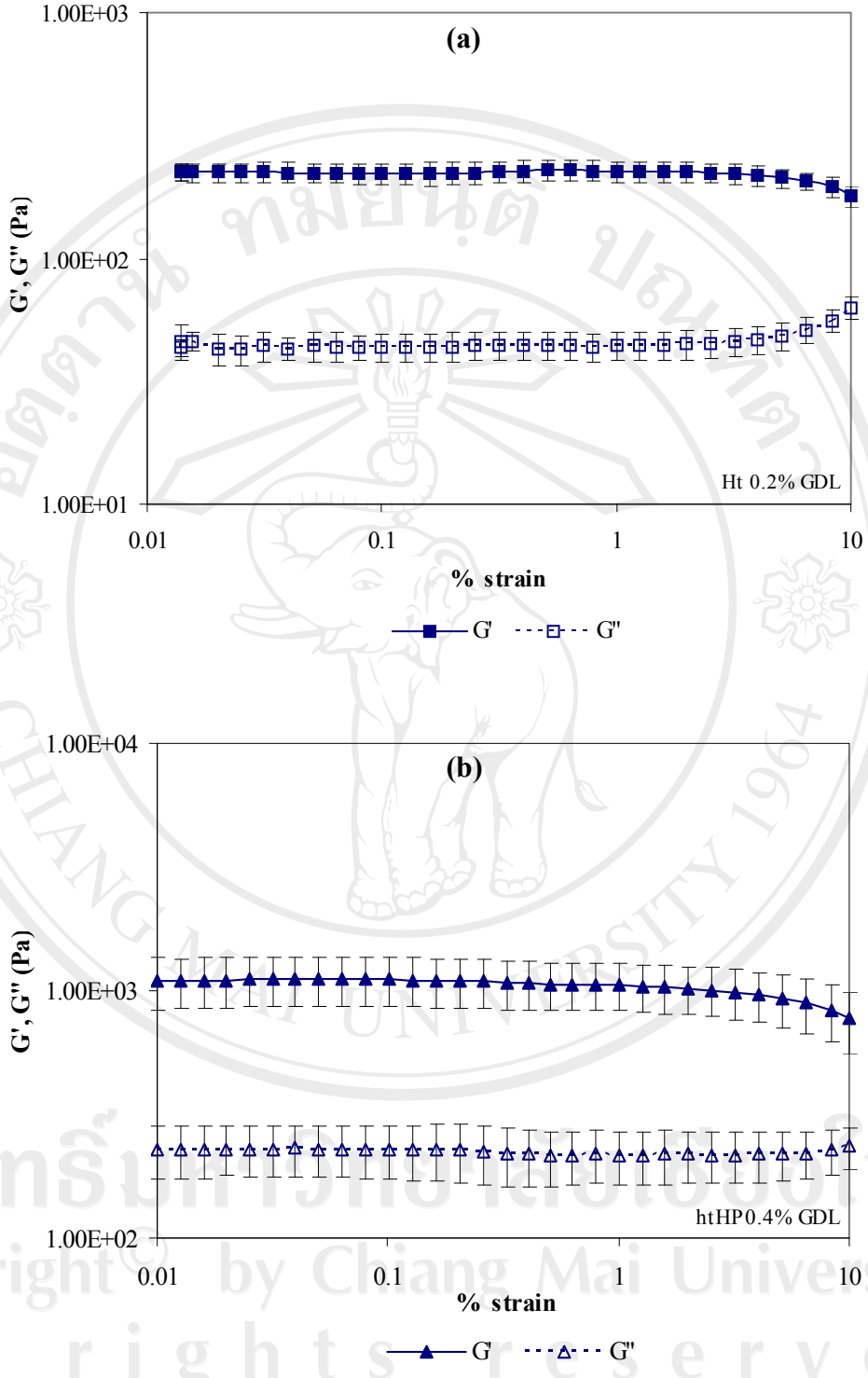

Figure 4.1 The storage (G') and loss (G") moduli of GDL set tofu samples as a function of strain amplitude (0.01 to 10% strain) at an angular frequency of 1 rad/sec (mean, n = 6) for: (a) 0.2% w/v GDL heat set tofu gel; (b) 0.4% w/v GDL preheated soymilk following pressure set tofu gel.

4.2.5 Scanning electron microscopy (SEM) of tofu gels

A Scanning electron microscope (JEOL JSM-5910LV, Jeol Ltd., Tokyo, Japan) was used to examine the microstructure of treated tofu. Since this chapter was conducted at CMU where the CSLM was not available, whereas SEM is applicable and could similarly detected the difference of microstructure among treatment conditions.

The small piece (approximate 3 mm cube) of tofu samples were fixed with 2.5% glutaaldehyde for 4 hr. After two washings with 0.1 M phosphate buffer at 15 min intervals, the samples were post fixed with 1% osmium tetraoxide for 1 hr. The post fixed samples then were rinsed two times with phosphate buffer at 15 min intervals and dehydrated in a graded 'ethanol series' (15%, 30%, 50%, 70%, 80%, 95% and 100% w/w, 30 min in each solution). The samples were then rinsed with acetone. Critical point drying (CPD) was conducted using CO_2. The dried sample was finally mounted on a stub and sputter-coated with gold using SPI-MODULE sputter coater (SPI Supplies, Division of Structure Probe, Inc., Canada). The observations were carried out at 10 kV and a magnification of 2000× and 7000×, respectively.

4.2.6 Water holding capacity of tofu gels

Water holding capacity (WHC) was determined following the method of Pandey *et al*. (2000) and Molina *et al*. (2002). A sample size of 25-30 g with approximately 25 mm diameter and 50 mm length was centrifuged with a force of 1500 g for 30 min at 15°C (Rotina 46 R, Hettich Zentrifugen, Germany). The fluid was separated from the solid mass and weighted. WHC was calculated as the percentage of released water per gram of initial sample. The measurement was carried out for six replications.

4.2.7 Native polyacrylamide gel electrophoresis of tofu gels

Method used was as described in section 3.2.8

4.2.8 Statistical analysis

Methods used were as described in section 3.2.9. Statistical analysis design for factorial (Law and Kelton, 2000) was set in the experiment of rheological measurements on tofu materials.

4.3 Results and discussion

4.3.1 Analysis of soybean, soymilk, and tofu samples

4.3.1.1 Chemical composition of CM-soybean and soymilk

Table 4.1 The chemical composition of CM-soybean, MF-soybean, and their soymilk products.

Determination	Soybean CM	Soybean MF	Soymilk CM	Soymilk MF
seed weight (g/100 seeds)	15.25 ± 0.14	13.34 ± 0.37	-	-
moisture content (%)	9.75 ± 0.74	9.80 ± 0.16	92.09 ± 0.44	91.32 ± 0.34
total solid (%)	-	-	7.91 ± 0.44	8.68 ± 0.34
pH value	-	-	6.5-6.6	6.4-6.6
total protein (%) (N × 6.25)[a]	36.77 ± 0.54	36.44 ± 0.49	3.69 ± 0.13	4.43 ± 0.10
total fat (%) [a]	15.41 ± 1.54	11.14 ± 0.32	1.04 ± 0.11	0.90 ± 0.03
ash (%)[a]	4.45 ± 0.05	4.65 ± 0.02	0.42 ± 0.027	0.39 ± 0.004
total carbohydrate (%)	33.61	37.96	2.75	2.96
water uptake (%)	220	240	-	-

[a] wet weight basis, n = 3

Table 4.1 shows the chemical composition of CM-soybean and its soymilk comparing with the previous MF-soybean (chapter 3). The seed weight of CM-soybean per 100 seeds was heavier than those of the MF-soybean indicating CM seed is larger than the MF seed. In contrast the smaller size of seed (MF-soybean) up took water more than the equivalent weight of CM-seed. Nevertheless these particular

properties from different soybeans did not appear to contribute to the overall consistency of final tofu product. The chemical compositions of both soybean varieties were relatively similar, except that the fat content CM-soybean seemed to be higher. This could be easily adjusted after making the soymilk (Table 4.1, column 4 and 5).

4.3.1.2 Activity of the trypsin inhibitors in the soybean and soymilks

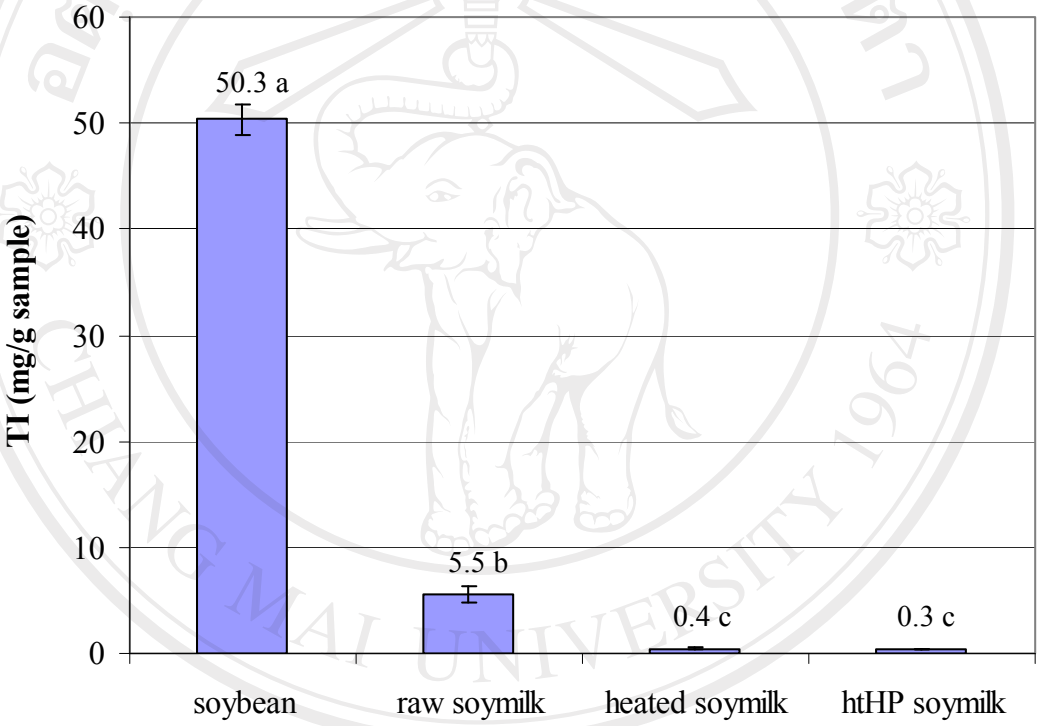

Figure 4.2 Activity of trypsin inhibitors in CM-soybean, raw soymilk, heated soymilk, and pressurised heated (htHP) soymilk. Means followed by the same letters (a, b, c) are not significantly different ($p<0.05$).

Figure 4.2 shows drastic reduction of TI activity by roughly 90% from the values recorded in the raw soymilk after passing through a series of processes. The materials preheated at 97-100°C for 7 min and then high pressure treated at 600 MPa and 50°C for 20 min displayed significant decreases in the values of the measured TI

activity ($p<0.5$) when compared with raw soymilk. However the effect of heat and a combination of heat and pressure on TI activity displayed no significant differences between the two treatment conditions. These results suggested that high pressure plus heat at 50°C could not inactivate this enzyme on its own without an associated preheating process. The results agree with activity of trypsin inhibitors in MF-soybean, raw soymilk, heated (Ht) soymilk, and pressurised (400 MPa, 600 MPa) soymilks reported earlier (section 3.3.1.2). It is worth noting that the original TI in MF-soybean (33.4 mg/g sample, Figure 3.3) was lower than that in CM-soybean (50.3 mg/g sample, Figure 4.2) while Hafez (1983, cited in Anderson and Wolf, 1995) found that 19 varieties and strains of soybean contained 17-27 mg of TI/g.

4.3.1.3 Visual appearance and chemical properties of tofu gel materials

4.3.1.3.1 Heat set tofu gels

The heat set (Ht) tofu gels from CM soybean with the addition of 0.2%-0.6% w/v GDL, 0.2%-0.6% w/v $CaSO_4.2H_2O$, and 0.12%-0.16% w/v $CaCl_2.2H_2O$ appeared to be both smooth and homogenous in texture and were essentially the same as those gels set from MF-soybean material (section 3.3.1.2.1).

4.3.1.3.2 Preheated soymilk, pressure set tofu gels (htHP)

Preheated (97-100°C for 7 min) soymilk without added coagulant did not form gel, even pressurised up to 600 MPa. However, by adding various coagulants for example 0.4% w/v GDL, 0.4% w/v calcium sulphate and 0.14% w/v calcium chloride, very smooth more 'elastic' and 'compact' gel structure could be observed (Figure A2 in appendix A). The higher concentrations of coagulants used in these studies gave rise to some sample curding before the final pressure setting. This eventually created very different 'lumpy' final products. Preheated soymilk with subsequently added 0.4% w/v GDL or 0.4% w/v $CaSO_4.2H_2O$ then pressurised at 400 MPa or 600 MPa and 30 or 50°C for 20 min gave 'self-standing' gel. While added 0.14% w/v $CaCl_2.2H_2O$ gave 'self-standing' gel only at the pressure treatment of 600 MPa and 50°C for 20 min.

Table 4.2 Chemical properties of heat set (Ht) tofu, preheated soymilk following high pressure treatment (htHP) tofus made by adding 0.4% w/v GDL, 0.4% w/v $CaSO_4.2H_2O$, and 0.14% w/v $CaCl_2.2H_2O$.

Tofu type	% Moisture content [a]	% Total protein [a]	pH range [b]
Ht-GDL	92.25 ± 0.3	3.82 ± 0.14	5.3-5.6
htHP-GDL	91.95 ± 0.1	4.05 ± 0.34	5.3-5.4
Ht-$CaSO_4$	91.43 ± 0.7	3.86 ± 0.27	6.0-6.1
htHP-$CaSO_4$	90.87 ± 0.3	4.02 ± 0.18	6.0
Ht-$CaCl_2$	92.31 ± 0.2	3.84 ± 0.30	6.2-6.3
htHP-$CaCl_2$	92.61 ± 0.1	3.93 ± 0.38	6.1-6.2

[a] wet weight basis for mean ± sd, n = 3

[b] n = 6

Table 4.2 illustrates that the moisture content, total protein and pH range of the tofu set by either heat or high pressure using various coagulants gave similar values to those reported in a study by Saio *et al* in 1979.

4.3.2 Rheological properties of heat set and preheated soymilk, high pressure set tofu gels

4.3.2.1 Typical rheological behaviour of heat set tofu gels

4.3.2.1.1 Heat set tofu gels with added GDL

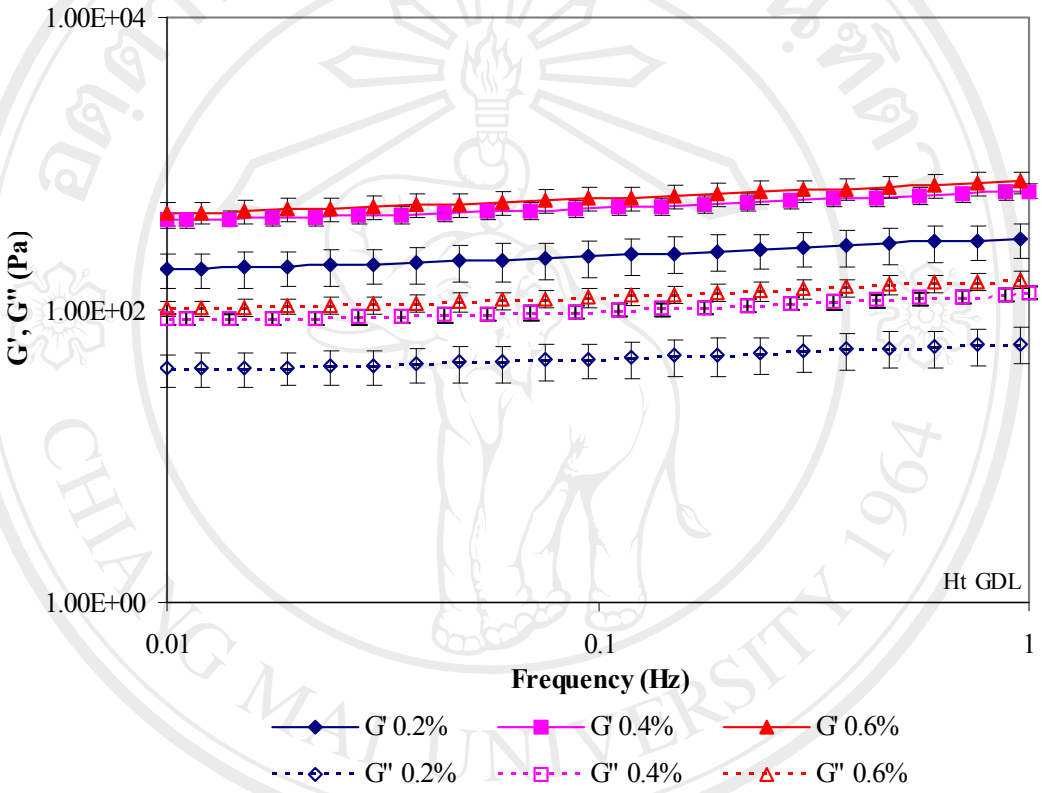

Figure 4.3 The storage (G') and loss (G") moduli of heat set tofu gels with added 0.2% to 0.6% w/v GDL as a function of frequency (0.01-1 Hz) at a strain amplitude of 0.1% (mean, n = 6).

Figure 4.3 showed that the plots of the storage (G") and loss (G") moduli are similar in shape to each other, with the storage (G') moduli greater than the loss (G") moduli over all of the assessed frequency range. The heated tofu gels with 0.6% and 0.4% w/v GDL moduli were significantly higher for both the storage (G') and the loss (G'') moduli than those gels with only 0.2% w/v added GDL (Table 4.3), suggesting

that these tofu gels had higher 'cross link density' than those at the lower coagulant level (Apichartsrangoon, 2002). Therefore 0.4% w/v GDL was selected to be the optimum concentration for further high pressure treated materials.

Table 4.3 Viscoelastic values observed at 0.1 Hz for heat set tofu gels with added GDL (0.2% to 0.6% w/v).

Tofu type	G' (Pa)	G" (Pa)	tan δ
0.2% GDL	231.3 ± 64.9 a	46.2 ± 12.6 a	0.20 ± 0.01
0.4% GDL	503.1 ± 63.5 b	99.9 ± 10.4 b	0.20 ± 0.01
0.6% GDL	573.4 ± 104.2 b	121.8 ± 17.2 b	0.21 ± 0.01

The results are the means ± standard deviations of six replicates. Different letters (a, b) indicate significant ($p<0.05$) differences within a column.

The results in Table 4.3 for the heated tofu gels with added GDL all had essentially the same loss tangent (tan δ) of about 0.2, indicating the gelling behaviours were consistent with materials with weak non-specific interactions, giving rise to amorphous gel structures (Ferry, 1980).

4.3.2.1.2 Heat set tofu gels with added $CaSO_4.2H_2O$

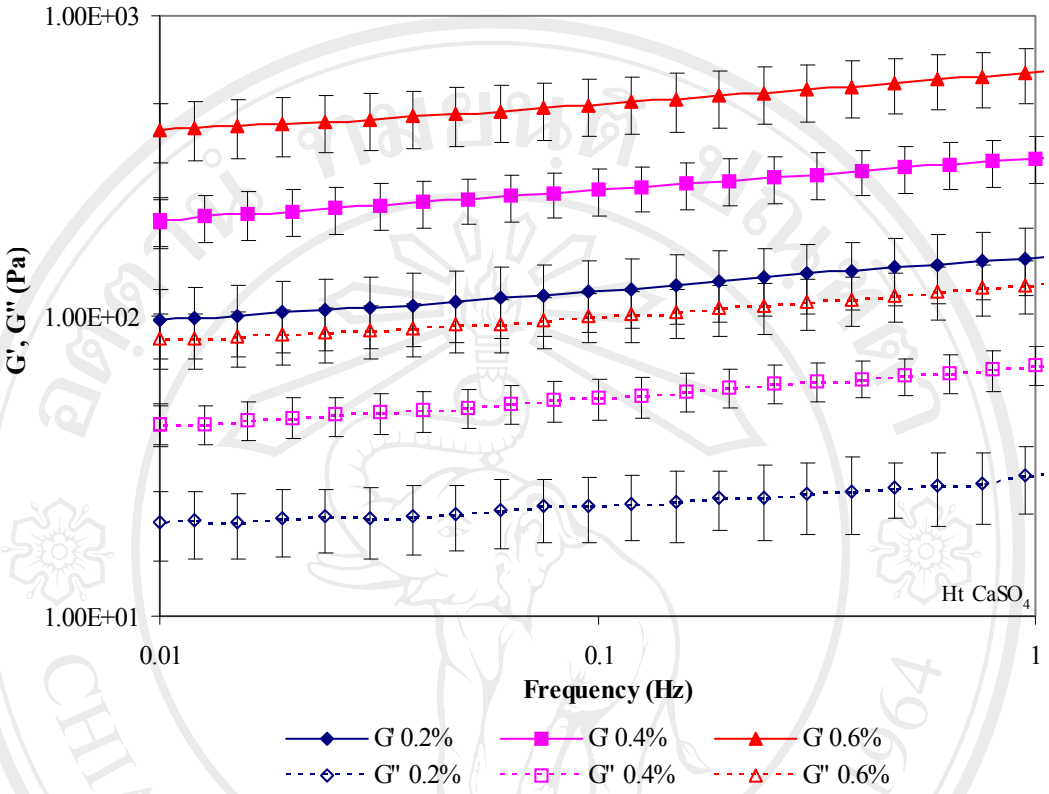

Figure 4.4 The storage (G') and loss (G") moduli of heat set tofu gels with added 0.2% to 0.6% w/v $CaSO_4.2H_2O$ as a function of frequency (0.01-1 Hz) at a strain amplitude of 0.1% (mean, n = 6).

Figure 4.4 shows that the plots of the storage (G") and loss (G") moduli are all similar in shape, with the storage (G') moduli greater than the loss (G") moduli over all of the assessed frequency range. The heated tofu gels with 0.6% added $CaSO_4.2H_2O$ gave significant higher storage (G') and loss (G'') moduli values than those gels made using 0.4% and 0.2% w/v $CaSO_4.2H_2O$, (Table 4.4). This suggested that these tofu gels with higher concentrations of $CaSO_4$ as a coagulant had a 'cross link density' higher than those at the lower coagulant levels (Apichartsrangoon, 2002).

Table 4.4 Viscoelastic values observed at 0.1 Hz for heat set tofu gels with added $CaSO_4.2H_2O$ (0.2% to 0.6% w/v).

Tofu type	G' (Pa)	G" (Pa)	tan δ
0.2% $CaSO_4.H_2O$	120.4 ± 31.9 a	23.3 ± 5.9 a	0.20 ± 0.02
0.4% $CaSO_4.H_2O$	262.5 ± 45.8 b	53.2 ± 8.3 b	0.20± 0.01
0.6% $CaSO_4.H_2O$	503.0 ± 109.1 c	100.1 ± 19.1 c	0.20± 0.01

The results are the means ± standard deviations of six replicates. Different letters (a, b, c) indicated significant ($p<0.05$) differences within a column.

Table 4.4 shows that the heat set tofu gels with added $CaSO_4.2H_2O$ (0.2% to 0.6% w/v) had similar loss tangent values (tan δ) of about 0.2, indicating that all of the gels examined behaved as 'weak' viscoelastic gels with the loss tangent approaching unity (Ferry, 1980).

4.3.2.1.3 Heat set tofu gels with added $CaCl_2.2H_2O$

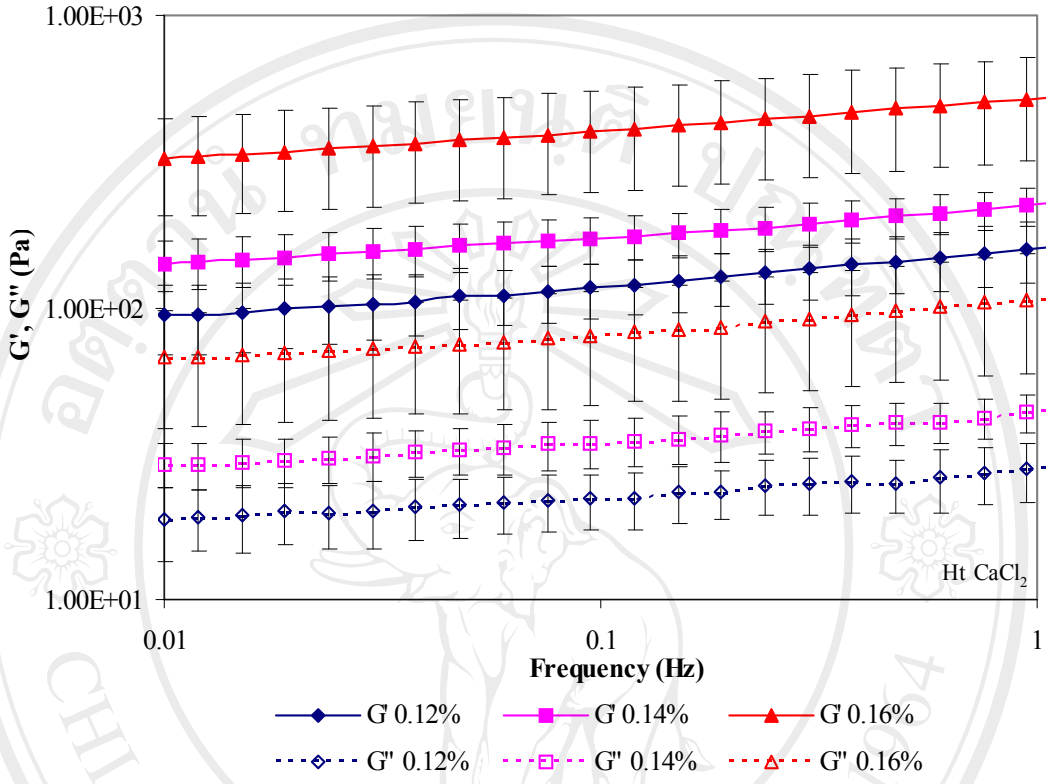

Figure 4.5 The storage (G') and loss (G") moduli of heat set tofu gels with added 0.12% to 0.16% w/v $CaCl_2.2H_2O$ as a function of frequency (0.01-1 Hz) at a strain amplitude of 0.1% (mean, n = 6).

As shown in figure 4.5 the plots of the storage (G") and loss (G") moduli are again all similar in shape, with the storage (G') moduli greater than the loss (G") moduli over all of the measured frequency range. The heated tofu gels with 0.16% $CaCl_2.2H_2O$ appeared to have significant higher values of both storage (G') and loss (G") moduli than those gels with 0.14% and 0.12% w/v $CaCl_2.2H_2O$, added (Table 4.5). This suggested that these tofu gels with the higher levels of $CaCl_2$ coagulant had increasing cross link densities than those of the lower coagulant levels (Apichartsrangoon, 2002).

Table 4.5 Viscoelastic values observed at 0.1 Hz for heat set tofu gels with added $CaCl_2.2H_2O$ (0.12% to 0.16% w/v).

Tofu type	G' (Pa)	G" (Pa)	tan δ
0.12% $CaCl_2.2H_2O$	116.6 ± 24.8 a	22.2 ± 4.72 a	0.19 ± 0.01
0.14% $CaCl_2.2H_2O$	172.1 ± 31.2 b	34.4 ± 6.48 a	0.20 ± 0.01
0.16% $CaCl_2.2H_2O$	365.2 ± 53.8 c	80.1 ± 33.8 b	0.20 ± 0.01

The results are the means ± standard deviations of six replicates. Different letters (a, b, c) indicated significant ($p<0.05$) differences within a column.

Table 4.5 showed that heated tofu gels made by adding 0.2% to 0.6% w/v $CaCl_2.2H_2O$ had essentially the same loss tangent (tan δ) value of 0.2, indicating behaviour of all of the gels was consistent with the behaviour expected from 'weak' viscoelastic gel systems, with the loss tangent approaching unity (Ferry, 1980).

4.3.2.2 Typical rheological behaviour of preheated soymilk, high pressure set tofu gels

4.3.2.2.1 Preheated soymilk, high pressure set tofu gels with added GDL

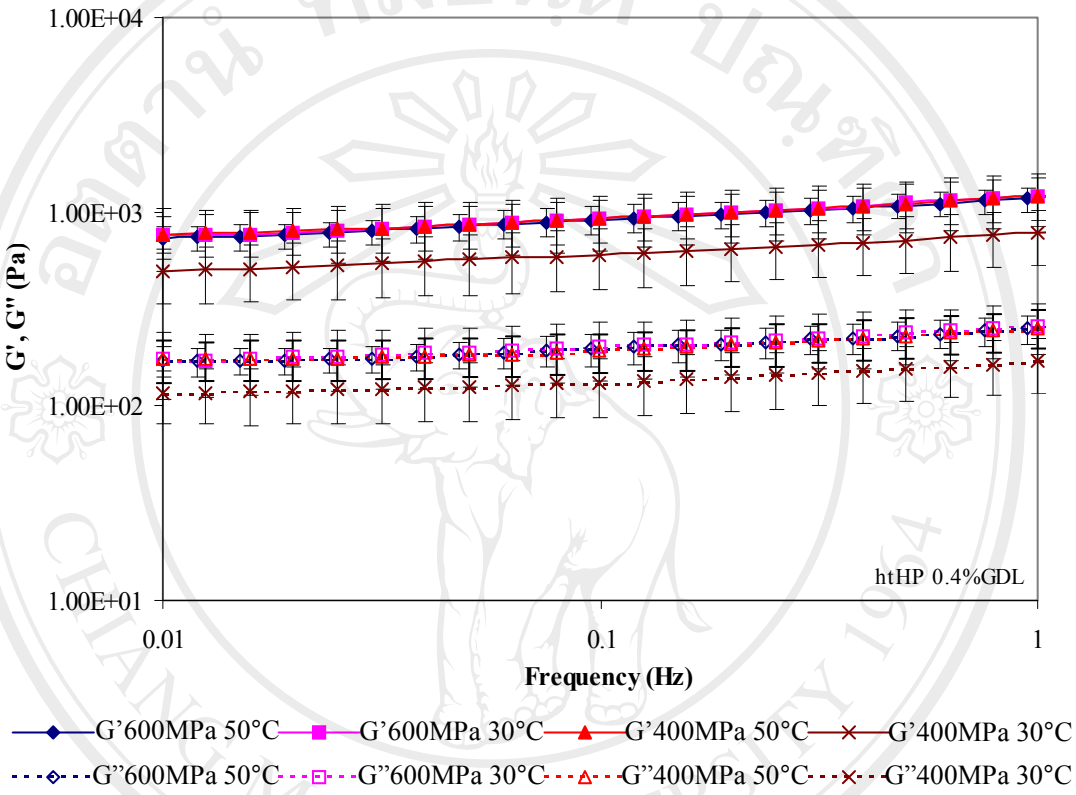

Figure 4.6 The storage (G') and loss (G") moduli of preheated soymilk, high pressure set tofu gels with added 0.4% w/v GDL as a function of frequency (0.01-1 Hz) at a strain amplitude of 0.1% (mean, n = 6).

As shown in figure 4.6 the plots of the storage (G") and loss (G") moduli as a function of frequency are again all similar in shape to one another, with the storage (G') moduli greater than the loss (G") moduli over all of the measured frequency range. The moduli of tofu gels obtained from 600 MPa 50°C, 600 MPa 30°C, and 400 MPa 50°C are essentially in the same range and have significantly higher moduli values than those of the 400 MPa 30°C treated tofu sample. Those samples with

higher moduli values imply the stronger structure because of possible increase in the systems cross link density as suggested by Apichartsrangoon (2002). The statistical analysis for factorial design shows no combination effect between heat and high pressure set tofu gels.

Table 4.6 Viscoelastic values observed at 0.1 Hz for preheated soymilk, high pressure set (400, 600 MPa and 30, 50°C for 20 min) tofu gels with added 0.4% w/v GDL.

Tofu type	G' (kPa)	G" (kPa)	tan δ
600 MPa 50°C	0.91 ± 0.16 b	0.20 ± 0.04 b	0.22± 0.01
600 MPa 30°C	0.92 ± 0.29 b	0.20 ± 0.07 b	0.22± 0.01
400 MPa 50°C	0.92 ± 0.23 b	0.19 ± 0.04 b	0.21± 0.01
400 MPa 30°C	0.60 ± 0.21 a	0.13 ± 0.04 a	0.22± 0.01

The results are the means ± standard deviations of six replicates. Different letters (a, b) indicate significant ($p<0.05$) differences within a column.

Table 4.6 showed that the tan δ values for the 0.4% w/v GDL htHP tofu gels lay in the same range of 0.21-0.22 at 0.1 Hz, suggesting the gels behave viscoelastic property of a material with weak non-specific interactions giving rise to amorphous gel structures (Ferry, 1980).

4.3.2.2.2 Preheated soymilk, high pressure set tofu gels with added $CaSO_4.2H_2O$

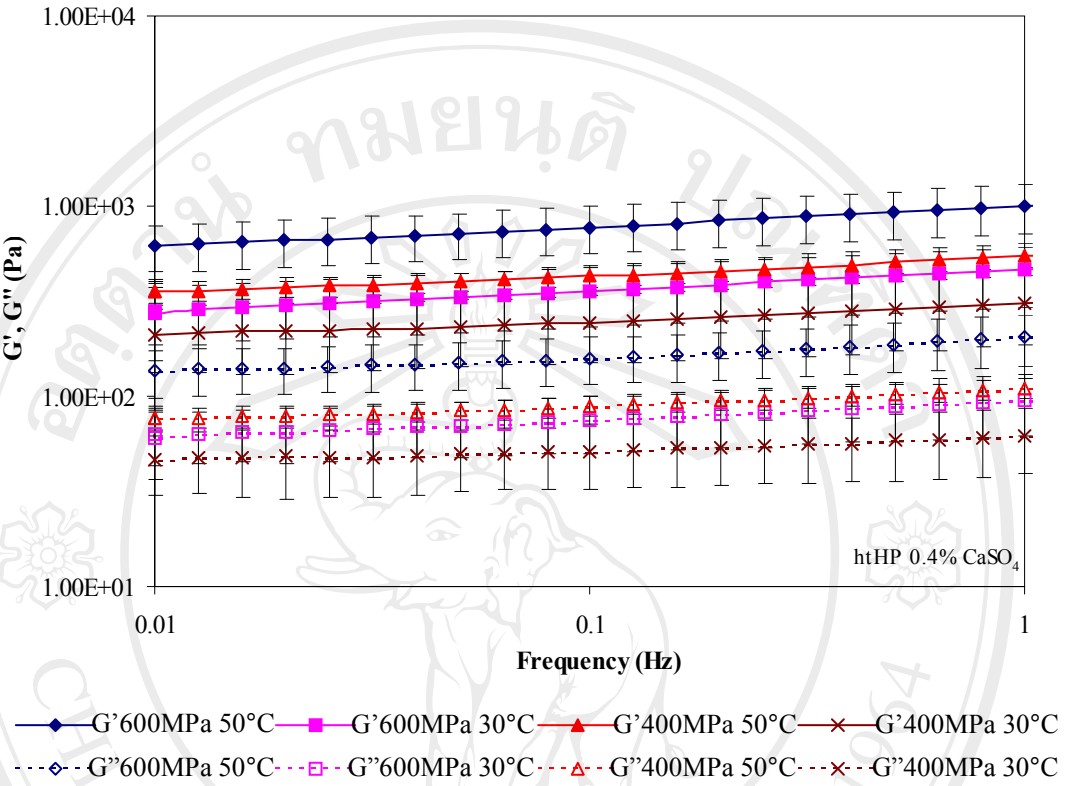

Figure 4.7 The storage (G') and loss (G") moduli of preheated soymilk, high pressure set tofu gels with added 0.4% w/v $CaSO_4.2H_2O$ as a function of frequency (0.01-1 Hz) at a strain amplitude of 0.1% (mean, n = 6).

Figure 4.7 shows that the plots of the storage (G') and loss (G") moduli were again all similar in their shape, with the values of the storage (G') moduli greater than the loss (G") moduli values over all of the measured frequency range. The moduli values of the material processed at 600 MPa and 50°C were significant greater than those of other treatment conditions, implying a higher cross link density (Apichartsrangoon, 2002) with a resulting stronger overall gel system. The gels obtained from 600 MPa 30°C, and 400 MPa 50°C were found not to be significantly different in their storage (G') moduli values. The statistical analysis for factorial

design shows combination effect between heat and preheated soymilk, high pressure set tofu gels.

Table 4.7 Viscoelastic values observed at 0.1 Hz for preheated soymilk, high pressure set (400, 600 MPa and 30, 50°C for 20 min) tofu gels with added $CaSO_4.2H_2O$ (0.4% w/v).

Tofu type	G' (kPa)	G" (kPa)	tan δ
600 MPa 50 °C	0.77 ± 0.22 c	0.159 ± 0.045 c	0.21± 0.004
600 MPa 30 °C	0.36 ± 0.13 ab	0.075 ± 0.025 ab	0.21± 0.01
400 MPa 50 °C	0.43 ± 0.05 b	0.089 ± 0.010 b	0.21± 0.01
400 MPa 30 °C	0.24 ± 0.95 a	0.052 ± 0.019 a	0.21± 0.01

The results are the means ± standard deviations of six replicates. Different letters (a, b, c) indicate significant ($p<0.05$) differences within a column.

As shown in table 4.7 the tan δ values for the 0.14% w/v $CaSO_4$ htHP tofu gels lay in the same range of 0.21 at 0.1 Hz, again suggesting that the gels behaviours are consistent with the materials with weak non-specific interactions giving rise to amorphous gel structures (Ferry, 1980).

4.3.2.2.3 Preheated soymilk, high pressure set tofu gels with added $CaCl_2.2H_2O$

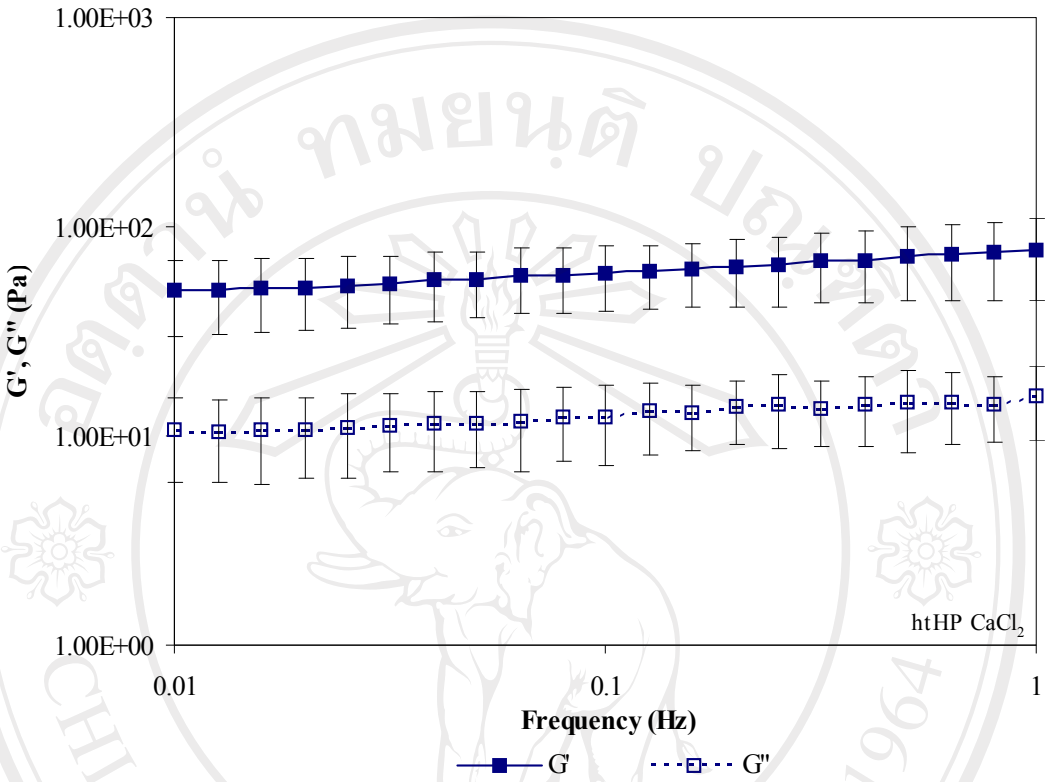

Figure 4.8 The storage (G') and loss (G") moduli values for preheated soymilk, high pressure (600 MPa 50°C 20 min) set tofu gel with 0.14% w/v added $CaCl_2.2H_2O$ as a function of frequency (0.01-1 Hz) at a strain amplitude of 0.1% (mean, n = 6).

. In figure 4.8 the plots of the values of the storage (G') and the loss (G") moduli of the htHP tofu obtained from processing at 600 MPa and 50°C for 20 min with added 0.14% w/v $CaCl_2$ show the storage (G') moduli greater than the loss (G") moduli over all of the measure frequency range with the tan δ value of 0.20 at 0.1 Hz suggesting that the $CaCl_2$ htHP tofu gels behaved as 'weak' viscoelastic gel type materials (Ferry, 1980).

4.3.2.2.4 Comparison of the rheological properties of the heat treated and the high pressure set tofu gels

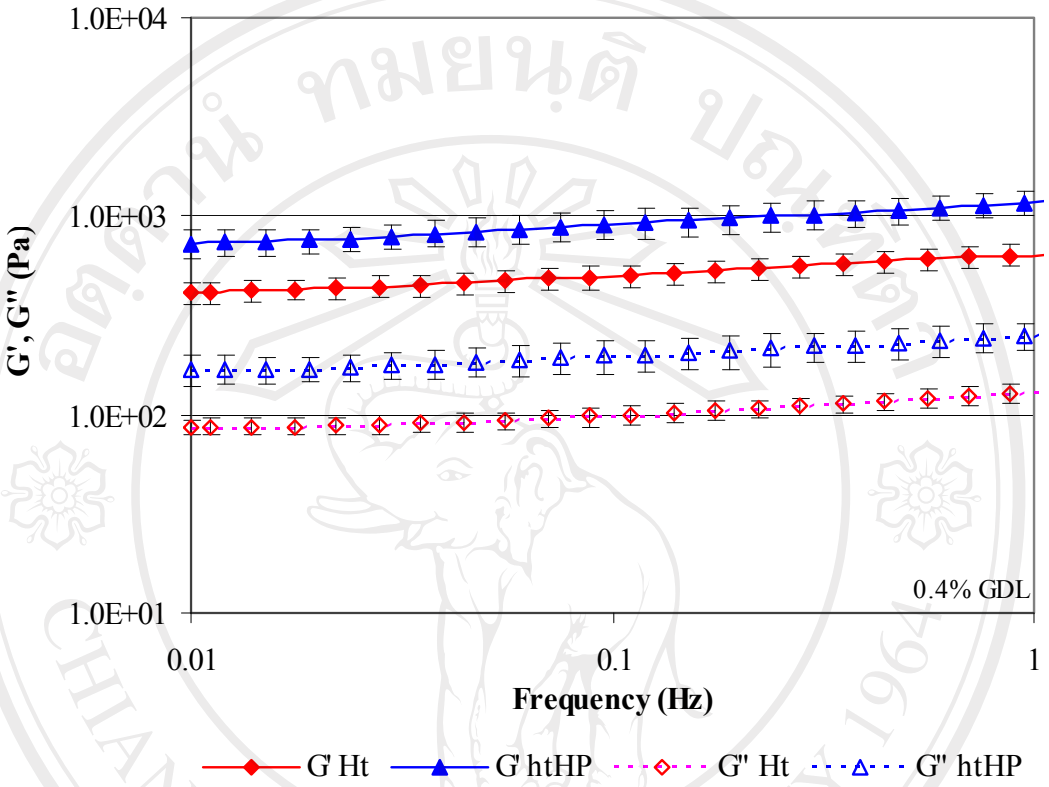

Figure 4.9 The storage (G') and loss (G") moduli as a function of frequency (0.01-1 Hz) for heat (Ht) and preheated soymilk, high pressure (htHP) (600 MPa 50°C 20 min) set tofu gels with added 0.4% w/v GDL (mean, n = 6).

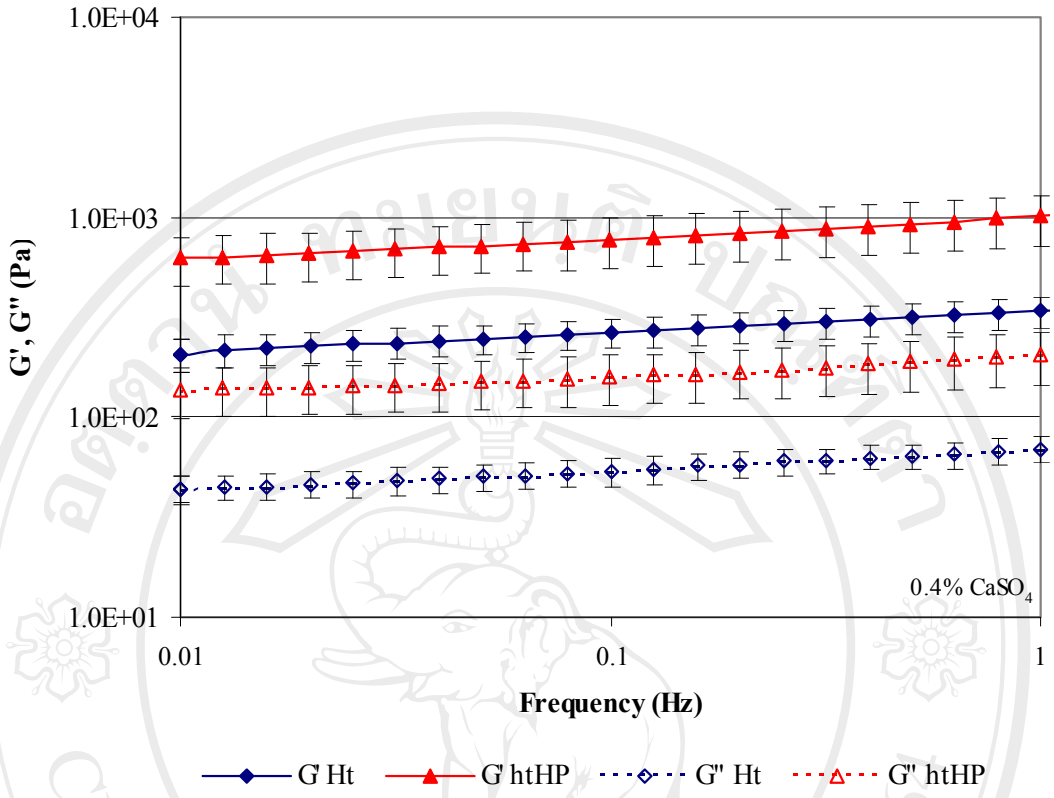

Figure 4.10 The storage (G') and loss (G") moduli shown as a function of frequency (0.01-1 Hz) for heat (Ht) and preheated soymilk, high pressure (htHP) (600 MPa 50°C 20 min) set tofu gels with added 0.4% w/v $CaSO_4.2H_2O$ (mean, n = 6).

Figure 4.9 and 4.10 show the same type of data plots of frequency dependence of the storage (G') and loss (G'') moduli for heat and pressure (600 MPa 50°C 20 min) set tofu gels by adding 0.4% w/v GDL and 0.4% w/v added $CaSO_4.2H_2O$, respectively. Both of the pressure treated storage and loss moduli values were higher than those of the heated samples, indicating pressure treatment had induced more 'elastic' structure than the equivalent heat processing had (Apichartsrangoon, 2002).

Table 4.8 Comparison of mean G' and G" values across frequency range 0.01-1 Hz between heat treated (Ht) and preheated soymilk, high pressure (htHP) (600 MPa 50°C 20 min) set tofu gels with added 0.4% w/v GDL, 0.4% w/v $CaSO_4.2H_2O$, and 0.14% w/v $CaCl_2.2H_2O$.

Coagulants	**Paired means G' (kPa)**		**Paired means G" (kPa)**	
	Ht	**htHP**	**Ht**	**htHP**
GDL	0.513	0.935 s	0.102	0.204 s
$CaS0_4.2H_2O$	0.266	0.789 s	0.054	0.163 s
$CaCl_2.2H_2O$	0.173	0.060 s	0.035	0.012 s

s indicates a significant ($p<0.05$) difference between the paired means.

Table 4.8 shows that the htHP tofu with GDL or calcium sulphate gave significantly higher storage (G') and loss (G") moduli than those produced by Ht alone, whereas for tofu produced with the addition of calcium chloride the moduli of Ht set gels were higher than those of the equivalent pressure set gels. Overall storage and loss moduli of GDL treated materials gave the highest modular values, while calcium chloride gave the lowest. Since the loss tangent of htHP tofu with GDL significantly higher than that of the Ht tofu of the equivalent coagulant this suggests that the the htHP tofu with GDL behave a more 'liquid' viscoelastic system.

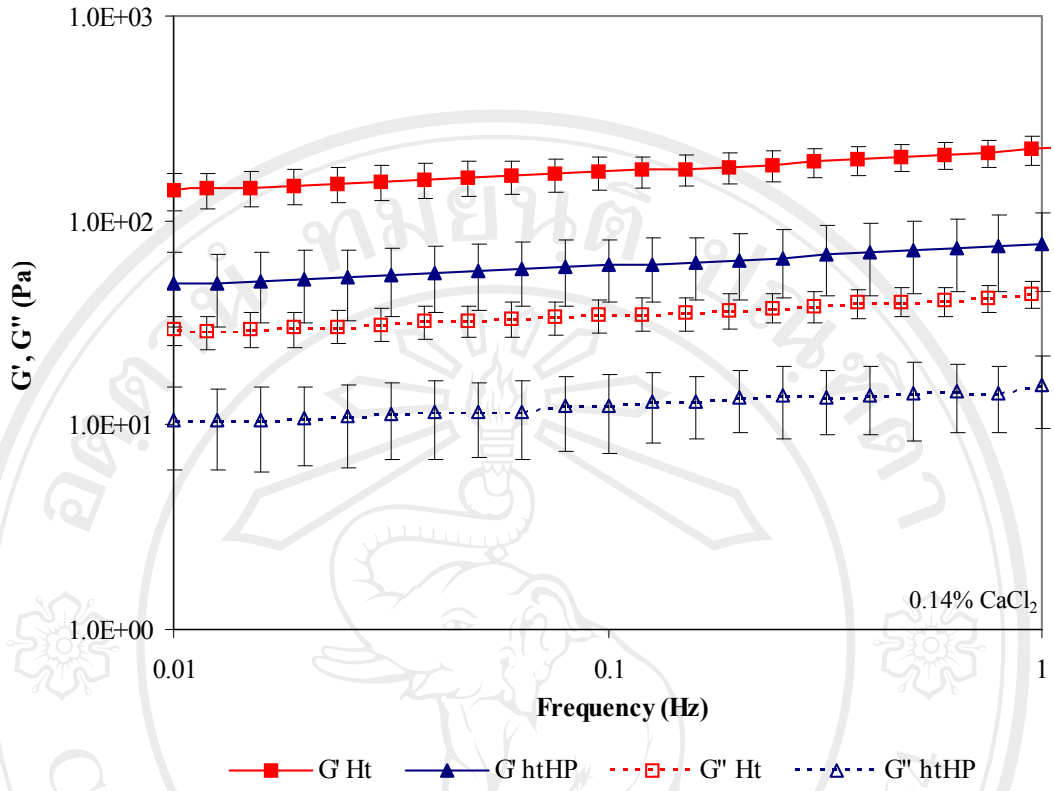

Figure 4.11 The storage (G') and loss (G") moduli as a function of frequency (0.01-1 Hz) for heat (Ht) treated and preheated soymilk, high pressure (htHP) (600 MPa 50°C 20 min) set tofu gels with added 0.14% w/v $CaCl_2.2H_2O$ (mean, n = 6).

Figure 4.11: unlike GDL and $CaSO_4.2H_2O$ processed materials, the $CaCl_2.2H_2O$ processed samples displayed a somewhat different rheological behaviour than that of the heat induced gels. These illustrated higher G' and G" values than those processed at a high pressure did. Moreover, gel with $CaCl_2.2H_2O$ showed lower G' and G" values when compared with the other two coagulants. This is an indication of soft gel system with a lower cross linking density (Apichartsrangkoon, 2002). With regards to the linkage in soymilk gel, Molina and Ledward (2003) suggested that in the heat-set gels the structure will be primarily maintained by disulphide bonds and hydrophobic interactions. However, on subsequent pressure treatment, these hydrophobic linkages will be broken but the disulphide bonds will be maintained, and

may even increase in number. On the release of the pressure, hydrogen bonds will form prior to there being any chance of any hydrophobic interaction being set up, and thus, the texture will be different. It is likely that it is the relative ratio of the hydrophobic interaction and hydrogen bonds within these gels that dictate their different properties (Molina and Ledward, 2003).

4.3.3 Microstructure of heat treated (Ht) and high pressure set (htHP) tofu gels as evidenced using scanning electron microscopy (SEM)

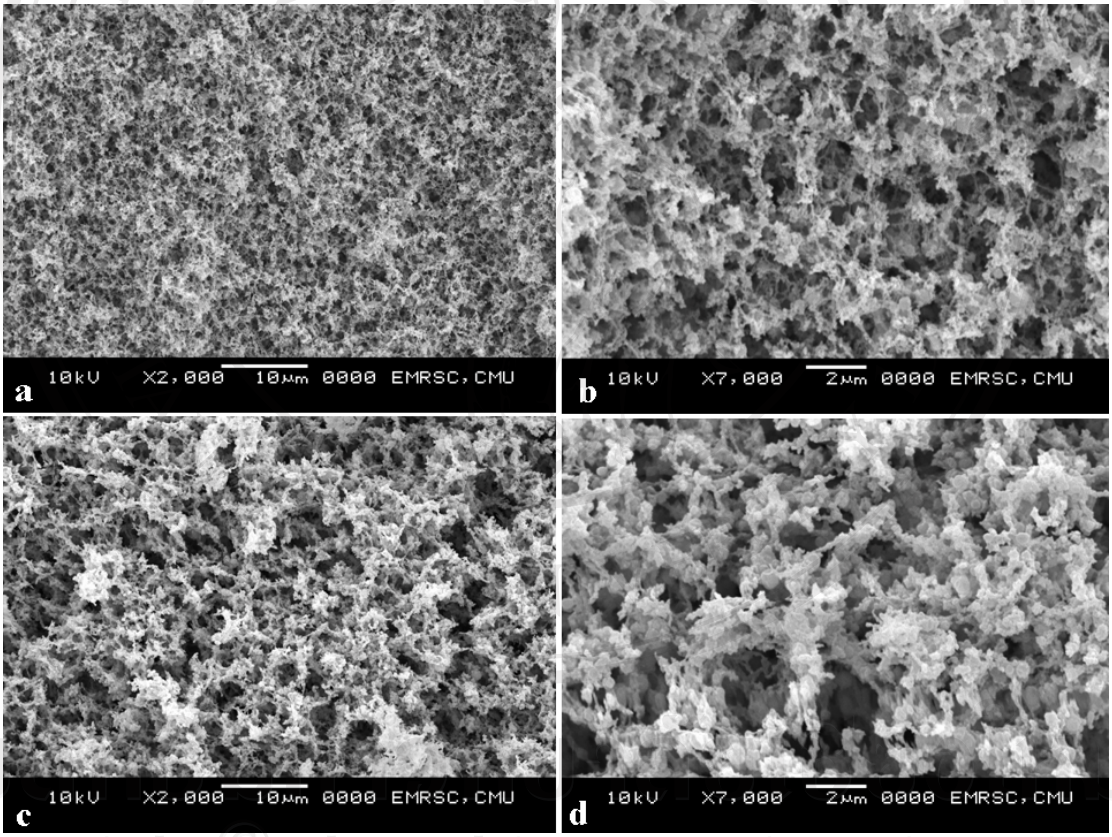

Figure 4.12 Typical SEM images of heat treated (Ht) and preheated soymilk, high pressure set (htHP) tofu with added 0.4% w/v GDL: (a) Ht GDL tofu ×2,000; (b) Ht GDL tofu ×7,000; (c) htHP GDL tofu ×2,000 (d) htHP GDL tofu ×7,000.

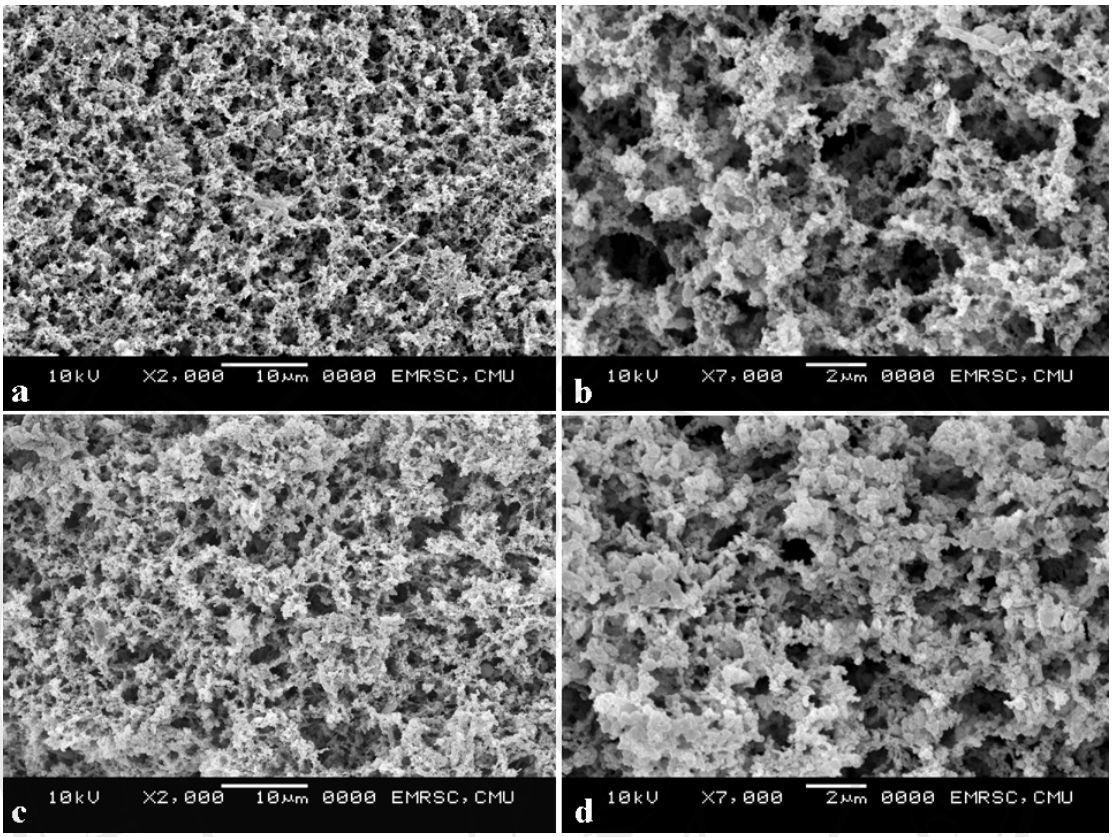

Figure 4.13 Typical SEM images of heat treated (Ht) and preheated soymilk, high pressure set (htHP) tofu with added 0.4% w/v $CaSO_4.2H_2O$: (a) Ht $CaSO_4$ tofu ×2,000; (b) Ht $CaSO_4$ tofu ×7,000; (c) htHP $CaSO_4$ tofu ×2,000; (d) htHP $CaSO_4$ tofu ×7,000.

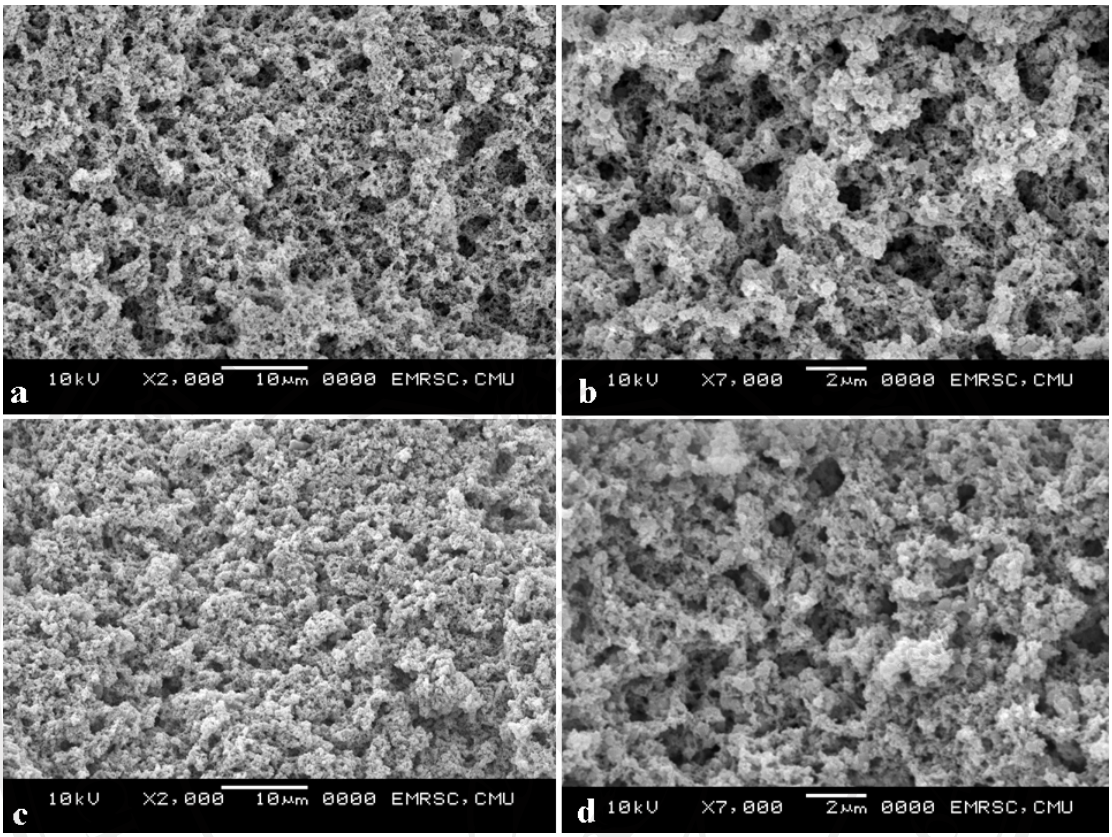

Figure 4.14 Typical SEM images of heat treated (Ht) and preheated soymilk, high pressure set (htHP) tofu with added 0.14% w/v $CaCl_2.2H_2O$: (a) Ht $CaCl_2$ tofu ×2,000; (b) Ht $CaCl_2$ tofu ×7,000; (c) htHP $CaCl_2$ tofu ×2,000; (d) htHP $CaCl_2$ tofu ×7,000.

Figures 4.12, 4.13, and 4.14 show typical SEM images of Ht and htHP tofu samples made by adding 0.4% w/v GDL, 0.4% w/v $CaSO_4.2H_2O$, and 0.14% w/v $CaCl_2.2H_2O$, respectively. The surface microstructures of Ht GDL tofu (Figure 4.12a and b), $CaSO_4.2H_2O$ (Figure 4.13a and b) samples, and the $CaCl_2.2H_2O$ treated materials (Figure 4.14a, and b) were even with a uniform distribution of small holes. These differed from the networks formed using $CaCl_2.2H_2O$ which produced larger and less consistent voids than those produced using the other two coagulants to give set tofu gels.

The microstructures of the htHP tofu produced by adding GDL (Figure 4.12c and d), $CaSO_4.2H_2O$ (Figure 4.13c, and d), and $CaCl_2.2H_2O$ (Figure 4.14c and d) were

similar to those observed for the Ht tofu but the networks of the htHP tofu samples are 'thicker' than those observed for the equivalent Ht tofu made using the same coagulant.

Chang and Yuan (2005) reported that the observed structure as evidenced by SEM of tofu samples, varied widely. The protein-lipid coagulum particulates were observed to be linked together to form a three-dimensional network. Various sizes of waterholes/voids were surrounded by this three-dimensional network. Coagulant was observed to be one of the factors to affect the size of these waterholes/voids. GDL in combination with calcium sulphate was reported to produced tofu with significantly larger holes than that produced using a combination of GDL and calcium chloride. This is regarded as a 'fast-action' coagulant and may be responsible for forming finer overall structures.

Results published by de Man *et al*. (1986) suggested that "pressed" tofu obtained using $CaCl_2.2H_2O$ was course, granular and hard, whereas those formed using GDL and $CaSO_4.½H_2O$ gave a smooth, soft and uniform curd. Saio (1979) stated that the network of GDL tofu consisted of flocculated aggregates, and that of Ca coagulated tofu showed a spongy structure. S-S bonds play important roles in both GDL coagulated tofu and also in Ca coagulated tofu. Pore size has been observed in soy protein isolate gel made using $CaCl_2$ and found to increase with $CaCl_2$ concentration and decreased as protein concentration raised (Maltais *et al*., 2005).

4.3.4 Water holding capacity of heat treated (Ht) and preheated soymilk, high pressure set (htHP) tofu gels

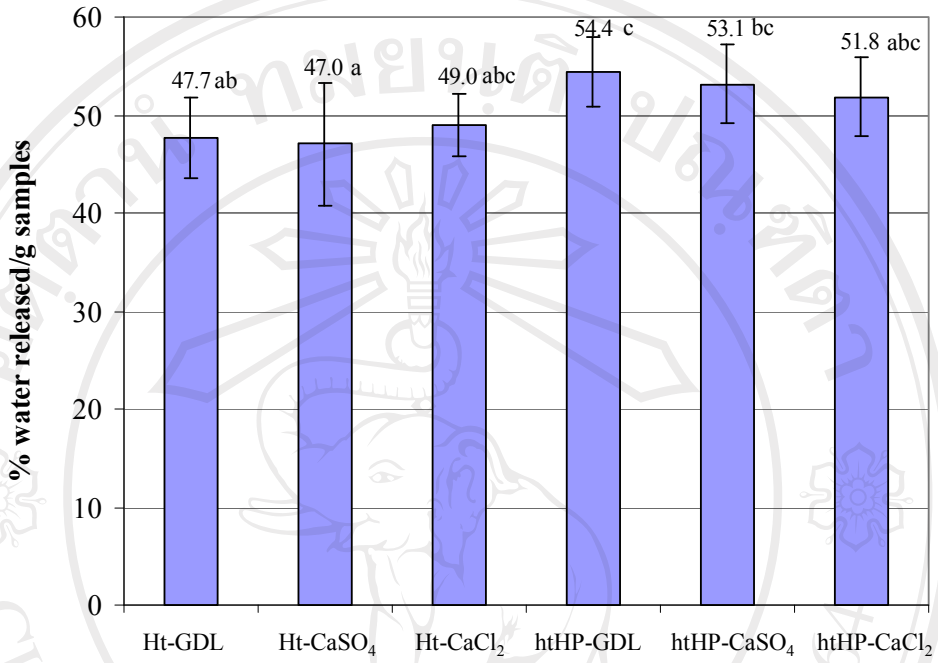

Figure 4.15 Mean water holding capacity (WHC) of heat set (Ht) and preheated soymilk, high pressure set (htHP) tofu gels with added GDL, $CaSO_4.2H_2O$, and $CaCl_2.2H_2O$ (mean ± sd of 6 replicates). Mean followed by the same letters (a, b, c) are not significantly different ($p<0.05$).

Figure 4.15 shows the mean WHC of Ht and htHP processed tofu samples with added 0.4% w/v GDL, 0.4% w/v $CaSO_4.2H_2O$, and 0.14% w/v $CaCl_2.2H_2O$ as total water released per gram of sample (as measured in section 4.2.6). The results show no differences ($p<0.05$) among samples prepared from the same Ht or htHP treatment whichever coagulant was used. Water released of the htHP tofu gels with added GDL and $CaSO_4.2H_2O$ were found to be higher than those of the Ht gels produced by using both GDL and $CaSO_4.2H_2O$ ($p<0.05$). However, differences were not observed between $CaCl_2.2H_2O$ treated tofu samples obtained from either the Ht or the htHP treatment.

Hermansson (1994) suggested that pore size distribution determines the overall WHC of a gel. In general, the more open the structure the lower WHC and coarsely aggregated gels tend to have a lower WHC than those with a finer structure.

Puppo and Anon (1998) reported that WHC of soy protein gels prepared with either NaCl or $CaCl_2$ decreased with increasing salt concentration due to the fact that at high ionic strength a more open matrix was formed. Maltais *et al*. (2005) supported the idea that WHC of soy protein isolate gels with $CaCl_2$ improved with higher protein concentrations and lower $CaCl_2$ concentrations as the pore size increased with increasing $CaCl_2$ concentrations and decreased with increasing protein concentration.

Molina *et al*. (2002) reported that the unfolding of soy protein enhanced its water binding capacity, thus favouring the WHC of HP soy protein systems. The partial unfolding of the protein will allow greater interaction between the protein subunits and help form a flexible network in which water is entrapped, however, at higher pressures, aggregation and precipitation are enhanced which will tend to decrease the WHC.

4.3.5 Typical gel electrophoregrams of heat treated (Ht) and preheated soymilk, high pressure set (htHP) tofu gels: native polyacrylamide gel electrophoresis (native PAGE)

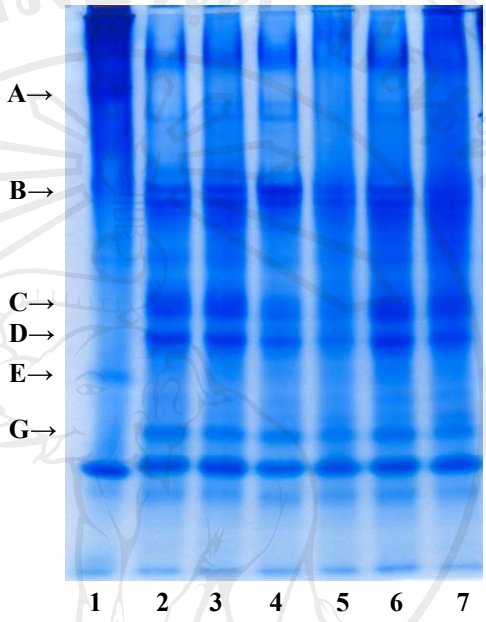

Figure 4.16 Typical native PAGE pattern of heat (Ht) and preheated soymilk, high pressure set (htHP) tofu gels with added 0.4% w/v GDL, 0.4% w/v $CaSO_4.2H_2O$, and 0.14% w/v $CaCl_2.2\ H_2O$: lane 1, raw soymilk (control); lane 2, Ht GDL tofu; lane 3, htHP GDL tofu; lane 4, Ht $CaSO_4$ tofu; lane 5, htHP $CaSO_4$ tofu; lane 6, Ht $CaCl_2$ tofu; lane 7, htHP $CaCl_2$ tofu.

Figure 4.16 illustrates the typical electrophoretic profiles under native conditions of Ht and htHP tofu gels. Raw soymilk (lane 1) as a control sample shows not many bands of protein and is similar to the MF-soymilk (Figure 3.17). The protein pattern of Ht and htHP samples (lane 2 to lane 7) show more protein bands than those found for the raw soymilk, indicating that protein denaturation had occurred both in Ht and htHP tofu samples.

The protein pattern observed for the Ht and htHP tofu gels (lane 2 to lane 7) were similar, indicating that heat treatment at 97-100°C denatured the soy protein stronger than the subsequence treatment, heated at 70°C for 60 min or high pressure (600 MPa 50°C 20 min) on coagulation process. These results also suggest that the tofu samples prepared with difference coagulants show no difference in the overall soluble protein profile on either heat treatment or high pressure treatment.

Band A and E, which were shown in raw soymilk (lane 1), showed a lesser intensity in the Ht and htHP tofu samples (lane 2 to lane 7). Band B, C, D, and G are the new bands which appeared in the Ht and htHP samples and indicated that protein denaturation had occurred in these samples.

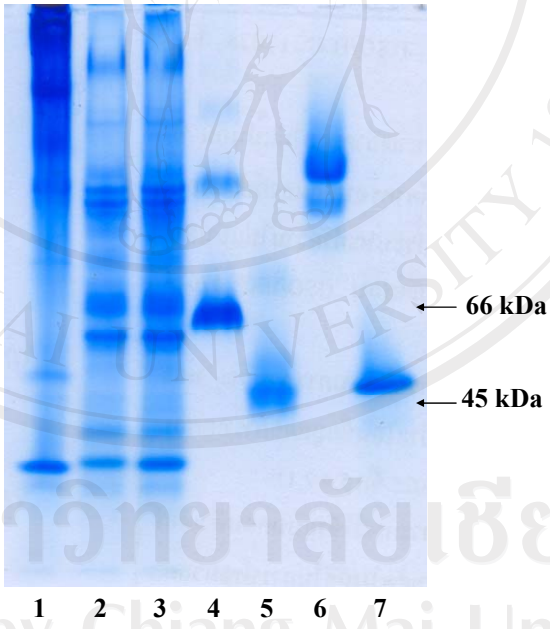

Figure 4.17 Typical native PAGE pattern of heat treated (Ht) and preheated soymilk, high pressure set (htHP) tofu gels with added 0.4% w/v GDL: lane 1, raw soymilk (control); lane 2, Ht GDL tofu; lane 3, htHP GDL tofu; lane 4-7, standards.

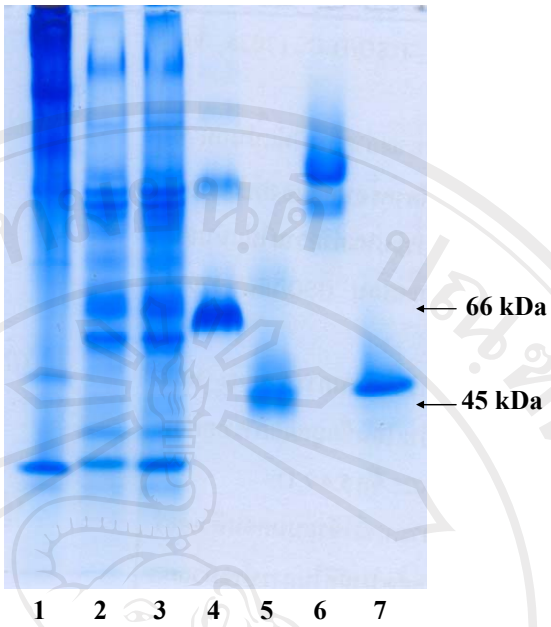

Figure 4.18 Typical native PAGE pattern of heat treated (Ht) and preheated soymilk, high pressure set (htHP) tofu gels with added 0.4% w/v $CaSO_4.2H_2O$: lane 1, raw soymilk (control); lane 2, Ht $CaSO_4$ tofu; lane 3, htHP $CaSO_4$ tofu; lane 4-7, standards.

Figure 4.17 and Figure 4.18 shows typical native PAGE patterns for Ht and htHP induced GDL and $CaSO_4$ tofu gels respectively. Both showed the appearance of 'new' bands in the Ht and htHP samples at about 66 kDa. However, the tofu samples which were prepared using the other various different coagulants show no difference in the soluble protein patterns of soy proteins as a result of any of these treatments.

4.4 Conclusions

The presented results suggested that high pressure can be used to produce filled tofu from preheated soymilk by adding coagulants such as GDL, $CaSO_4.2H_2O$, and $CaCl_2.2H_2O$. The overall appearance of the preheated soymilk, high pressure (htHP) set tofu gels are similar to those samples produced by heat setting (Ht). Other

investigations have suggested some similarities and some differences between these materials. Although the Ht and htHP tofu gels both gave frequency profiles consistent with a solid-like material having a weak gel structure (Ferry, 1980; Steffe, 1996), the htHP tofu products differ significantly from those the Ht products. The htHP tofu gels with added 0.4% GDL and 0.4% $CaSO_4.2H_2O$ were stronger elastically than those for the equivalent Ht tofu gels (section 4.3.2.2.4). The htHP tofu gel with added 0.14% $CaCl_2.2H_2O$ was 'softer' than the equivalent Ht 0.14% sample. However, the htHP tofu sample presents similar tan δ value as the Ht induced sample using the equivalent coagulant indicating the same underlying viscoelastic behaviour. The SEM images showed the structure of htHP and Ht tofu samples are continuous and uniform structures with small holes or voids (section 4.3.3). The htHP tofu gels with added GDL and $CaSO_4.2H_2O$ showed a structure of thicker strands than those of the equivalent Ht samples, resulted in the lower water holding capacity of the htHP tofu gels (section 4.3.4). Native PAGE showed that proteolysis occurred in the htHP and Ht tofu gels with further protein bands appearing when compare to the profiles of the raw soymilks.