CHAPTER 4
RESULTS AND DISCUSSIONS

4.1 The optimal amount of sodium tripolyphosphate (STPP) and sodium chloride (NaCl) incorporating yor formula

The yor formulas were added various levels of STPP (0, 0.25 and 0.50%, w/w) and NaCl (1, 2 and 3%, w/w). The heated samples were subjected to water holding capacity measurements and sensory evaluation for choosing the optimal levels which gave the "best" product.

4.1.1 Water holding capacity

Released water is generally assessed to as the water retained in the casing and at the surface, whereas expressible water is the water remaining in the sample, which can be released when compression is applied (Funami *et al.*, 1998). Therefore, water holding capacity has a close correlation with cooked yield of the product.

Result obtained from analysis of variance indicated that the individual or the combined effects of NaCl and STPP had a positive impact on amount of released plus expressible water ($P \leq 0.05$) (Table 4.1).

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Table 4.1 Amount of released plus expressible water of ostrich-meat yors formulas added different levels of NaCl and STPP

NaCl (%, w/w)	Released plus expressible water (%)
1	12.68 ± 3.01^{A}
2	9.92 ± 2.06^{B}
3	8.10 ± 2.06^{C}

STPP (%, w/w)	Released plus expressible water (%)
0.00	13.33 ± 2.67^{a}
0.25	9.17 ± 1.38^{b}
0.50	8.21 ± 1.97^{c}

NaCl (%, w/w)	STPP (%, w/w)	Released plus expressible water (%)
1	0.00	16.70 ± 0.15^{a}
2	0.00	12.62 ± 0.22^{b}
3	0.00	10.67 ± 0.02^{cd}
1	0.25	10.82 ± 0.01^{c}
2	0.25	9.04 ± 0.02^{e}
3	0.25	7.65 ± 0.04^{g}
1	0.50	10.53 ± 0.01^{d}
2	0.50	8.12 ± 0.05^{f}
3	0.50	5.98 ± 0.04^{h}

All values are the means±sd of three replications.

Means followed by the same letters within each column are significantly different, $P \leq 0.05$ (analysed using Duncan's multiple range test).

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Table 4.1 showed the effect of various concentrations of NaCl and STPP on the amount of released plus expressible water significantly ($P \leq 0.05$) decreased with increasing addition of NaCl and/or STPP, in vice versa water holding capacity of yor formulas increased with increasing concentration of NaCl and/or STPP. This may be caused by distribution of the extra sodium and phosphate ions leading to an increase in the osmotic pressure within the myofibrils. This may well result in the swelling of the filament structure. Therefore, with added NaCl and STPP there is a marked

increase in water holding capacity (Miyaguchi *et al.*, 2004). While Hsu and Chung (1998) found that the higher salt addition in meatball products caused more salt-soluble protein which helped in forming a stable meat emulsion, which in turn lower the cooking losses. Gordon and Barbut (1992) also noted that increasing NaCl from 1.5 to 2.5% (w/w) resulted in differing protein extraction profiles when examined by gel electrophoresis for chicken breast meat batters. This resulted in increased network formation among some proteins and increasing juiciness of the final product (Tseng *et al.*, 2000). Sofos (1986) also found that the effect of phosphates in improving water holding capacity of meat products is greatly enhanced when used in combination with salt.

4.1.2 Sensory evaluation

BIB design was used to determine the sensory evaluation of ostrich-meat yors with various levels of added NaCl and STPP.

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Table 4.2 Sensory attributes of ostrich-meat yors added various NaCl and STPP concentrations

NaCl (%, w/w)	STTP (%, w/w)	Appearance	Colour	Firmness	Saltiness	Juiciness	Overall acceptability
1	0.00	5.16 ± 0.89^{e}	7.50 ± 0.53^{ns}	4.71 ± 0.71^{d}	3.25 ± 0.89^{c}	5.07 ± 0.99^{d}	5.38 ± 0.74^{d}
2	0.00	5.67 ± 0.74^{cde}	7.50 ± 0.53^{ns}	5.75 ± 0.71^{c}	7.50 ± 0.53^{a}	6.01 ± 0.92^{c}	6.38 ± 0.74^{c}
3	0.00	6.46 ± 0.74^{bc}	7.25 ± 0.46^{ns}	6.05 ± 0.92^{c}	6.62 ± 0.52^{b}	6.25 ± 0.71^{c}	6.88 ± 0.35^{abc}
1	0.25	5.61 ± 0.92^{de}	7.38 ± 0.52^{ns}	5.77 ± 0.71^{c}	3.62 ± 0.52^{c}	6.50 ± 0.76^{c}	6.38 ± 0.92^{c}
2	0.25	7.37 ± 0.52^{a}	7.12 ± 0.35^{ns}	7.20 ± 0.83^{a}	8.12 ± 0.83^{a}	7.56 ± 0.53^{a}	7.50 ± 0.53^{a}
3	0.25	7.13 ± 0.83^{ab}	7.25 ± 0.46^{ns}	6.91 ± 0.83^{ab}	6.62 ± 0.74^{b}	7.44 ± 0.52^{ab}	7.38 ± 0.52^{ab}
1	0.50	6.04 ± 0.64^{cd}	7.50 ± 0.53^{ns}	6.22 ± 0.46^{bc}	3.88 ± 0.83^{c}	6.71 ± 0.71^{bc}	6.62 ± 0.52^{bc}
2	0.50	6.95 ± 0.64^{ab}	7.50 ± 0.53^{ns}	7.08 ± 0.83^{a}	8.00 ± 0.76^{a}	7.34 ± 0.52^{ab}	6.88 ± 0.83^{abc}
3	0.50	6.86 ± 0.83^{ab}	7.38 ± 0.52^{ns}	6.95 ± 0.76^{ab}	6.25 ± 0.89^{b}	7.50 ± 0.53^{a}	6.12 ± 0.83^{c}

All values are the means±sd of eight replications.

Means followed by the same letters within each column are significantly different, $P \leq 0.05$ (analysed using Duncan's multiple range test).

ns, no significant difference ($P>0.05$) for any treatment.

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Table 4.2 showed the influence of NaCl and STPP on sensory attributes of ostrich-meat yors. It appeared that NaCl and STPP produced significant ($P \leq 0.05$) changes in appearance, firmness, saltiness, juiciness and overall acceptability whereas, the colour of all formulas were all quite similar ($P > 0.05$). The scores of firmness and juiciness significantly increased with increasing levels of NaCl and STPP ($P \leq 0.05$). These results were in accord with the released plus expressible water (Table 4.1) which higher NaCl and STPP addition gave more salt-soluble proteins on extraction.

In summary, the results of sensory evaluation indicated that yor formula added 2% (w/w) of NaCl and 0.25% (w/w) of STPP gave the highest acceptability. Therefore, these optimized levels of NaCl and STPP (2 and 0.25% (w/w), respectively) were added to the subsequent yor formulations.

4.2 The optimal amount of pepper and garlic added in yor formula

Traditional "yor" is usually made from fresh of pork or fish, but fresh ostrich-meat trimmings, which has strong flavour, was used in this research. Therefore, pepper and garlic were incorporated in the formula to mask the strong ostrich-meat flavour.

The results of sensory evaluation of the yor formulations with various levels of pepper and garlic showed in Table 4.3.

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Table 4.3 Sensory attributes of yor formulas added three levels of pepper or garlic

Pepper (%, (w/w)	Garlic (%, w/w)	Appearance	Pepper flavour	Garlic flavour	Overall acceptance
1	3	7.81±0.46[ns]	5.73±0.46[b]	5.70±0.46[b]	5.81±0.46[c]
2	3	7.46±0.52[ns]	7.85±0.64[a]	5.87±0.35[b]	5.82±0.35[c]
3	3	7.58±0.53[ns]	3.46±0.53[c]	5.64±0.52[b]	5.35±0.52[d]
1	5	7.46±0.53[ns]	5.48±0.53[b]	8.26±0.46[a]	6.40±0.52[b]
2	5	7.69±0.52[ns]	8.06±0.76[a]	8.50±0.53[a]	8.23±0.46[a]
3	5	7.58±0.53[ns]	3.24±0.46[c]	8.11±0.35[a]	6.81±0.46[b]
1	7	7.40±0.53[ns]	5.39±0.52[b]	3.28±0.46[c]	4.76±0.46[e]
2	7	7.46±0.53[ns]	7.70±0.52[a]	3.15±0.35[c]	4.30±0.52[f]
3	7	7.34±0.53[ns]	3.09±0.35[c]	2.99±0.01[c]	2.40±0.35[g]

All values are the means±sd of eight replications.

Means followed by the same letters within each column are significantly different, $P \leq 0.05$ (analysed using Duncan's multiple range test).

ns, no significant difference ($P > 0.05$) for any treatment.

Three levels of added pepper or garlic in the yor formula did not significantly change ($P > 0.05$) the appearance of the products. On the other hand, the flavour of pepper, garlic and the overall acceptance were significantly different among of each formulation ($P \leq 0.05$).

Overall the formulation with 2% (w/w) of pepper and 5% (w/w) of garlic showed the highest scores for all of the measured attributes (Table 4.3). This formulation was used for the subsequent experiments.

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4.3 The optimal condition of pressure, temperature and holding time for yor processing

The concentration of 0.25% (w/w) of STPP, 2% (w/w) of NaCl, 2% (w/w) of pepper and 5% (w/w) of garlic were used for preparation of pressurised ostrich-meat yors. The samples were subjected to different pressures, temperatures and holding times as follows: pressure of 300, 500 and 700 MPa at both 40 and 60°C for both 40 and 60 min. Subsequently, these processed samples were analysed for their various physicochemical properties.

4.3.1 Measurement of colour

Analysis of variance indicated that the individual and combined effects of pressure, temperature and time influenced the L^* (lightness), a^* (redness) and b^* (yellowness) values ($P \leq 0.05$) (Table 4.4).

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Table 4.4 L^*, a^* and b^* values of ostrich-meat yors after treatment at 300, 500, 700 MPa and 40 or 60°C with holding times of 40, 60 min

Pressure (MPa)	Temperature (°C)	Time (min)	L^{*1}	a^{*2}	b^{*3}
300	40	40	43.29 ± 0.31^{g}	20.18 ± 0.26^{a}	8.36 ± 0.01^{i}
300	40	60	44.92 ± 0.25^{f}	19.89 ± 0.02^{a}	8.44 ± 0.02^{i}
300	60	40	44.75 ± 0.42^{f}	19.87 ± 0.58^{a}	8.67 ± 0.19^{h}
300	60	60	45.93 ± 0.20^{e}	19.35 ± 0.35^{b}	9.02 ± 0.06^{g}
500	40	40	49.13 ± 0.91^{d}	18.28 ± 0.30^{c}	9.14 ± 0.02^{fg}
500	40	60	48.57 ± 0.22^{d}	17.81 ± 0.33^{d}	9.21 ± 0.06^{f}
500	60	40	51.39 ± 0.14^{c}	16.79 ± 0.40^{e}	9.29 ± 0.02^{ef}
500	60	60	51.79 ± 0.60^{bc}	16.44 ± 0.19^{e}	9.38 ± 0.06^{de}
700	40	40	52.18 ± 0.03^{ab}	12.60 ± 0.06^{f}	9.51 ± 0.05^{d}
700	40	60	52.35 ± 0.03^{ab}	12.21 ± 0.02^{f}	9.81 ± 0.14^{c}
700	60	40	52.40 ± 0.01^{ab}	11.48 ± 0.01^{g}	9.99 ± 0.05^{b}
700	60	60	52.68 ± 0.01^{a}	11.27 ± 0.03^{g}	10.35 ± 0.17^{a}

$^{1}L^*$ lightness

$^{2}a^*$ redness

$^{3}b^*$ yellowness

All values are the means±sd of three replications.

Means followed by the same letters within each column are significantly different, $P \leq 0.05$ (analysed using Duncan's New multiple range test).

L^* values increased significantly with increasing pressure and temperature. This may reflect the degree of denaturation of the proteins, including the myoglobin (Cheah and Ledward, 1997; Chéret, *et al.*, 2005; Chevalier *et al.*, 2001; Hugas *et al.*, 2002) and the myosin (Mor-Mur and Yuste, 2003). The a^* values decreased significantly with increasing pressure ($P \leq 0.05$) (Table 4.4). This may also be indicative of the oxidation/denaturation of myoglobin under pressure (Cheah and Ledward, 1997; Chéret, *et al.*, 2005; Jung *et al.*, 2003). The b^* values increased significantly with pressure. Tabilo-Munizaga and Barbosa-Cánovas (2004a) reported

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that L^* and b^* values of surimi gels containing potato starch and/or egg white were increased with increasing pressure (400 and 650 MPa for 10 min at 20°C). Moreover, Yuste *et al.* (1999) treated poultry meat sausages at 500 MPa and 50, 60, 70 and 75°C for 30 min and also found that the products increased in lightness but decreased in redness and yellowness at the higher temperatures.

4.3.2 Water holding capacity

Decreased amounts of released plus expressible water indicates an increase in cooking yield since more water is retained in the products, that is they have better water holding capacity (Funami *et al.*, 1998; Visessanguan *et al.*, 2004). Released water is generally referred to as the water retained in the casing and at the surface, whereas expressible water is the water remaining in the sample, which can be released when compression is applied.

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Table 4.5 Amount of released plus expressible water of ostrich-meat yors after treatment at 300, 500, 700 MPa and 40 or 60°C for holding times of 40 and 60 min

Pressure (MPa)	Temperature (°C)	Time (min)	Released plus expressible water (%)
300	40	40	22.89±0.09[a]
300	40	60	22.68±0.10[b]
300	60	40	20.45±0.00[c]
300	60	60	20.24±0.03[d]
500	40	40	16.81±0.03[e]
500	40	60	16.72±0.05[f]
500	60	40	12.57±0.02[g]
500	60	60	12.44±0.03[h]
700	40	40	8.69±0.03[i]
700	40	60	8.63±0.05[i]
700	60	40	8.29±0.02[j]
700	60	60	8.15±0.02[k]

All values are the means±sd of three replications.

Means followed by the same letters within each column are significantly different, $P \leq 0.05$ (analysed using Duncan's New multiple range test).

Table 4.5 showed the effects of pressure, temperature and time on the amount of released plus expressible water in the ostrich-meat yors. The amount of released plus expressible water significantly decreased with increasing pressure, temperature and time ($P \leq 0.05$), the effects were most marked at 500 MPa and above. This improved water holding probably reflects denaturation of the proteins, especially myosin. Since this is the major gel forming protein in meat (Jiménez-Colmenero *et al.*, 1998).

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4.3.3 Gel strength from penetration tests

The factorial design for the combined effect of pressure, temperature and time used to analyse the gel strength results showed that the conditions that significantly affect the treated samples were as follows: pressure, temperature and time as well as the combined effect of both pressure and temperature, pressure and time.

Table 4.6 Gel strength of ostrich-meat yors after treatment at 300, 500, 700 MPa and 40 or 60°C for holding times of 40 and 60 min

Pressure (MPa)	Temperature (°C)	Time (min)	Gel strength (kg.mm)
300	40	40	0.85±0.00[g]
300	40	60	0.85±0.01[g]
300	60	40	1.80±0.00[f]
300	60	60	1.80±0.00[f]
500	40	40	2.53±0.00[e]
500	40	60	2.53±0.00[e]
500	60	40	3.25±0.00[d]
500	60	60	3.25±0.00[d]
700	40	40	4.37±0.00[c]
700	40	60	4.38±0.00[b]
700	60	40	5.00±0.00[a]
700	60	60	5.00±0.00[a]

All values are the means±sd of three replications.

Means followed by the same letters within each column are significantly different, $P \leq 0.05$ (analysed using Duncan's New multiple range test).

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Table 4.6 showed that the gel strength increased sharply with increasing severity of the treatment. This was as expected since several authors had shown that increasing temperatures increased gel strength as did increasing pressure (Apichartsrangkoon *et al.*, 1998; Cheftel and Culioli, 1997; Fernández-Martín *et al.*, 1997; Ma and Ledward, 2004; Zamri *et al.*, 2006). The present results suggested that

the effects in this system were synergistic, which was not always the case in others gel systems (Apichartsrangkoon *et al.*, 1998). Not unexpectedly there was a good correlation between the released plus expressible water and gel strength ($r^2 = 0.974$, $P \leq 0.001$).

4.3.4 Stress relaxation test

The stress relaxation data, subjected to non-linear regression analysis, indicated that the relaxation curves for the pressurised ostrich-meat yors (Figs. 4.1 and 4.2) could be fitted to an equation consisting of a five element model which contains four Maxwell elements connected in parallel with a free spring as shown in Fig. 4.3 (Steffe, 1996). The following equation represented the stress relaxation behavior of the pressurised ostrich-meat yor:

$$\sigma(t) = A_1 \exp(-t/\lambda_1) + A_2 \exp(-t/\lambda_2) + A_3 \exp(-t/\lambda_3) + A_4 \exp(-t/\lambda_4) + \sigma_e$$

Where σ = stress (kPa)

t = time (sec)

λ = relaxation time of each Maxwell element (sec)

σ_e = equilibrium stress (kPa)

A = initial stress of each Maxwell element; this is an indication of predominance of viscous behaviour by the amount of added Maxwell elements (Steffe, 1996)

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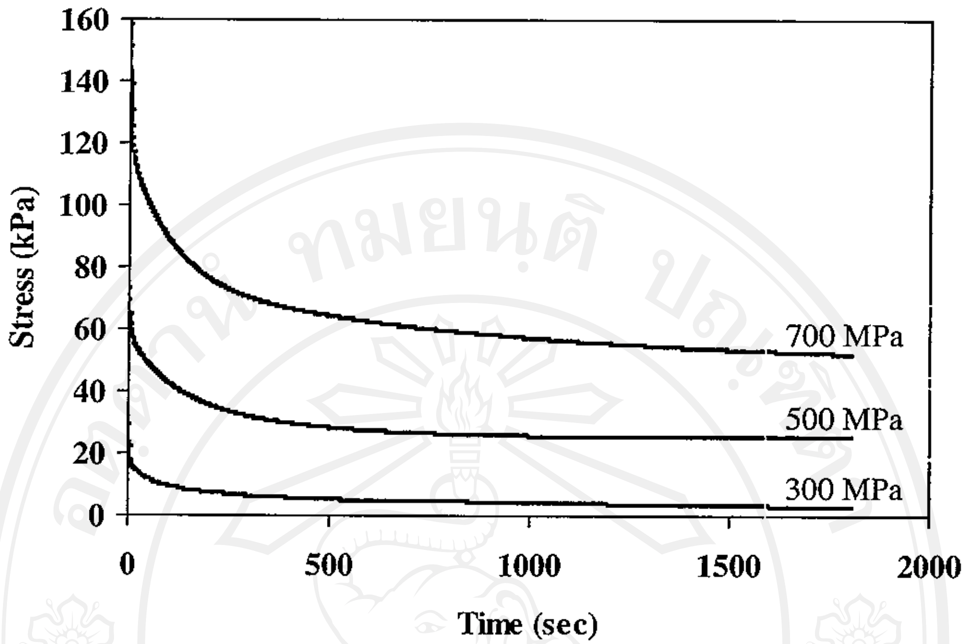

Figure 4.1 Stress relaxation curves of ostrich-meat yors after treatment at 300, 500, 700 MPa and 40°C with a holding time for 60 min.

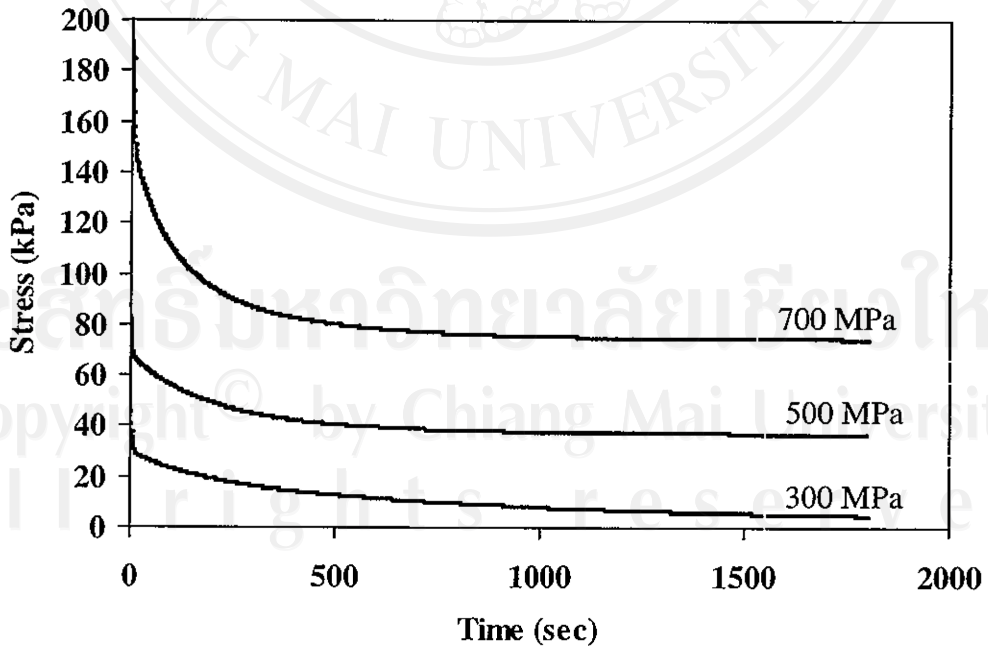

Figure 4.2 Stress relaxation curves of ostrich-meat yors after treatment at 300, 500, 700 MPa and 60°C with a holding time for 60 min.

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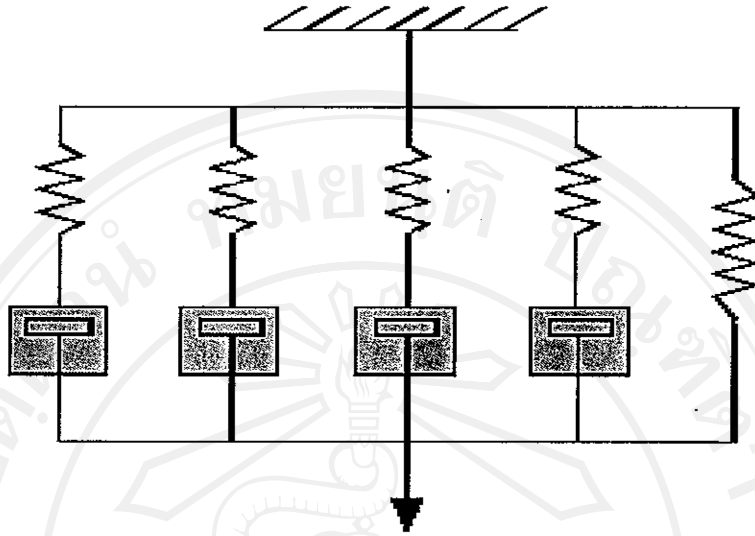

Figure 4.3 Model developed from the stress relaxation curves of pressurised ostrich-meat yor.

Figures 4.1 and 4.2 showed the stress relaxation curves of the samples treated at 300-700 MPa for 60 min at 40 and 60°C, respectively. Samples treated at the higher pressures and temperatures displayed both greater initial and equilibrium stress than those treated under milder conditions (Table 4.7). This was probably due to the combined effect of pressure and heat giving rise to a firmer structure with more elasticity or cross-link density (Apichartsrangkoon, 2002).

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Table 4.7 Relaxation time and equilibrium stress of ostrich-meat yors after treatment at 300, 500, 700 MPa and 40 or 60°C for holding times of 40 and 60 min

Pressure (MPa)	Temperature (°C)	Time (min)	Relaxation time (sec)	Equilibrium stress (kPa)
300	40	40	38 ± 2^{j}	1.62 ± 0.21^{j}
300	40	60	58 ± 2^{i}	2.62 ± 0.20^{i}
300	60	40	144 ± 3^{h}	4.10 ± 0.17^{h}
300	60	60	268 ± 5^{g}	4.27 ± 0.17^{h}
500	40	40	561 ± 9^{f}	13.63 ± 0.19^{g}
500	40	60	606 ± 17^{e}	25.01 ± 0.17^{f}
500	60	40	676 ± 14^{d}	35.12 ± 0.16^{e}
500	60	60	720 ± 22^{c}	36.63 ± 0.17^{d}
700	40	40	727 ± 20^{c}	49.98 ± 0.16^{c}
700	40	60	865 ± 11^{b}	51.76 ± 0.18^{b}
700	60	40	885 ± 8^{a}	73.75 ± 0.01^{a}
700	60	60	903 ± 2^{a}	73.89 ± 0.02^{a}

All values are the means±sd of three replications.

Means followed by the same letters within each column are significantly different, $P\leq0.05$ (analysed using Duncan's New multiple range test).

Equilibrium stress refers to the viscoelastic materials relaxing to the end point which depends on the molecular structure of the material being tested (Steffe, 1996). Relaxation time, the time it takes for the stress to decay to 1/e (approximately 36.8%) of its initial stress value, also increased with the severity of treatment (Table 4.7) as would be expected for the firmer gels. Shellhammer *et al.* (1997) stated that a material that has a short relaxation time infers that the stress imposed by a strain dissipates quickly and therefore the material is more viscous than elastic in nature.

Analysis of variance in factorial design showed that pressure, temperature and time as well as the combination of pressure and temperature, pressure and time as well as pressure, temperature and time significantly influenced both the relaxation time and equilibrium stress values ($P\leq0.05$). As with the gel strength (Table 4.6) these

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values increased with increase in treatment severity. Thus the elasticity gradually increased with increasing severity as a result of increasing cross-link density (Apichartsrangkoon, 2002; Apichartsrangkoon and Ledward, 2002).

4.3.5 Differential scanning calorimetry (DSC)

Differential scanning calorimetry is a powerful technique that has been used to study the structural and thermal properties of natural polymers such as proteins and it has been used to relate the denaturation of individual muscle proteins to the textural changes in meat caused by cooking and pressurization (Ma and Ledward, 2004).

Figures 4.4 and 4.5 showed the DSC thermograms of pressurised ostrich-meat yors from 300, 500 and 700 MPa for 60 min at temperature 40 (Fig. 4.4) and 60°C (Fig. 4.5) compared with untreated sample.

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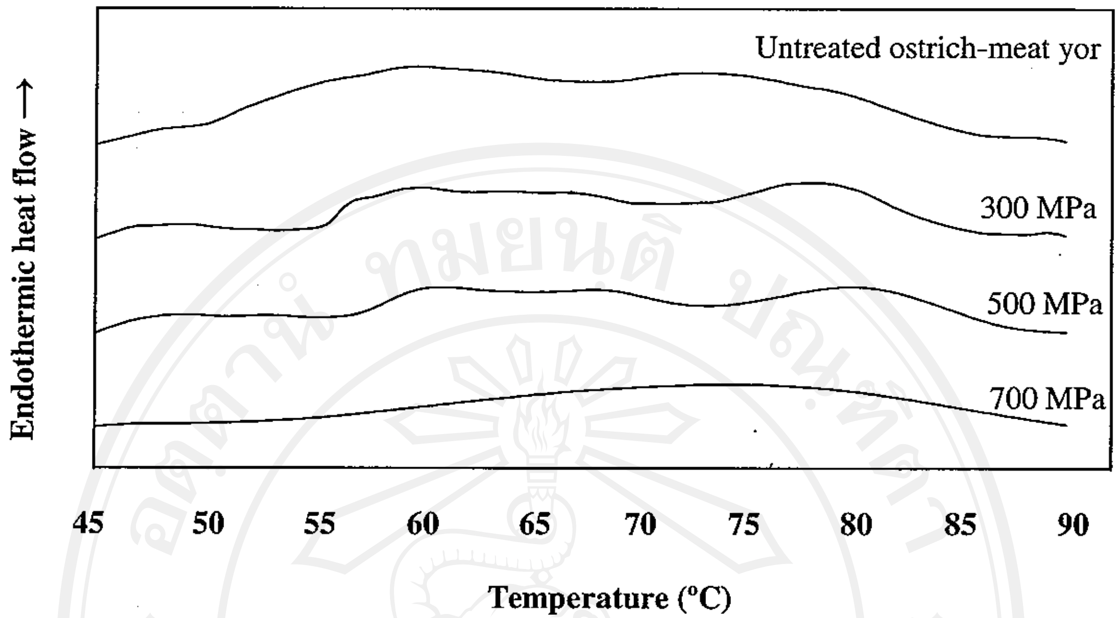

Figure 4.4 DSC thermograms of pressurised ostrich-meat yor at 300, 500 and 700 MPa 40°C for 60 min and untreated sample.

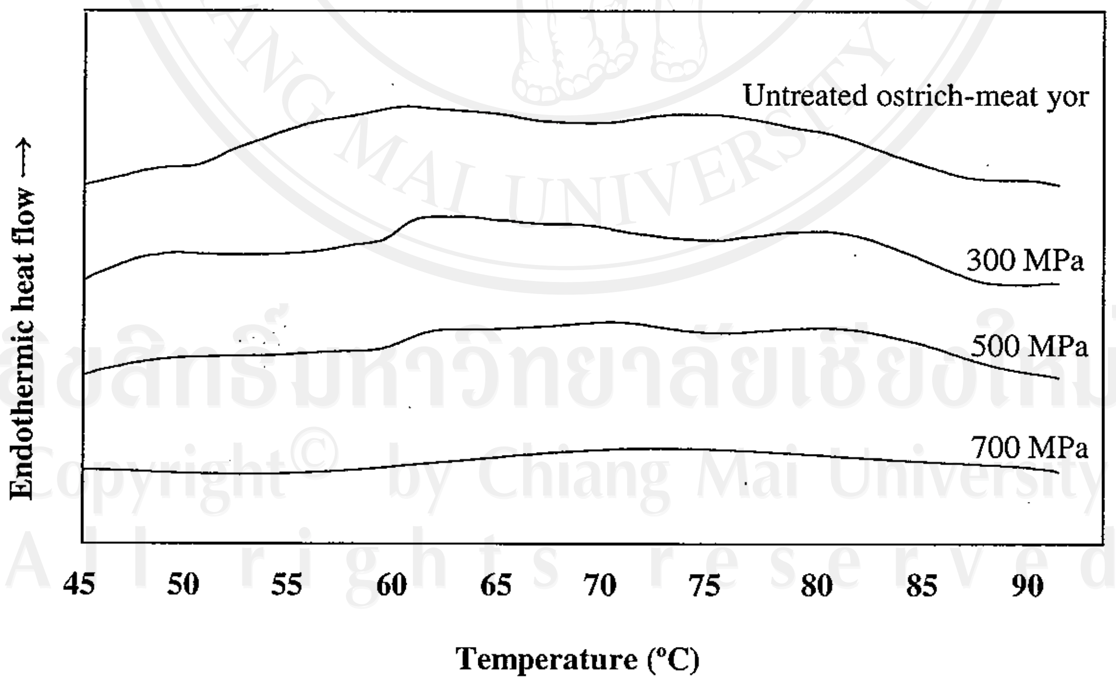

Figure 4.5 DSC thermograms of pressurised ostrich-meat yor at 300, 500 and 700 MPa 60°C for 60 min and untreated sample.

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Two discernible peaks for myosin and actin of untreated sample could be observed at transition temperature 59.09 (T_{max1}) and 75.81°C (T_{max2}), respectively. Amako and Xiong (2001) revealed that typical transition temperatures for muscle proteins could range from 43 to 67°C for myosin and 71 to 81°C for actin. For example endothermic transition peaks for myosin and actin of beef muscle were 54.6 and 77.3°C, respectively (Ma and Ledward, 2004). In the samples treated at 300 and 500 MPa, these two peaks still remain (Fig. 4.4) but with reduce enthalpies of protein denaturation (Fig. 4.5 and Table 4.8). The endothermic peaks disappeared after pressurisation above 700 MPa (Figs. 4.4- 4.5 and Table 4.8). It had been shown that higher pressure enhances denaturation and gelation of the structural proteins of muscle (myosin and actomyosin) (Cheftel and Culioli, 1997). In addition, the denaturation temperature of these two peaks shifted to higher temperature with severe treatment conditions (Table 4.8). Similar result was observed by Fernández-Martín *et al.* (1997) who found that in pork meat batters pressurised at 200-400 MPa and 10-70°C for 30 min, the enthalpy of protein denaturation decreased with increased severity of treatment.

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Table 4.8 Transition temperatures and enthalpies of protein denaturation for myosin and actin (T_{max1}, T_{max2}, ΔH_1, ΔH_2) of pressurised samples at 300, 500, 700 MPa temperature 40, 60°C holding time for 40, 60 min compared with untreated sample

Pressure (MPa)	Temperature (°C)	Time (min)	T_{max1} for myosin (°C)	ΔH_1 (J/g)	T_{max2} for actin (°C)	ΔH_2 (J/g)
Raw ostrich-meat yor			59.09 ± 0.55^{d}	0.53 ± 0.06^{a}	75.81 ± 0.27^{d}	0.46 ± 0.02^{a}
300	40	40	59.75 ± 0.35^{c}	0.46 ± 0.02^{b}	78.94 ± 0.05^{c}	0.43 ± 0.02^{b}
300	40	60	59.57 ± 0.16^{cd}	0.38 ± 0.01^{c}	78.75 ± 0.14^{c}	0.38 ± 0.03^{c}
300	60	40	60.95 ± 0.10^{ab}	0.35 ± 0.00^{d}	80.83 ± 0.49^{a}	0.35 ± 0.01^{c}
300	60	60	61.29 ± 0.34^{a}	0.30 ± 0.01^{d}	79.87 ± 0.15^{b}	0.31 ± 0.02^{d}
500	40	40	60.81 ± 0.10^{ab}	0.28 ± 0.01^{e}	80.02 ± 0.00^{b}	0.29 ± 0.01^{de}
500	40	60	60.54 ± 0.00^{b}	0.18 ± 0.01^{e}	80.53 ± 0.00^{a}	0.28 ± 0.01^{de}
500	60	40	61.03 ± 0.29^{ab}	0.17 ± 0.00^{e}	80.87 ± 0.00^{a}	0.27 ± 0.01^{e}
500	60	60	61.24 ± 0.19^{a}	0.15 ± 0.02^{g}	80.53 ± 0.35^{a}	0.26 ± 0.01^{e}
700	40	40	np	np	np	np
700	40	60	np	np	np	np
700	60	40	np	np	np	np
700	60	60	np	np	np	np

np, no peak was observed. All values are the means±sd of three replications.

Means followed by the same letters within each column are significantly different, $P\leq0.05$ (analysed using Duncan's multiple range test).

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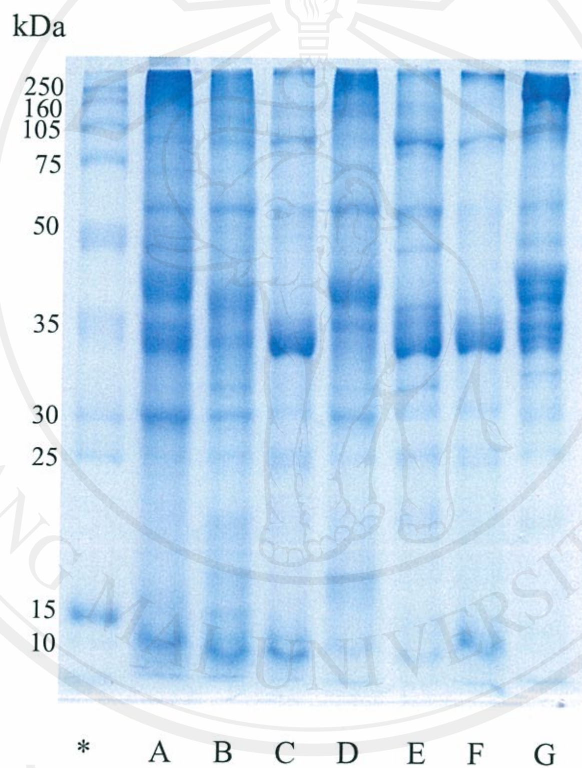
4.3.6 Electrophoretic characterisation

Figures 4.6-4.9 presented the electrophoretic patterns of ostrich-meat yor after treatment at 300, 500, 700 MPa and 40 or 60°C for holding times of 40 or 60 min compared to the untreated ostrich yor in the absence and presence of 2-mercaptoethanol. The gels were stained using Coomassie Brilliant Blue G-250. Following Figs. 4.10-4.13 showed the same samples which were using stained silver.



A = 300 MPa, 40°C B = 500 MPa, 40°C C = 700 MPa, 40°C
D = 300 MPa, 60°C E = 500 MPa, 60°C F = 700 MPa, 60°C
G = Raw ostrich yor * = Molecular weight markers

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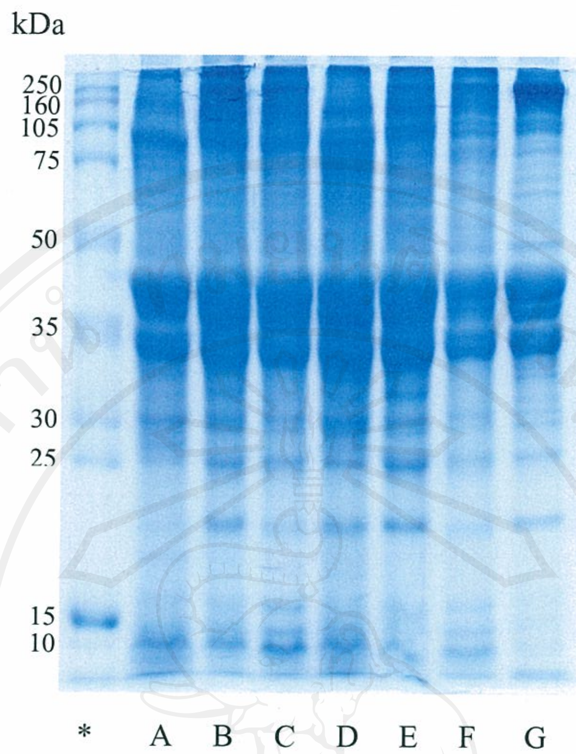
Figure 4.6 The electrophoretic patterns of ostrich-meat yor after treatment at 300, 500, 700 MPa and 40 or 60°C for holding times of 40 min compared to raw ostrich yor in the absence of 2-mercaptoethanol; the samples were stained using the Coomassie Brilliant Blue G-250 method.



A = 300 MPa, 40°C B = 500 MPa, 40°C C = 700 MPa, 40°C
D = 300 MPa, 60°C E = 500 MPa, 60°C F = 700 MPa, 60°C
G = Raw ostrich yor * = Molecular weight markers

Figure 4.7 The electrophoretic patterns of ostrich-meat yor after treatment at 300, 500, 700 MPa and 40 or 60°C for holding times of 40 min compared to raw ostrich yor in the presence of 2-mercaptoethanol; the samples were stained using the Coomassie Brilliant Blue G-250 method.

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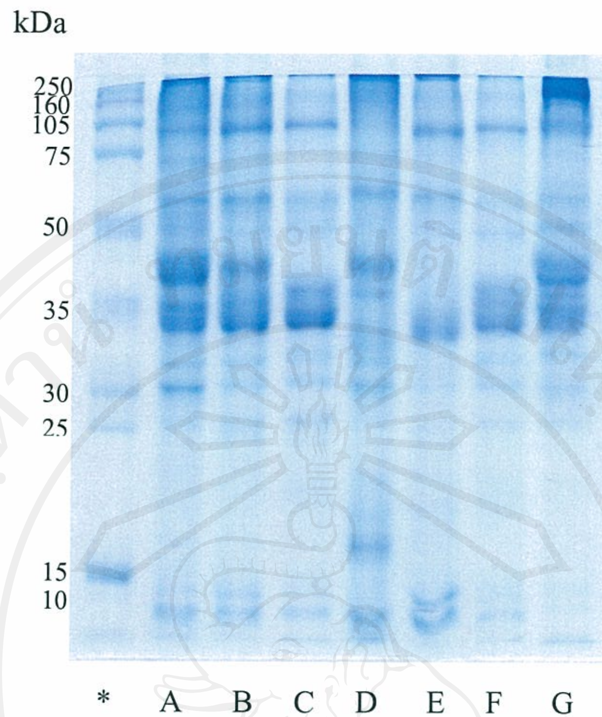

A = 300 MPa, 40°C B = 500 MPa, 40°C C = 700 MPa, 40°C

D = 300 MPa, 60°C E = 500 MPa, 60°C F = 700 MPa, 60°C

G = Raw ostrich yor * = Molecular weight markers

Figure 4.8 The electrophoretic patterns of ostrich-meat yor after treatment at 300, 500, 700 MPa and 40 or 60°C for holding times of 60 min compared to raw ostrich yor in the absence of 2-mercaptoethanol; the samples were stained using the Coomassie Brilliant Blue G-250 method.

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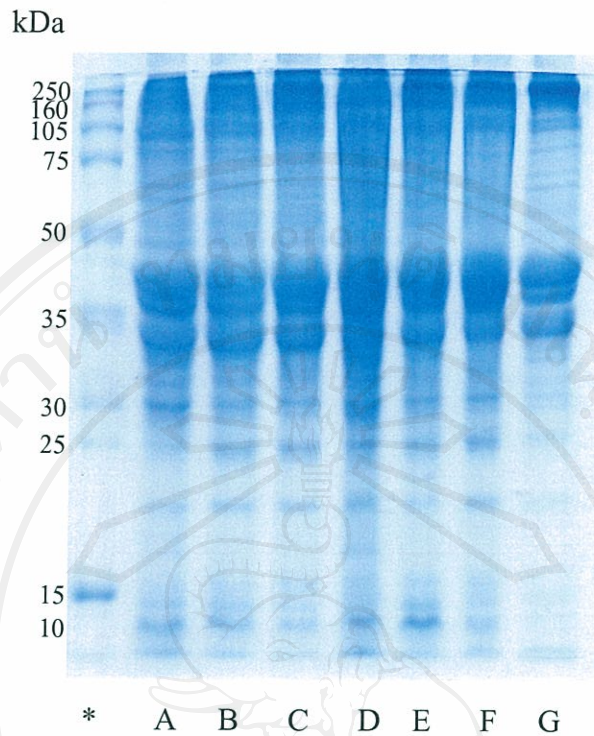

A = 300 MPa, 40°C B = 500 MPa, 40°C C = 700 MPa, 40°C
D = 300 MPa, 60°C E = 500 MPa, 60°C F = 700 MPa, 60°C
G = Raw ostrich yor * = Molecular weight markers

Figure 4.9 The electrophoretic patterns of ostrich-meat yor after treatment at 300, 500, 700 MPa and 40 or 60°C for holding times of 60 min compared to raw ostrich yor in the presence of 2-mercaptoethanol; the samples were stained using the Coomassie Brilliant Blue G-250 method.

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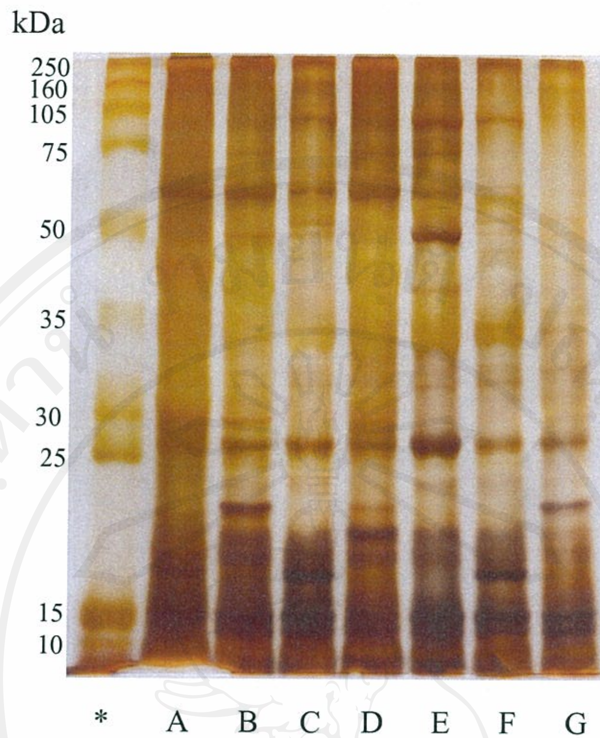

A = 300 MPa, 40°C B = 500 MPa, 40°C C = 700 MPa, 40°C

D = 300 MPa, 60°C E = 500 MPa, 60°C F = 700 MPa, 60°C

G = Raw ostrich yor * = Molecular weight markers

Figure 4.10 The electrophoretic patterns of ostrich-meat yor after treatment at 300, 500, 700 MPa and 40 or 60°C for holding times of 40 min compared to raw ostrich yor in the absence of 2-mercaptoethanol; the samples were using a silver stain method.

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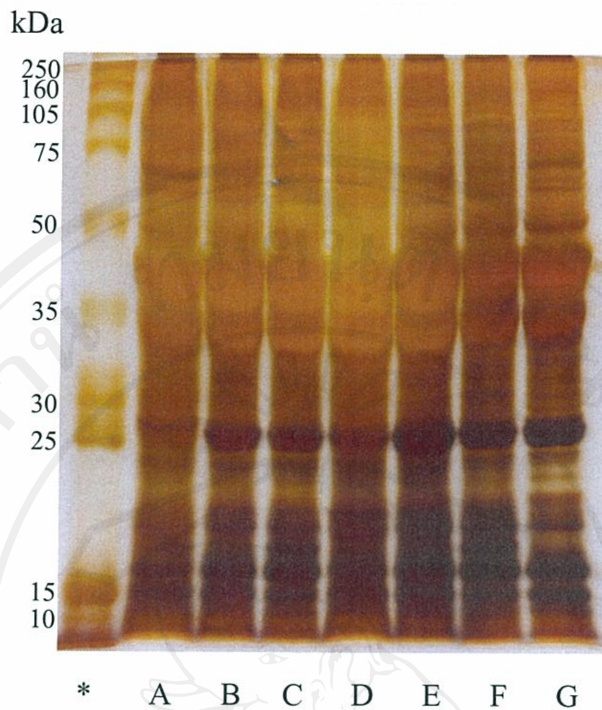

A = 300 MPa, 40°C　　B = 500 MPa, 40°C　　C = 700 MPa, 40°C

D = 300 MPa, 60°C　　E = 500 MPa, 60°C　　F = 700 MPa, 60°C

G = Raw ostrich yor　　* = Molecular weight markers

Figure 4.11　The electrophoretic patterns of ostrich-meat yor after treatment at 300, 500, 700 MPa and 40 or 60°C for holding times of 40 min compared to raw ostrich yor in the presence of 2-mercaptoethanol; the samples were using a silver stain method.

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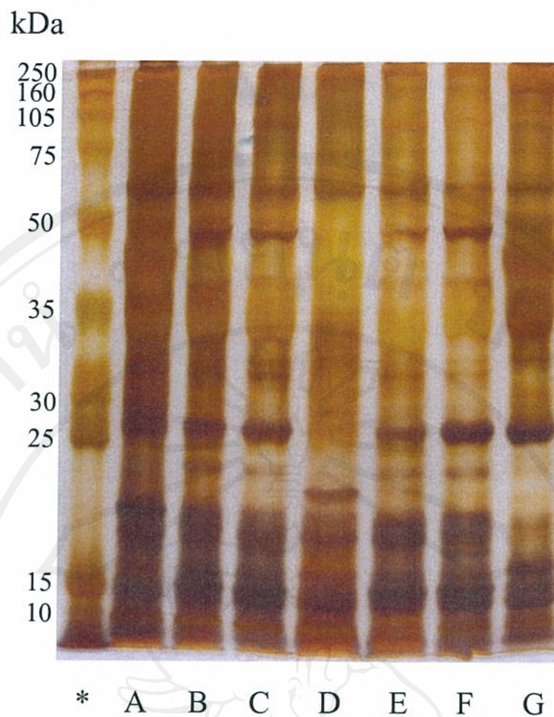

A = 300 MPa, 40°C   B = 500 MPa, 40°C   C = 700 MPa, 40°C
D = 300 MPa, 60°C   E = 500 MPa, 60°C   F = 700 MPa, 60°C
G = Raw ostrich yor   * = Molecular weight markers

Figure 4.12 The electrophoretic patterns of ostrich-meat yor after treatment at 300, 500, 700 MPa and 40 or 60°C for holding times of 60 min compared to raw ostrich yor in the absence of 2-mercaptoethanol; the samples were using a silver stain method.

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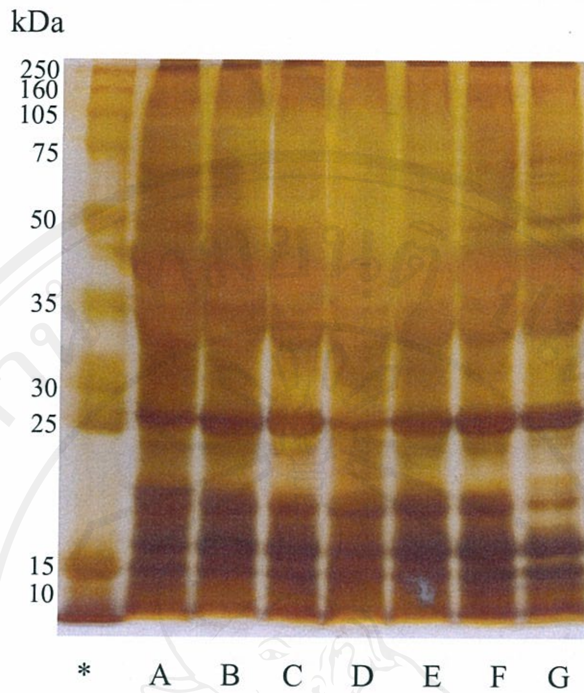

A = 300 MPa, 40°C B = 500 MPa, 40°C C = 700 MPa, 40°C
D = 300 MPa, 60°C E = 500 MPa, 60°C F = 700 MPa, 60°C
G = Raw ostrich yor * = Molecular weight markers

Figure 4.13 The electrophoretic patterns of ostrich-meat yor after treatment at 300, 500, 700 MPa and 40 or 60°C for holding times of 60 min compared to raw ostrich yor in the presence of 2-mercaptoethanol; the samples were using a silver stain method.

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The electrophoretic patterns of the samples treated at the same pressure and temperature for 40 min (Fig. 4.6) were similar to those of treated for 60 min (Fig. 4.8). This means that holding time for 40 and 60 min did not affect the protein profiles. However, the electrophoretic patterns of the samples treated at various pressures and temperatures showed different protein profiles. The electrophoregrams of the samples treated at 300 (A and D) and 500 MPa (B and E) displayed some slight reduction in the intensity of some of the bands, especially the lower molecular masses (30-50 kDa). It is markedly that the electrophoregrams of the samples treated at 700 MPa (C and F) are more reduction in the intensity of some bands at the same of those molecular mass regions. This tended to suggest that high pressure treatment at 700 MPa made the ostrich-meat proteins less soluble in the SDS due to formation of covalent (presumably disulphide) bonds (Apichartsrangkoon *et al.*, 1998). The addition of a reducing agent, 2-mercaptoethanol, which ruptured any disulphide bonds present and solubilised any aggregates so that the electrophoretic patterns were similar to those of the raw ostrich-meat yor (Figs. 4.7 and 4.9). These results confirm that the higher pressure and temperature enhance the gel strength and viscoelastic properties of ostrich-meat yors.

The electrophoregram of pressurised ostrich-meat yor using silver staining (Figs. 4.10-4.13) were similar to those of the samples stained using Coomassie Brilliant Blue G-250 (Figs. 4.6-4.9). However, the silver staining produces much sharper bands and a higher band resolution than Coomassie Brilliant Blue G-250, with silver staining a sensitivity of 15 pg per band can be achieved (Westermeier, 2001). Such electrophoregrams using silver staining consequently display more protein bands than those stained using Coomassie Brilliant Blue.

It is concluded that combined pressure and temperature will texturise comminuted ostrich-meat system yielding product with a variety of colours and textures. Thermal analysis showed that extreme treatment conditions (700 MPa, 40°C, 40 min) can induce complete denaturation of ostrich-meat proteins. The colour measurement results also agreed with DSC thermogram in that the L^* and b^* values increased but a^* value decreased with increasing severity of treatment which reflected the degree of protein modification. Gel strength and stress relaxation determination also confirm by electrophoregrams and display similar results as DSC and colour

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measurement. Thus these treatments may be a useful means of producing products with different eating qualities to help satisfy the diverse requirements of the market-place.

4.4 Investigate the interaction of soy protein isolate (SPI), whey protein isolate (WPI) and wheat gluten (WG) on pressurised ostrich-meat yor

An optimum condition of section 4.3 was assigned for this experiment, subsequently mixed with various concentrations of SPI, WPI and WG at levels of 0-4% (w/w). The processed samples were subjected to several physicochemical analysis as follows:

4.4.1 Transient measurement for Creep test

Viscoelastic behaviour of a gel network of pressurised ostrich-meat yor under constant stress (50 Pa, Fig. 3.3) following creep curves as shown in Figs. 4.14 and 4.15.

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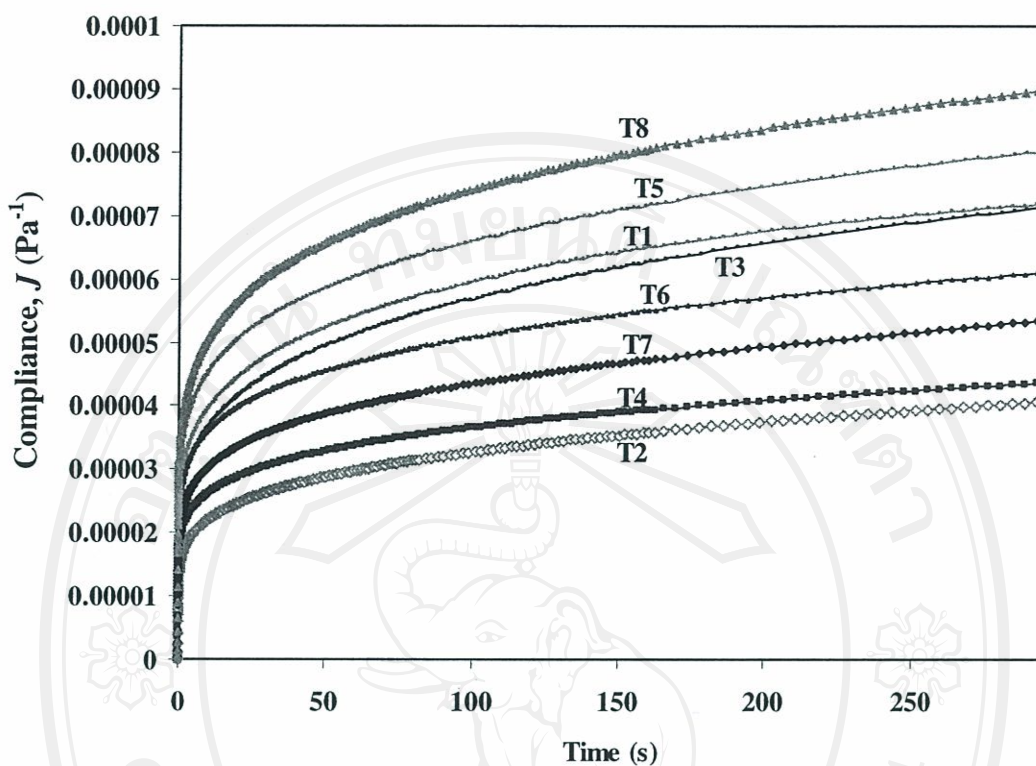

T1 = 4% (w/w) SPI | T2 = 4% (w/w) WPI

T3 = 4% (w/w) WG | T4 = 2% (w/w) SPI, 2% (w/w) WPI

T5 = 2% (w/w) SPI, 2% (w/w) WG | T6 = 2% (w/w) SPI, 2% (w/w) WG

T7 = 1.33% (w/w) SPI, 1.33% (w/w) WPI, 1.33% (w/w) WG

T8 = Control

Figure 4.14 Time-dependent creep compliance of pressurised ostrich-meat yors mixed with various concentrations of SPI, WPI and WG at 0-4% (w/w).

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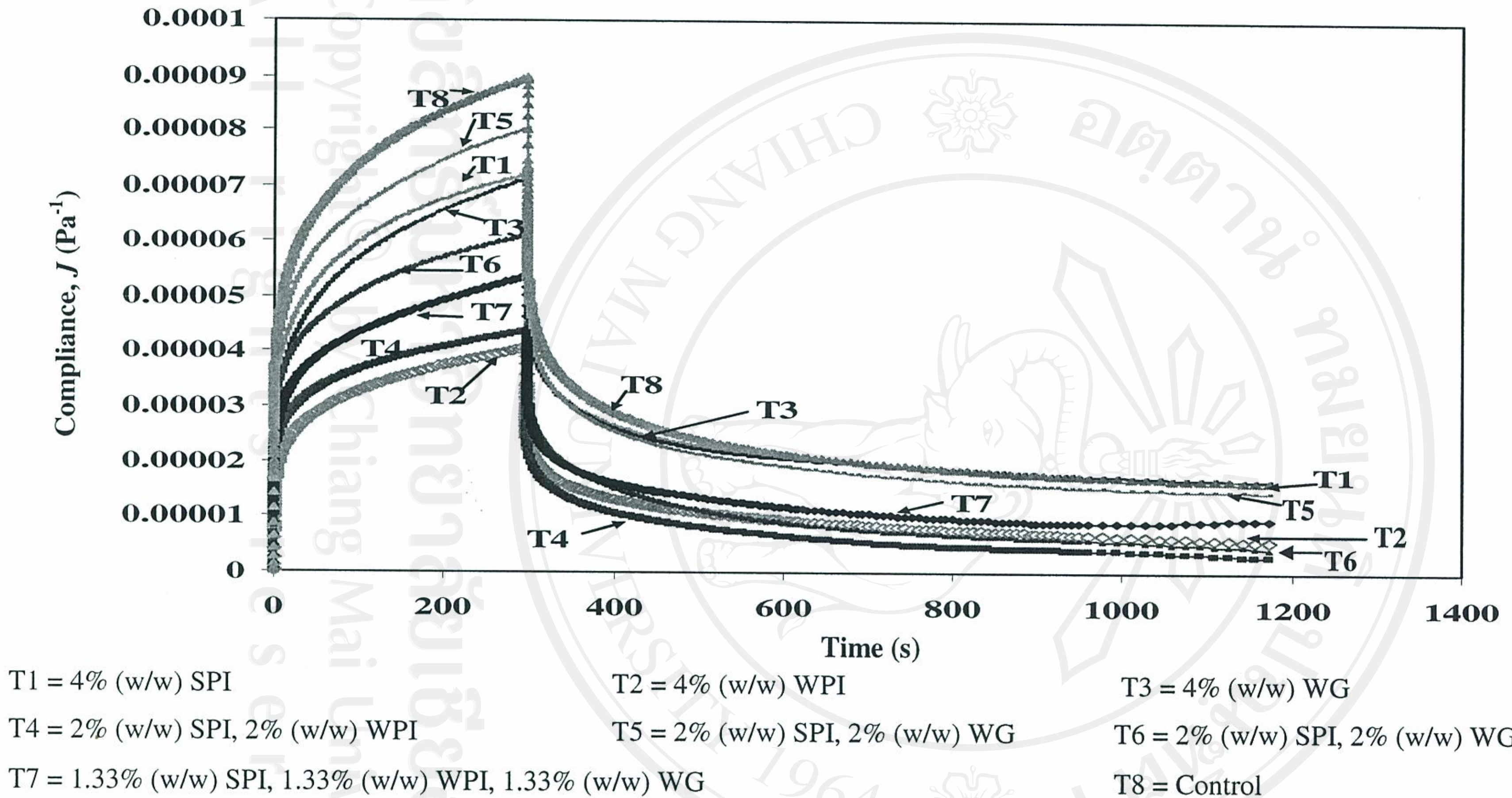

T1 = 4% (w/w) SPI T2 = 4% (w/w) WPI T3 = 4% (w/w) WG

T4 = 2% (w/w) SPI, 2% (w/w) WPI T5 = 2% (w/w) SPI, 2% (w/w) WG T6 = 2% (w/w) SPI, 2% (w/w) WG

T7 = 1.33% (w/w) SPI, 1.33% (w/w) WPI, 1.33% (w/w) WG T8 = Control

Figure 4.15 Time-dependent creep/recovery compliance of pressurised ostrich-meat yors mixed with various concentrations of SPI, WPI and WG at 0-4% (w/w).

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A creep curve is basically divided into three distinct regions. Following applied constant stress, there is an immediate strain, referred to an instantaneous elastic compliance (represented by the spring unit of the Maxwell element). Secondly the subsequent deformation of both elastic and viscous elements, referred to a retarded elastic compliance (represented by three Kelvin-Voight elements), finally at a sufficient large time scales, the resultant deformation is due solely to viscous flow, referred to a Newtonian compliance (represented by a dashpot of the Maxwell element). The recovery compliance could also be divided into three regions: the instantaneous elastic recovery; the retarded elastic recovery; and the nonrecoverable strain (Chen *et al.*, 2000; Steff, 1996).

All creep data in Figs. 4.14 and 4.15, were fitted with an equation consisting eight-element Burgers model (Fig. 4.16) as follows

$$J(t) = J_0 + \sum_{i=1}^{3} [Ji\ (1\text{-}\exp(-t\,/\,\lambda_j))] + t\,/\mu_0$$

Creep parameters of the model were illustrated in Table 4.9, where $J(t)$ represented the compliance, J_0 represented the instantaneous compliance, J_i and λ_i represented retardation compliance and time of the Kelvin-Voight component, μ_0 represented Newtonian viscosity, J_{total} represented J_0 plus J_i also referred to the steady state compliance (Steff, 1996).

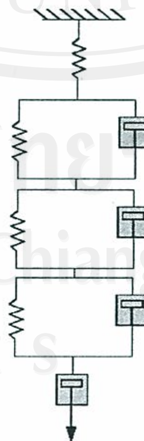

Figure 4.16 Viscoelastic model developed from the creep curves of pressurised ostrich-meat yor.

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Table 4.9 Creep parameters of pressurised ostrich-meat yors incorporating non-meat proteins

SPI (%)	WPI (%)	WG (%)	$J_o \times 10^{-6}$ (Pa^{-1})	$J_1 \times 10^{-6}$ (Pa^{-1})	$J_2 \times 10^{-6}$ (Pa^{-1})	$J_3 \times 10^{-6}$ (Pa^{-1})	$J_{total} \times 10^{-6}$ (Pa^{-1})	λ_1 (s)	λ_2 (s)	λ_3 (s)	Viscosity (μ_0) (1×10^6 Pa.s)
0	0	0	30.47 ± 0.09^{a}	17.33 ± 1.38^{bc}	11.80 ± 0.46^{a}	10.90 ± 0.92^{a}	70.50 ± 0.00^{a}	2.33 ± 0.09^{e}	34.56 ± 2.01^{f}	46.18 ± 0.32^{f}	15.00 ± 0.15^{e}
4	0	0	23.50 ± 2.23^{b}	19.57 ± 2.05^{b}	9.37 ± 0.90^{b}	7.17 ± 0.25^{b}	59.60 ± 0.89c	10.28 ± 0.24^{b}	62.87 ± 0.13^{c}	76.87 ± 0.53^{a}	24.00 ± 0.92^{c}
0	4	0	13.77 ± 1.16^{d}	8.47 ± 1.96^{e}	4.73 ± 0.39^{d}	3.73 ± 0.31^{e}	30.70 ± 1.85^{h}	5.57 ± 0.15^{d}	44.52 ± 0.34^{e}	50.07 ± 1.53^{e}	28.00 ± 0.69^{b}
0	0	4	22.53 ± 2.50^{b}	16.53 ± 0.61^{c}	8.77 ± 0.15^{b}	6.33 ± 0.12^{c}	54.17 ± 1.95^{d}	9.96 ± 0.89^{b}	64.04 ± 2.11^{c}	66.69 ± 1.69^{b}	17.00 ± 2.61^{d}
2	2	0	17.93 ± 0.93^{c}	10.63 ± 0.49^{de}	3.67 ± 0.31^{e}	3.27 ± 0.15^{e}	35.50 ± 1.76^{g}	13.53 ± 0.22^{a}	80.50 ± 1.71^{b}	60.60 ± 0.78^{d}	36.00 ± 0.90^{a}
2	0	2	29.90 ± 1.65^{a}	22.30 ± 0.70^{a}	6.53 ± 0.23^{c}	5.47 ± 0.21^{d}	64.20 ± 2.23^{b}	14.02 ± 1.61^{a}	88.04 ± 2.19^{a}	65.70 ± 3.30^{bc}	19.00 ± 1.76^{d}
0	2	2	25.17 ± 1.12^{b}	11.00 ± 1.67^{d}	5.33 ± 0.29^{d}	6.37 ± 0.38^{c}	47.87 ± 0.64^{e}	6.12 ± 0.12^{d}	52.70 ± 1.49^{d}	63.54 ± 1.43^{cd}	24.00 ± 0.26^{c}
1.33	1.33	1.33	19.43 ± 1.44^{c}	9.93 ± 0.85^{de}	5.30 ± 0.36^{d}	5.90 ± 0.53^{cd}	40.57 ± 2.17^{f}	8.20 ± 0.28^{c}	50.72 ± 0.21^{d}	49.70 ± 2.20^{e}	26.00 ± 2.99^{bc}

All values are means±sd of three replications.

Means followed the same letters within each column are significantly different, $P \leq 0.05$ (analysed using Duncan's New multiples range test).

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Most J_0 and J_{total} of the samples added individual or combination of SPI, WPI and WG were significantly ($P \leq 0.05$) lower than those of the control, except μ_0 appeared higher than those control sample, suggesting an increase in elastic or solid-like structure (Ojijo *et al.*, 2004). This was probably due to the combined effect of pressure (700 MPa) and heat (40ºC) transformed sol of non-meat proteins some of which might interact with meat proteins to create a gel-like product given rise to more crosslink density (Apichartsrangkoon, 2002; Supavititpatana and Apichartsrangkoon, 2007). As a consequence, the samples added non-meat proteins were more rigid or elastic than the control sample. Nagano and Nishinari (2001) who studied the rheology of high-gelling egg white and standard egg white gels using creep measurement. They found that the standard egg white gels are higher compliance possessed lower viscoelastic constants than high-gelling egg white gel, indicating that high-gelling egg white gel was more rigid and less fluid than standard egg white. Regression coefficient analysis of pressurised samples added non- meat protein also showed the same trends (Appendix B).

4.4.2 Dynamic viscoelastic behaviour for oscillation measurement

Dynamic viscoelastic measurement for storage modulus (G'), loss modulus (G'') and loss tangent ($\tan \delta$) are among parameters that characterise the structure of samples generated by high pressure treatment. The G' and G'' are measure of the energy stored and dissipated in the material whereas $\tan \delta$ is a ratio of G'' over G' (Jiménez-Avalos *et al.*, 2005).

Figures 4.17 and 4.18 illustrates the effect of added individual or combination of SPI, WPI and WG for pressurised samples 700 MPa at 40ºC for holding time 40 min. on G' (a) and G'' (b) plots against frequency (0.01-10 Hz.) compared with non-protein added sample (control sample).

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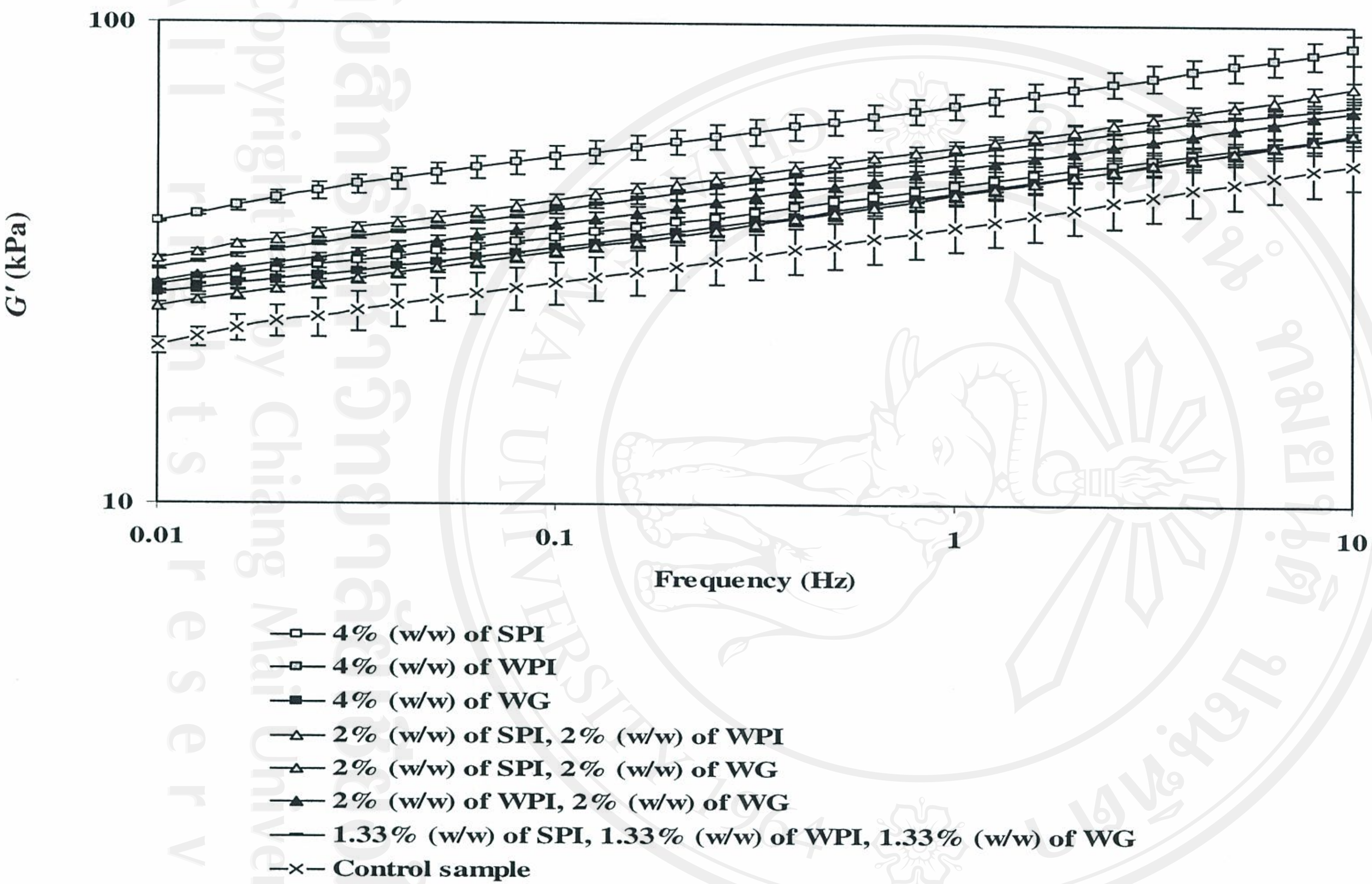

Figure 4.17 Storage modulus as a function of frequency for pressurised ostrich-meat yors added individual or combination of non-meat proteins.

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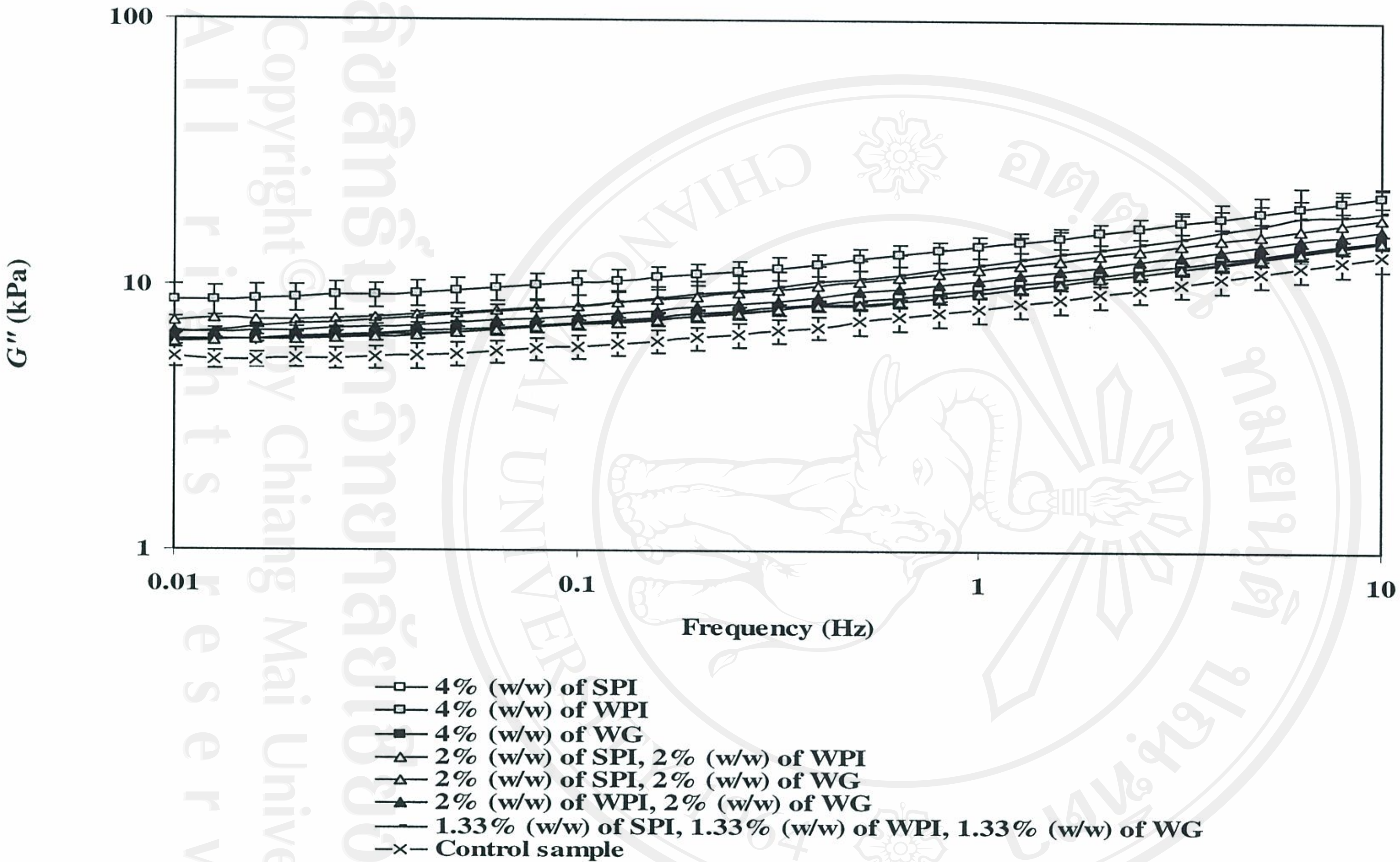

Figure 4.18 Loss modulus as a function of frequency for pressurised ostrich-meat yors added individual or combination of non-meat proteins.

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The G' and G'' plots of pressurised samples with added WIP was the highest moduli, following by samples added SPI and WG respectively. Whereas the combination of non-meat proteins added in the samples caused reduction of both moduli, being the weakest viscoelastic structure, however, those were still stronger structure than that of the control pressurised yor.

Table 4.10 Storage (G') and loss (G'') moduli, and tan δ measured at a frequency 1 Hz of pressurised ostrich-meat yors incorporating non-meat proteins

SPI (%, w/w)	WPI (%, w/w)	WG (%, w/w)	G' (kPa)	G'' (kPa)	tan δ
0	0	0	37.82 ± 4.21^{d}	8.19 ± 0.94^{d}	0.22 ± 0.00^{ns}
4	0	0	45.91 ± 2.20^{c}	9.75 ± 0.57^{c}	0.21 ± 0.01^{ns}
0	4	0	67.74 ± 4.14^{a}	14.00 ± 1.23^{a}	0.21 ± 0.01^{ns}
0	0	4	44.27 ± 2.04^{c}	9.40 ± 0.18^{cd}	0.21 ± 0.01^{ns}
2	2	0	55.38 ± 1.85^{b}	11.52 ± 0.45^{b}	0.21 ± 0.00^{ns}
2	0	2	43.96 ± 1.03^{c}	9.39 ± 0.29^{cd}	0.21 ± 0.01^{ns}
0	2	2	49.65 ± 2.44^{bc}	10.50 ± 0.69^{bc}	0.21 ± 0.00^{ns}
1.33	1.33	1.33	48.28 ± 6.17^{c}	10.09 ± 0.85^{c}	0.21 ± 0.01^{ns}

All values are means$\pm$sd of three replications.

Means followed by the same letters within each column are significantly different, $P \leq 0.05$ (analysed using Duncan's New multiples range test).

ns, no significant difference ($P > 0.05$) for any treatment.

Further consideration into each viscoelastic parameter i.e. G', G'' and tan δ measured at a frequency of 1 Hz displaying on Table 4.10, all pressurised non-meat protein treated samples gave significantly ($P \leq 0.05$) greater G' and G'' than those of the control sample with tan δ between 0.21 to 0.22. These might be an indication of more cross-link formation created by the interaction of non-meat proteins and meat proteins moieties imparted to these weak viscoelastic gel structures (Apichartsrangkoon *et al.*, 1999; Apichartsrangkoon, 2002; Apichartsrangkoon and Ledward, 2002; Apichartsrangkoon, 2003)

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Whey protein isolate generally composed of two main components, β-lactoglobulin and α-lactalbumin (Hinrichs and Rademacher, 2004) might interact well with meat proteins while the other two non-meat proteins might impart less or no interaction with the meat protein moieties. Apichartsrangkoon *et al.* (1999) and Apichartsrangkoon (2002) could not found any interaction between SPI and WG in the pressurised and heated mixtures of these binary system. Comfort and Howell (2003) also agreed that heated mixtures of salt-soluble meat proteins and WG at a ratio of 1:1 created a phase separation of these two proteins as shown by phase contrast microscopy, and consequently led to lower G' values in the mixed gels.

It is surprisingly noted that the combination of non-meat proteins caused reducing both moduli (Figs. 4.17-4.18 and Table 4.10). This was quite analogous with results of Ngarize *et al.* (2005) who studied high pressure induced gels of whey and egg albumen as well as their binary mixtures, suggesting that these mixtures were not favourable to interaction under pressure and hence gave weaker gel. Comfort and Howell (2002) also reported that the storage modulus of mixtures of SPI and WPI gels decreased as comparison to those of individual WPI gel.

The results of this experiment were conformed well to those of creep measurement. Regression coefficient analysis of pressurised samples added non- meat protein also showed the same trends (Appendix B).

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4.4.3 Gel strength from penetration tests

Table 4.11 Gel strength of pressurised ostrich-meat yors incorporating non-meat proteins

SPI (%, w/w)	WPI (%, w/w)	WG (%, w/w)	Gel strength (kg.mm)
0	0	0	4.02 ± 0.009^{h}
4	0	0	6.70 ± 0.002^{e}
0	4	0	10.62 ± 0.008^{a}
0	0	4	6.23 ± 0.004^{f}
2	2	0	8.03 ± 0.004^{b}
2	0	2	4.91 ± 0.006^{g}
0	2	2	6.78 ± 0.016^{d}
1.33	1.33	1.33	6.96 ± 0.009^{c}

All values are means±sd of three replications.

Means followed by the same letters within each column are significantly different, $P \leq 0.05$ (analysed using Duncan's New multiples range test).

Gel strength of the pressurised samples showed in Tables 4.11, all samples containing mixtures of non-meat proteins showed significant ($P \leq 0.05$) increase in gel strength as comparison to those control yors. Similar to the experiment of creep as well as G' and G'', WPI gave the strongest gel strength following by SPI, WG and the various combinations of them imparted those to a lesser extent. This was agreed with Van Camp and Huyghebaert (1995); Van Camp *et al.* (1996) and Kanno *et al.* (1998) who found that gel strength, storage and loss moduli of pressurised heamoglobin and whey protein increased with increasing protein concentration. In addition, Mourtzinos and Kiosseoglou (2005) revealed that non-meat proteins could be acted as an emulsifiers as well as an emulsion stabilizer to combine the salt soluble myofibrillar proteins and fat particle in the formulation to form a cohesive final products. The regression coefficient of the gel strength of pressurised ostrich-meat yors incorporating non-meat proteins also were analysed (Appendix B).

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4.4.4 Water holding capacity

Table 4.12 Released plus expressible water of pressurised ostrich-meat yors incorporating non-meat proteins

SPI (%, w/w)	WPI (%, w/w)	WG (%, w/w)	Released plus expressible water (%)
0	0	0	8.80±0.02[a]
4	0	0	1.59±0.02[g]
0	4	0	2.02±0.01[e]
0	0	4	2.15±0.01[c]
2	2	0	2.00±0.01[e]
2	0	2	1.93±0.02[f]
0	2	2	2.28±0.01[b]
1.33	1.33	1.33	2.10±0.01[d]

All values are means±sd of three replications.

Means followed by the same letters within each column are significantly different, $P \leq 0.05$ (analysed using Duncan's New multiples range test).

Table 4.12, all treatments of the pressurised samples added individual or combination of non-meat protein displayed lower values of released plus expressible water than those control sample, suggesting better water holding capacity in those non-meat protein treated (Funami *et al.*, 1998). Since these two properties were antagonistic to each other. According to Chen and Trout (1991); Gujral *et al.* (2002); Mourtzinos and Kiosseoglou (2005); indicated that non-meat protein such as SPI, WPI, WG *etc.* played a vital role in the modification of functional properties such as water holding capacity and textural properties of meat products. Especially, the sample added 4% (w/w) SPI showed the highest water holding capacity. Rhee (1994) also suggested that SPI incorporated into emulsion of meat products, at level up to 4% (w/w), to improve product quality through its moisture binding ability. Regression coefficients analysis also showed in Appendix B.

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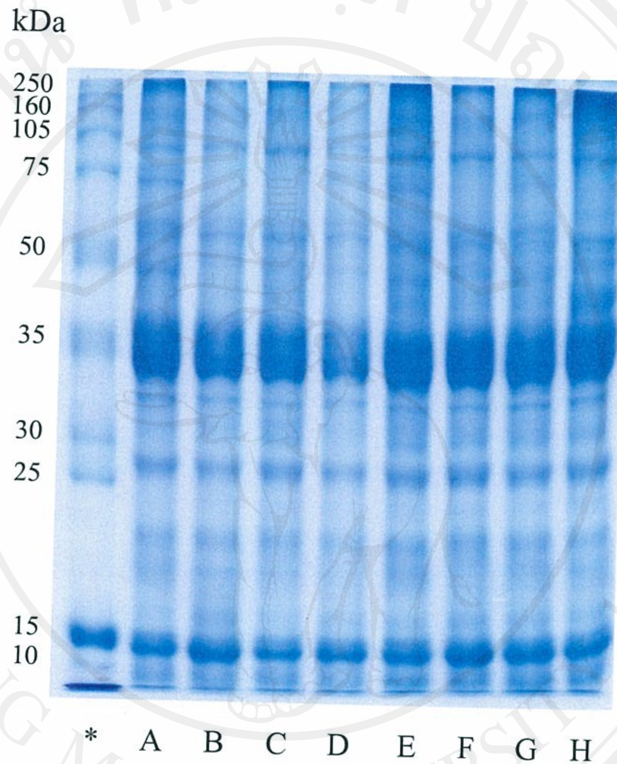
4.4.5 Electrophoretic characterisation

Figures 4.19 and 4.20 exhibited SDS-PAGE electrophoregrams of the samples added various concentrations of individual or combination of SPI, WPI and WG and compared with the control sample.



A = 4% (w/w) of SPI B = 4% (w/w) of WPI
C = 4% (w/w) of WG D = 2% (w/w) of SPI, 2% (w/w) of WPI
E = 2% (w/w) of SPI, 2% (w/w) of WG F = 2% (w/w) of SPI, 2% (w/w) of WG
G = 1.33% (w/w) of SPI, 1.33% (w/w) of WPI, 1.33% (w/w) of WG
H = Control sample * = Molecular weight markers

Figure 4.19 SDS-PAGE electrophoregrams of pressurised ostrich-meat yors incorporating non-meat proteins; the samples were dissolved in 2% SDS.

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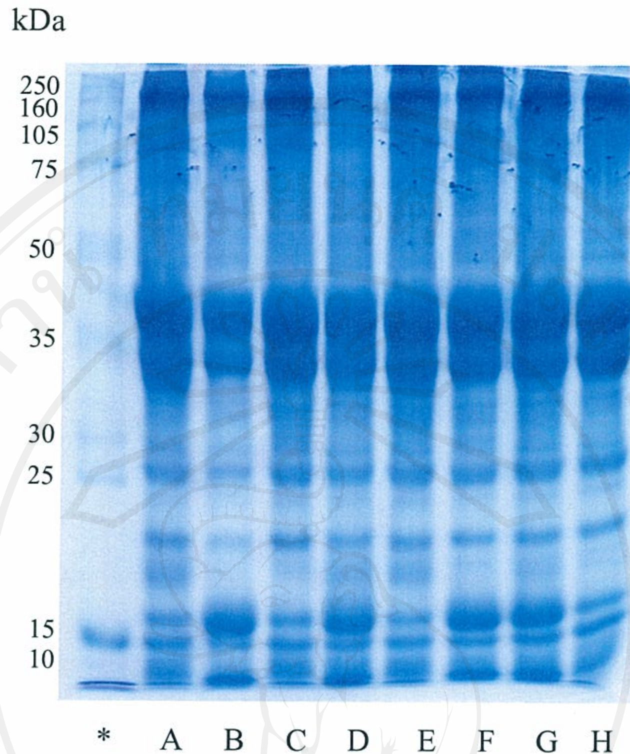

A = 4% (w/w) of SPI
B = 4% (w/w) of WPI
C = 4% (w/w) of WG
D = 2% (w/w) of SPI, 2% (w/w) of WPI
E = 2% (w/w) of SPI, 2% (w/w) of WG
F = 2% (w/w) of SPI, 2% (w/w) of WG
G = 1.33% (w/w) of SPI, 1.33% (w/w) of WPI, 1.33% (w/w) of WG
H = Control sample
* = Molecular weight markers

Figure 4.20 SDS-PAGE electrophoregrams of pressurised ostrich-meat yors incorporating non-meat proteins; the samples were dissolved in 2% SDS plus 2% 2-mercaptoethanol.

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Figure 4.19 displayed samples added non-meat protein (A-G) exhibited loss of protein bands at the regions of around 35-50 kDa as comparison to untreated sample (H). This tended to confirm that non-meat proteins made the meat yors less soluble in SDS due to formation of covalent, presumably disulphide bonds (Apichartsrangkoon, 2003). Since the addition of the reducing agent, 2-mercaptoethanol, which disrupted disulphide bonds, dissolved the aggregates so that,

the electrophoregrams were not treatment sensitive in this solvent (Fig. 4.20). This experiment was relatively conformed to the oscillatory measurement of storage and loss modulus as well as the gel strength. It could be concluded that the formation of covalent disulfide bonds by the non-meat protein moieties influenced the gel elasticity and strength of meat yors (Alting *et al.*, 2000). According to Apichartsrangkoon (2003) suggested that disulphide bonds could be observed in the electrophoregrams of pressurised soy protein and wheat gluten and imparted to their rheological characteristics.

4.4.6 Differential scanning calorimetry (DSC)

This investigation used SPI,WPI and WG as non-meat proteins for developing texture of pressurised ostrich-meat yors 700 MPa at 40°C for holding time 40 min., apparently, DSC thermograms were used to confirm the complete denaturation of the meat yor formulas. Several researchers reported that either endothermic peaks or glassy state of SPI, WPI and WG appeared at temperature rang from 78-96 (Ahmed *et al.*, 2006; Molina *et al.*, 2002), 61-81 (Boye *et al.*, 1997; Ibanoglu, 2005) and 50-85°C (Léon *et al.*, 2003), respectively. Concerning all thermograms of the samples added individual or the combination of non-meat proteins compared with the control sample showed in Fig. 4.21.

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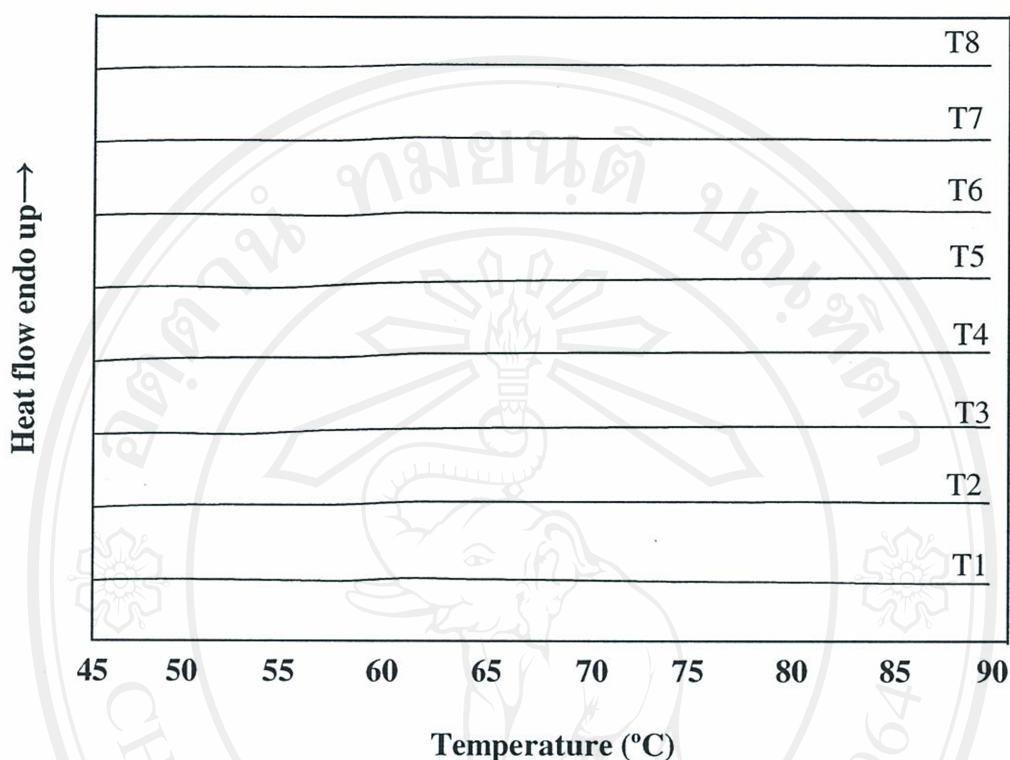

T1 = 4% (w/w) of SPI T2 = 4% (w/w) of WPI

T3 = 4% (w/w) of WG T4 = 2% (w/w) of SPI, 2% (w/w) of WPI

T5 = 2% (w/w) of SPI, 2% (w/w) of WG T6 = 2% (w/w) of SPI, 2% (w/w) of WG

T7 = 1.33% (w/w) of SPI, 1.33% (w/w) of WPI, 1.33% (w/w) of WG

T8 = Control sample

Figure 4.21 DSC thermograms of pressurised ostrich-meat yors incorporating non-meat proteins.

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Figure 4.21 illustrated that there were no endothermic peaks of denaturation protein appeared, suggesting that total proteins including those of meat and non-meat proteins were completely denatured at the previous state of pressurization, hence native protein left for further denaturation by DSC. Molina *et al.* (2002) observed the DSC thermogram of SPI following pressurisation at 700 MPa, but could not detect any discernible peaks. Therefore, the pressure 700 MPa at 40°C with holding time 40

min in this experiment could produce the meat yors incorporated non-meat proteins with complete denaturation state.

4.4.7 Confocal scanning laser microscopy (CSLM)

Ostrich-meat yor was an emulsified meat product of which protein was the main ingredients to provide a good emulsion due to their surface active properties. The capability of a protein to form and make an emulsion stable depended on two factors. Firstly, it had ability to reduce the interfacial tension by adsorption into an interface, and secondly, it had ability to form films which provided strong repulsive forces between droplets, and then preventing the droplets from coming close enough together to aggregate, (Mitidieri and Wagner, 2002; Sun *et al.*, 2007) hence, resisted to rupture due to their high viscoelasticity (Mitidieri and Wagner, 2002). Thus, the final droplet size distribution in an emulsion would be controlled by the detailed conditions of the balance between disruption and coalescence during emulsification. The occurrence of coalescence and bridging was an important mechanism governing the fat droplet size of protein stabilized emulsions (Tornberg *et al.*, 1990). Therefore, to determine fat droplet size in this emulsion was a prime investigation to characterise emulsion stability. Accordingly, the microstructure and fat droplets size of pressurised ostrich-meat yors as shown in Table 4.13, Figs. 4.22 and 4.23 was investigated.

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Table 4.13 Fat droplets size of pressurised ostrich-meat yors incorporating non-meat proteins

SPI (%)	WPI (%)	WG (%)	Fat droplet size (μm^2)
0	0	0	871.55$\pm$39.76[a]
4	0	0	232.09$\pm$6.08[e]
0	4	0	422.70$\pm$6.70[c]
0	0	4	610.03$\pm$33.12[b]
2	2	0	314.15$\pm$17.94[d]
2	0	2	606.66$\pm$30.04[b]
0	2	2	574.71$\pm$26.13[b]
1.33	1.33	1.33	426.93$\pm$42.11[c]

All values are means$\pm$sd of three replications.

Means followed by the same letters within each column are significantly different, $P \leq 0.05$ (analysed using Duncan's New multiples range test).

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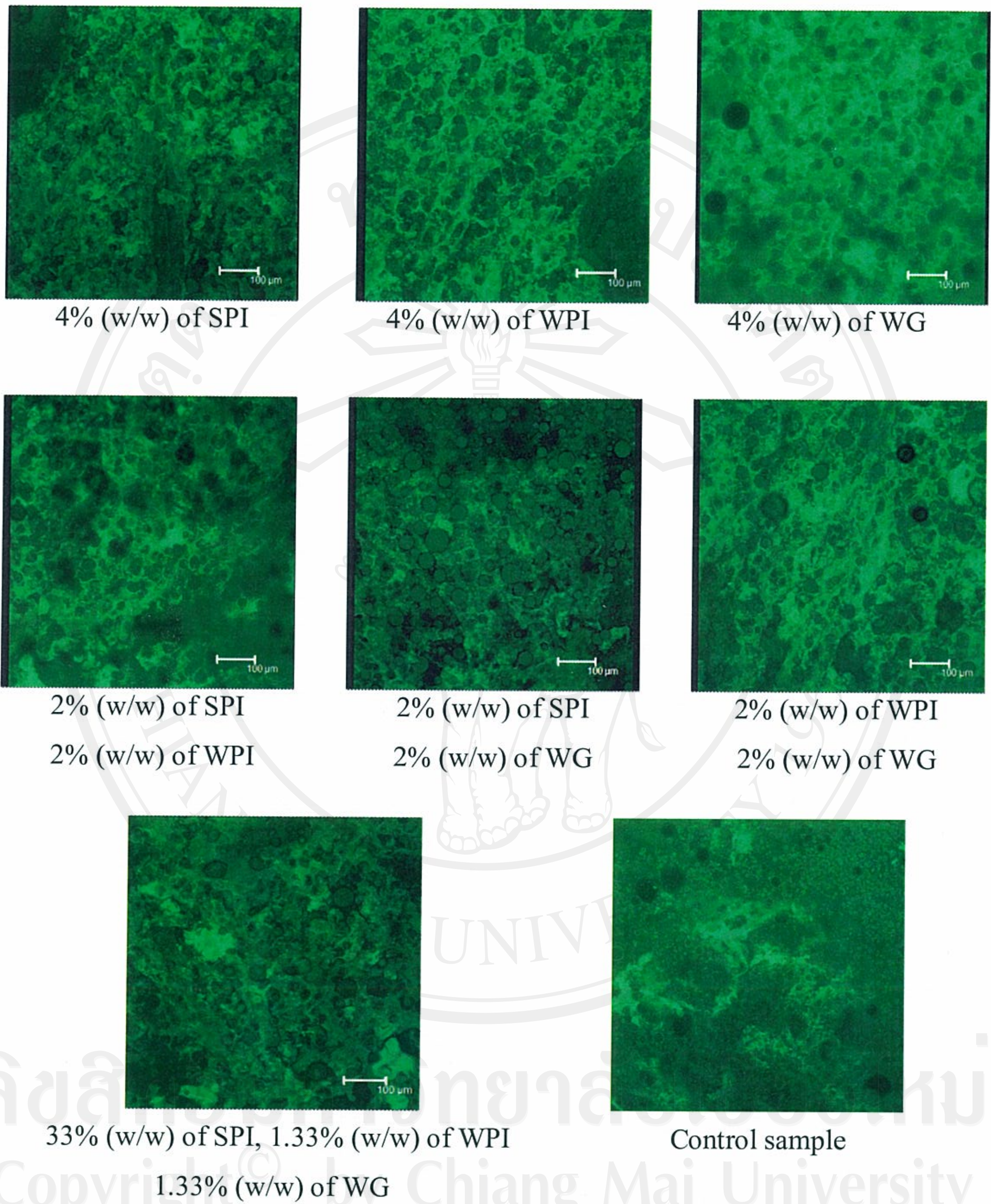

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Figure 4.22 Images of confocal scanning laser microscopy of the protein phase of pressurised ostrich-meat yors added different non-meat proteins.

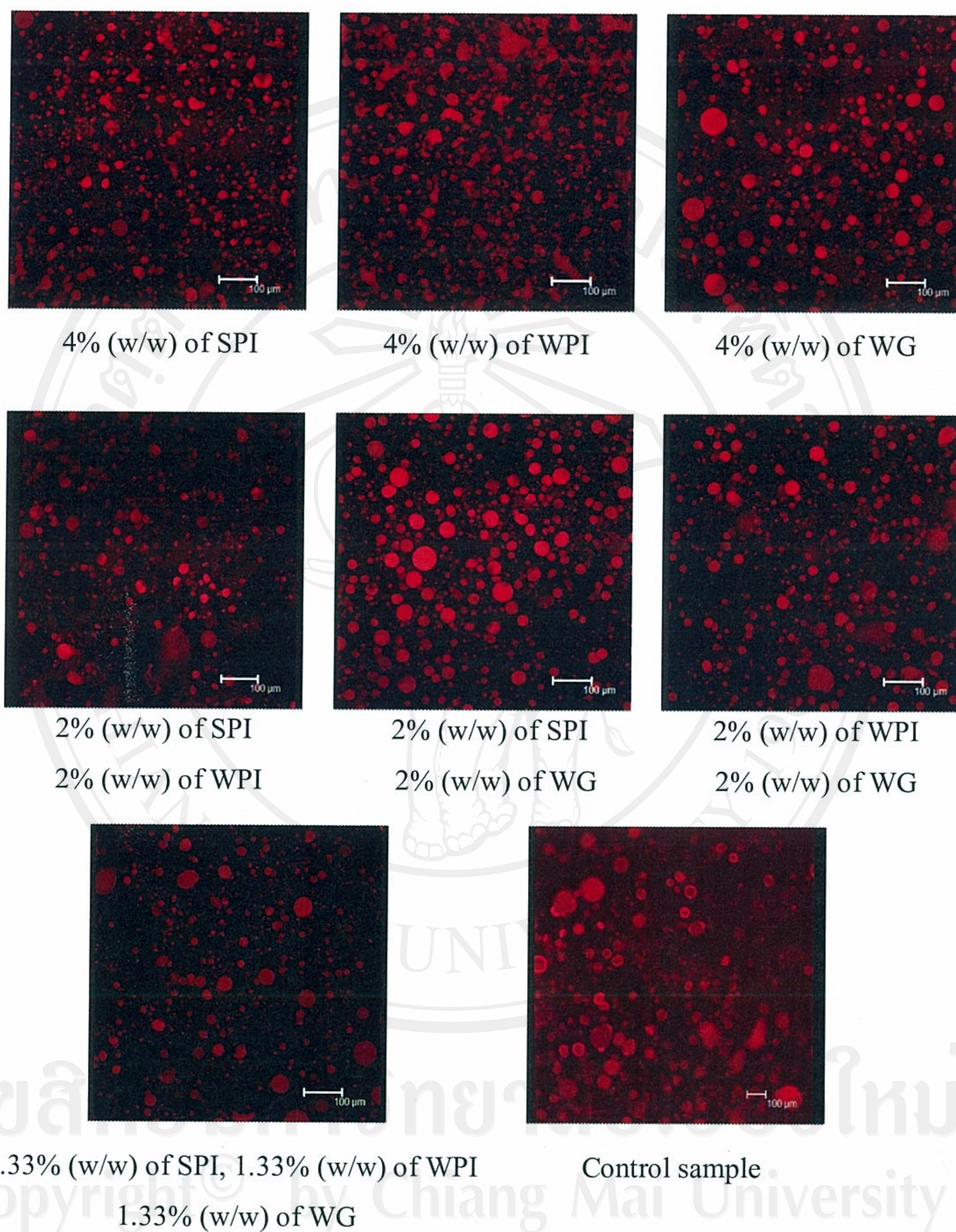

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Figure 4.23 Images of confocal scanning laser microscopy of the fat phase of pressurised ostrich-meat yors added different non-meat proteins.

The gel emulsions of pressurised ostrich-meat yor were characterized by CSLM which could visualize three-dimensional structure, with stained green (Fig. 4.21) for the protein phase and red droplet in back background (Fig. 4.22) for the fat phase.

The gels with added non-meat proteins had developed network structure characterized by thicker strands and smaller size of fat droplets than the control sample (Fig. 4.22, Fig 4.23 and Table 4.13). Sliwinski *et al.* (2003) stated that heating WPI stabilised emulsion, could promote intra-droplet protein-protein interactions, because hydrophobic groups due to complete unfolding of non-meat protein were able to rearrange themselves to be in contact with the fat phase thereby increasing the droplet surface hydrophobicity. As a consequence stabilized the fat-protein emulsion (Sun *et al.*, 2007).

It was essential that the sample addition of 4% (w/w) SPI gave the smallest sizes of evenly distributed fat droplets (Table 4.13 and Fig. 4.23), suggesting that those samples performed the most stability in this emulsified system, which also supported by the high water holding capacity (Table 4.12). Whereas WPI and the mixture of WPI and SPI gave moderate emulsification effect. Wheat gluten and samples incorporated with a mixture WG displayed highest and different fat droplet size, but still smaller than those control yor sample. Consequently, this result was in good agreement with the finding of Su *et al.* (2000), who stated that SPI contributed to formation of the protein network thus enhancing stability of fat and water in such emulsions. Moreover, Molina *et al.* (2001) supported that soy protein exhibited high emulsifying properties compared to other plant proteins.

To sum up, the pressurised ostrich-meat yor with added individual 4% (w/w) SPI and 4% (w/w) WPI were chosen for the next experiment on comparison of physicochemical, sensory qualities and rheological behaviour of pressurised and heat-treated ostrich yor, since these samples standed for the best emulsion stability and water holding capacity (Table 4.12), also gave the highest rheological characteristics as shown by their gel strength, G' and G'' plots (Table 4.11 and Figs. 4.17-4.18).

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4.5 Rheological, chemical and sensory qualities of pressurised and heated ostrich-meat yor

Ostrich-meat yors added either 4% (w/w) SPI or 4% (w/w) WPI were pressurised at 700 MPa, 40°C for holding time 40 min and compared with those steamed for 60 min. Subsequently, the processed samples were subjected to various physicochemical studies and sensory evaluations.

4.5.1 Small deformation measurement

To assure that the viscoelastic measurement were carried out within the linear viscoelastic region, stress amplitude sweeps of pressurised or heated meat yors were performed at frequency 1 Hz (Fig. 4.24).

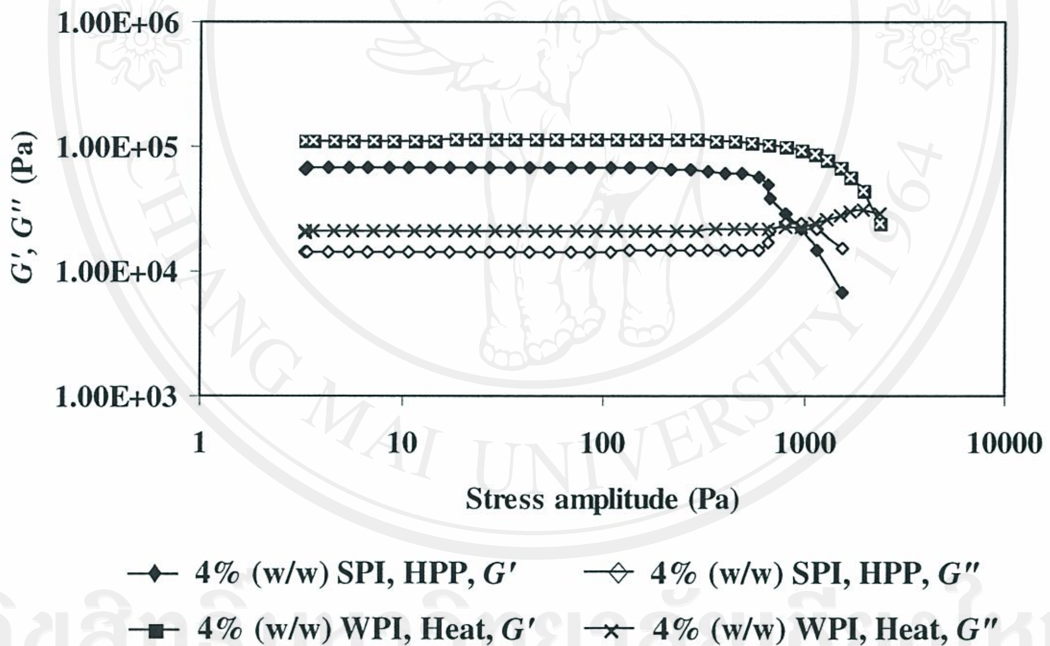

Figure 4.24 Stress amplitude sweeps at frequency 1 Hz of pressurised and heated ostrich-meat yors.

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Figure 4.24 showed stress amplitude sweeps at frequency 1 Hz of pressurised and heated ostrich-meat yors added either 4% (w/w) SPI or 4% (w/w) WPI. The G'

plot was apparently greater than those of G''. Based on these plots, stress amplitude of 50 Pa was chosen for the following transient creep and dynamic oscillation tests, since this was the most linear plot.

4.5.1.1 Transient creep test

The creep/recovery plots of pressurised or heated ostrich-meat yors incorporated either 4% (w/w) SPI or WPI as shown in Figs. 4.25 and 4.26, all creep curves were similarly fitted with an equation of eight-element Burgers model consisting a spring unit of the Maxwell element, three Kelvin-Voight elements and a dashpot of the Maxwell element.

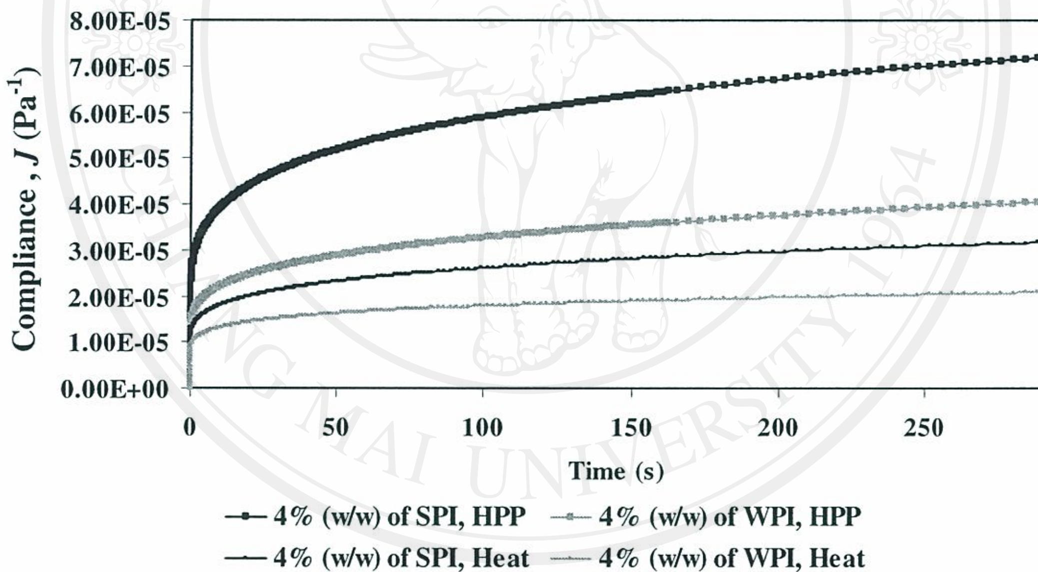

Figure 4.25 Time-dependent creep compliance of pressurised or heated ostrich-meat yors incorporating 4% (w/w) SPI or WPI.

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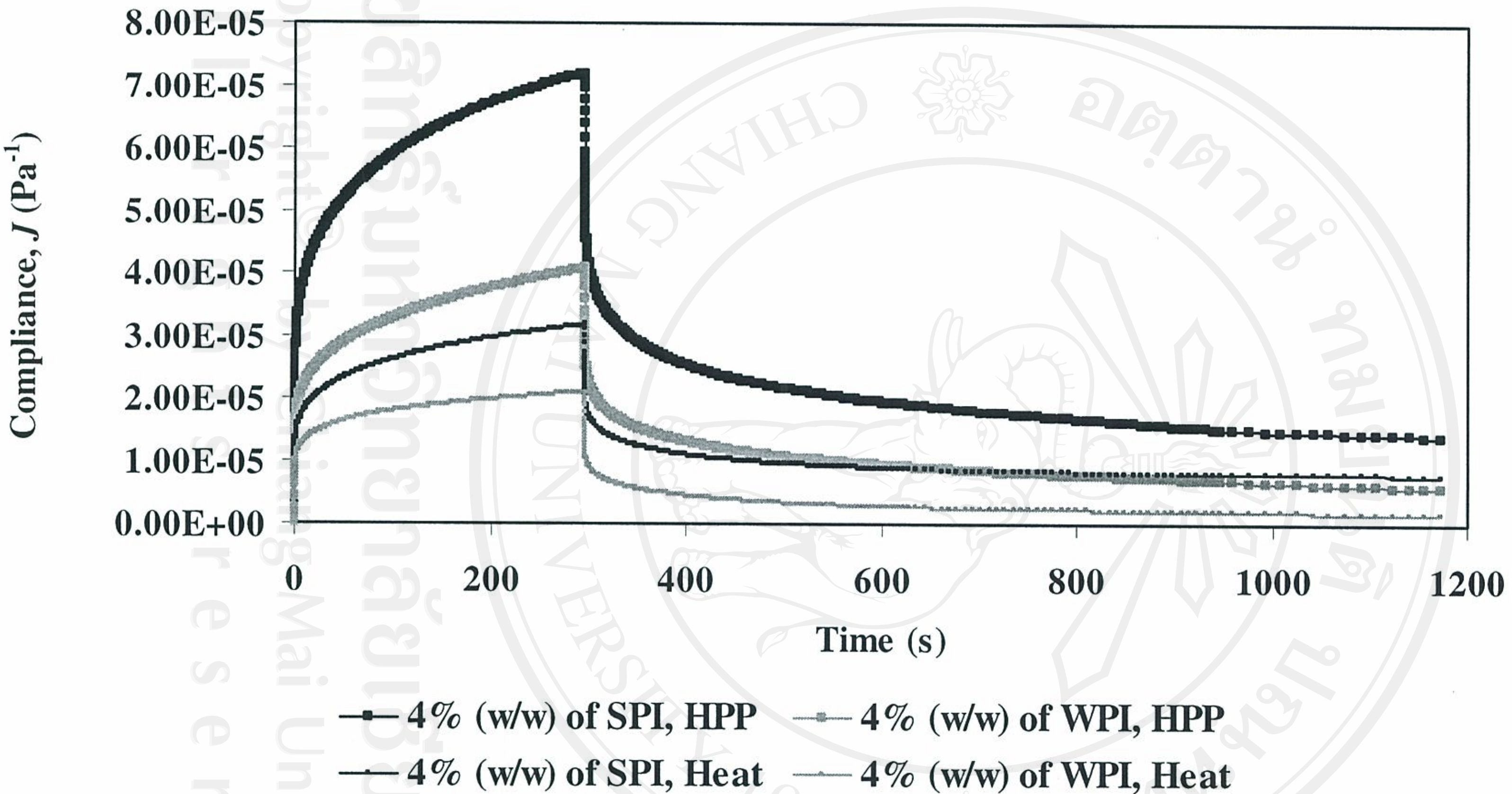

Figure 4.26 Time-dependent creep/recovery compliance of pressurised or heated ostrich-meat yors incorporating 4% (w/w) SPI or WPI.

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It was obvious that pressurised sample blended with 4% (w/w) SPI had the highest compliance, suggesting, this was the weakest sample and quite labile to be deformed by a constant stress. In vice versa heated sample with 4% (w/w) WPI displayed the lowest compliance, hence would give the strongest structure. The structures of the other two samples were in between the former two yors. Overall creep compliance of pressurised samples were greater than those heated samples which had the stronger linkage orientation (Apichartsrangkoon, 2003). Table 4.14 gave further elaboration on creep parameters. All compliance parameters (J_0, J_1, J_2, J_3 and J_{total}) of the heated samples were lower than those of the pressure treated yor, whereas μ_0 of the heated meat yor was greater than those of the pressurised samples. In particular, all samples added 4% (w/w) WPI had high viscosity, suggesting that these might be due to more cross-link formation to strengthen the viscoelastic structure (Apichartsrangkoon 2002, 2003; Ojijo *et al.*, 2004). All over the heated set of ostrich-meat yor was more elasticity than those pressurised set, as indicated by low J compliance and high μ_0, since these two parameters were antagonistic to each other (Steffe, 1996).

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Table 4.14 Creep parameters of pressurised or heated ostrich-meat yors incorporating 4% (w/w) SPI or WPI

Treatment	$J_0 \times 10^{-6}$ (Pa^{-1})	$J_1 \times 10^{-6}$ (Pa^{-1})	$J_2 \times 10^{-6}$ (Pa^{-1})	$J_3 \times 10^{-6}$ (Pa^{-1})	$J_{total} \times 10^{-6}$ (Pa^{-1})	λ_1 (s)	λ_2 (s)	λ_3 (s)	Viscosity (μ_0) (1×10^6 Pa.s)
4% (w/w) SPI, HPP	23.27 ± 2.53^{b}	18.87 ± 1.43^{a}	9.35 ± 0.92^{a}	6.43 ± 1.10^{a}	57.91 ± 3.33^{a}	10.15 ± 0.04^{a}	63.08 ± 0.28^{a}	76.67 ± 0.19^{a}	22.33 ± 4.70^{c}
4% (w/w) WPI, HPP	13.30 ± 0.36^{c}	8.93 ± 2.19^{b}	4.64 ± 0.35^{b}	3.82 ± 0.18^{b}	30.69 ± 1.84^{b}	5.40 ± 0.33^{b}	44.25 ± 0.36^{d}	49.76 ± 1.00^{d}	28.52 ± 0.49^{bc}
4% (w/w) SPI, Heat	10.70 ± 0.46^{d}	7.06 ± 0.69^{bc}	3.30 ± 070^{bc}	3.67 ± 0.59^{b}	24.73 ± 2.31^{c}	5.64 ± 0.49^{b}	51.18 ± 0.19^{b}	57.35 ± 0.18^{b}	42.08 ± 2.48^{b}
4% (w/w) WPI, Heat	7.90 ± 0.24^{a}	4.62 ± 0.18^{c}	2.53 ± 0.75^{c}	2.14 ± 0.56^{c}	17.18 ± 0.89^{d}	5.84 ± 0.19^{b}	49.44 ± 0.24^{c}	53.43 ± 2.859^{c}	82.33 ± 1.50^{a}

All values are means±sd of three replications.

Means followed by the same letters within each column are significantly different, $P \leq 0.05$ (analysed using LSD)

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4.5.1.2 Dynamic oscillation test

For oscillation test, storage (G') and loss (G'') moduli as well as loss tangent (tan δ) were among parameters that characterise the samples system generated by high pressure and heat treatments.

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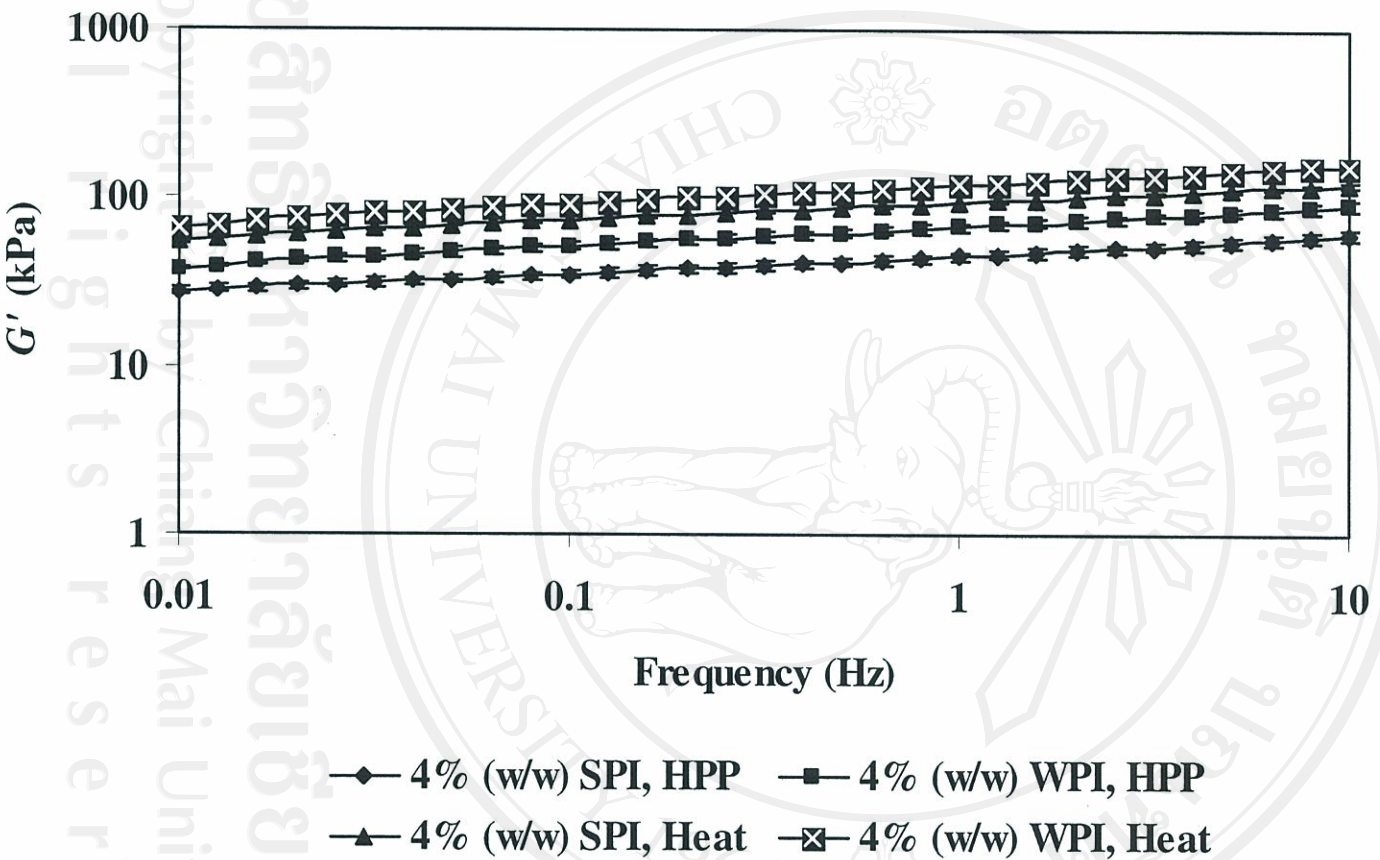

Figure 4.27 Storage modulus as a function of frequency for pressurised or heated ostrich-meat yors incorporating 4% (w/w) SPI or WPI.

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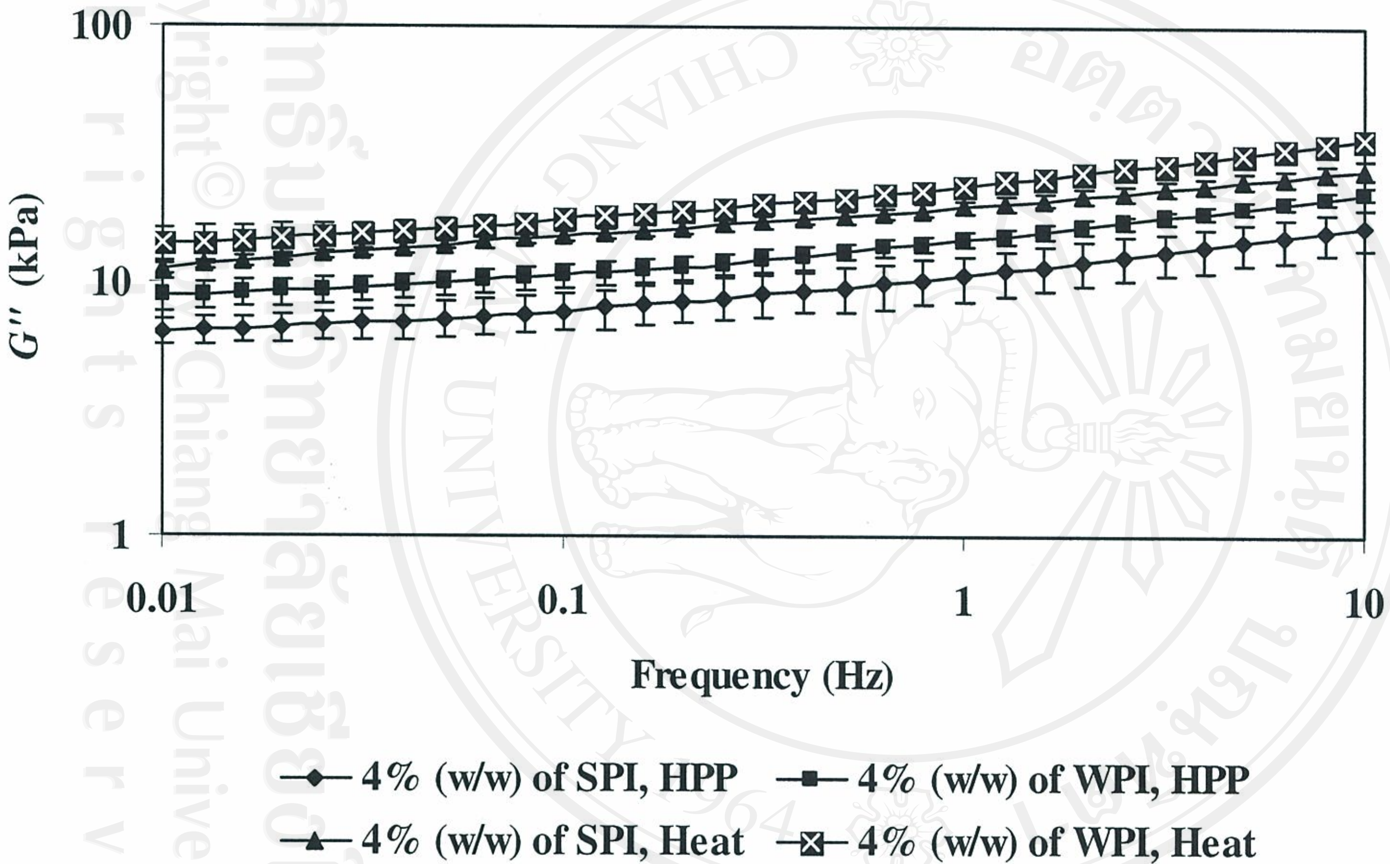

Figure 4.28 Loss modulus as a function of frequency for pressurised or heated ostrich-meat yors incorporating 4% (w/w) SPI or WPI.

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Table 4.15 Storage (G') and loss (G'') moduli, and tan δ at a frequency of 1 Hz for pressurised or heated ostrich-meat yors incorporating 4% (w/w) SPI or WPI

Treatments	G' (kPa)	G'' (kPa)	tan δ
4% (w/w) SPI, HPP	45.93 ± 2.67^{d}	10.55 ± 2.08^{d}	0.23 ± 0.03^{ns}
4% (w/w) WPI, HPP	68.01 ± 3.85^{c}	14.64 ± 1.05^{c}	0.22 ± 0.01^{ns}
4% (w/w) SPI, Heat	93.75 ± 0.75^{b}	19.78 ± 1.19^{b}	0.21 ± 0.01^{ns}
4% (w/w) WPI, Heat	119.37 ± 2.16^{a}	23.99 ± 1.26^{a}	0.20 ± 0.01^{ns}

All values are means±sd of three replications.

Means followed by the same letters within each column are significantly different, $P \leq 0.05$ (analysed using LSD).

ns, no significant difference ($P > 0.05$) for any treatment.

Figures 4.27-4.28 and Table 4.15, the G' of heated sample set was much more predominate than those of pressurised set, the G'' also exhibited the same trends. The loss tangent values were as low as between 0.20 to 0.23. These were characteristic of solid-like response with an elastic network (Apichartsrangkoon, 2003). Similar to creep experiment, G' and G'' of ostrich-meat yors added 4% (w/w) WPI were significantly ($P \leq 0.05$) higher than those added 4% (w/w) SPI subjected to the same treatment condition. According to Totosaus *et al.* (2002) revealed that pressure-induced denaturation leaded to very different gels to those produced by heat, especially, softer but resistant to breaking, and offered an additional means for modification of food texture (Hoover, 1993).

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4.5.2 Gel strength from penetration tests

Table 4.16 Gel strength of pressurised or heated ostrich-meat yors incorporating 4% (w/w) SPI or WPI

Treatments	Gel strength (kg.mm)
4% (w/w) SPI, HPP	6.70$\pm$0.01[d]
4% (w/w) WPI, HPP	10.62$\pm$0.01[b]
4% (w/w) SPI, Heat	7.19$\pm$0.02[c]
4% (w/w) WPI, Heat	12.24$\pm$0.01[a]

All values are means$\pm$sd of three replications.

Means followed by the same letters within each column are significantly different, $P \leq 0.05$ (analysed using LSD).

Unlike the creep, storage and loss moduli where the characteristic of treated samples were divided into two sets according to the processing condition, in this investigation, gel strength of the treated samples was also divided into two grouping but according to type of mixed non-meat protein either WPI or SPI. However the processing condition i.e. high pressure still significantly ($P \leq 0.05$) imparted weaker gel than heat (Table 4.16). Montero *et al.* (1997) mentioned that gel strength from penetration test measures the degree of compactness or density of actomyosin of the gel material. This could be implied that the heat treatment induced more dense gel than pressure did. Totosaus *et al.*, (2002) stated that heat treatment unfolded partially native protein to form a network, ordered matrix by aggregation of the molecules whereas pressure induced hydrophobic interaction and disulphide bonds between protein molecules, resulting in a rearrangement gel structure. Fernández-Martín *et al.* (1997) also found that pressurisation 200 or 400 MPa with five temperatures from 10-70ºC of pork meat batter gave lower gel strength than those of heat. Carlez *et al.*, (1995) also found the same trends with pressurised surimi 200-450 MPa/15 min/5-10ºC) and Okazaki *et al.* (1997) with pressurised Alaska Pollack gels at 500 MPa/10 min/ 0ºC.

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4.5.3 Water holding capacity

Table 4.17 Released plus expressible water of pressurised or heated ostrich-meat yors incorporating 4% (w/w) SPI or WPI

Treatment	Released plus expressible water (%)
4% (w/w) SPI, HPP	1.59±0.02[d]
4% (w/w) WPI, HPP	2.03±0.02[c]
4% (w/w) SPI, Heat	5.35±0.01[b]
4% (w/w) WPI, Heat	7.65±0.04[a]

All values are means±sd of three replications.

Means followed by the same letters within each column are significantly different, $P \leq 0.05$ (analysed using LSD).

Similar to the viscoelastic manners, the percentage of released plus expressible water could be divided into two main grouping according to the processing condition, pressure treatment gave lower released plus expressible water than those heat treatment which was an indication of higher water holding capacity in pressurised samples than heat. At relatively high pressure levels caused protein dissociation and unfolding and was probably triggered by the conversion of free water into a more compact water bond to the protein surface (Totosaus *et al.*, 2002) whereas in heating process the protein only absorbed free water on its surface (Funami *et al.*, 1998). Thus sample treated by pressure appeared more plasticizing effect in the gel network than heat did.

Overall the water holding capacity of every treated sample was significantly different ($P \leq 0.05$) which pressurised and added 4% (w/w) SPI was the highest, following by pressurised and added 4% (w/w) WPI as well as heated and added SPI. Whereas sample heated and added 4% (w/w) WPI gave the lowest water holding capacity.

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4.5.4 Electrophoretic characterization

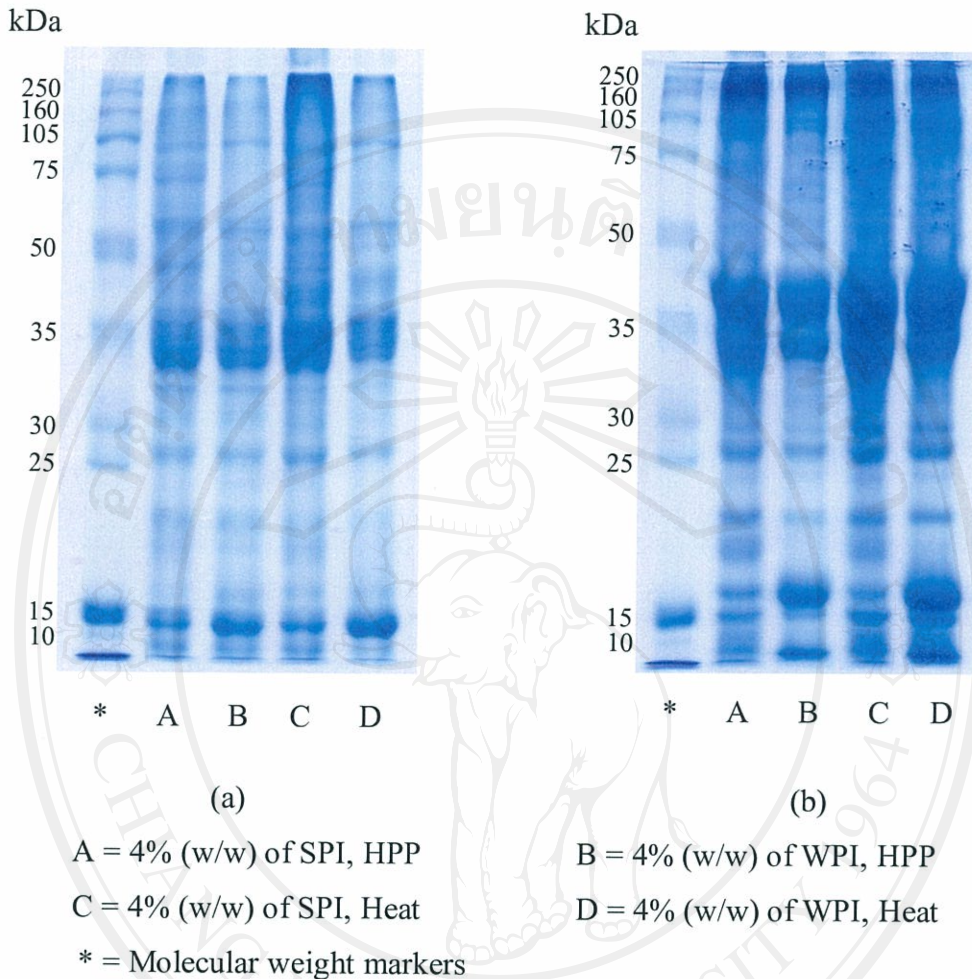

Figure 4.29 SDS-PAGE electrophoretic patterns of pressurised or heated ostrich-meat yors incorporating 4% (w/w) SPI or WPI in the absence (a) and presence of 2-mercaptoethanol (b).

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Figure 4.29 (a) showed SDS-PAGE electrophoregrams of the sample group added 4% (w/w) SPI displayed more solubilised protein bands at the region of 35-75 kDa than those of WPI grouping, suggesting that WPI induced meat protein less solubilised in hydrophobic disruption solvent such as SDS whereas either pressure or heat treatment conditions imparted lesser extent. With the addition of reducing agent, 2-mercaptoethanol (Fig. 4.29b) disrupted more protein bands made no difference of electrophoregrams in this solvent. These were supported that WPI created more disulphide cross-link with meat protein than those of SPI did. Apichartsrangkoon

(2002) found that heat induced more disulphide bonds formation than that pressure at the gluten/soy system.

4.5.5 Sensory evaluation

Table 4.18 Sensory attributes of pressurised and heated ostrich-meat yor incorporating 4% (w/w) SPI or WPI

Treatment	Colour	Flavour	Juiciness	Texture	Acceptability
4% (w/w) SPI, HPP	8.37 ± 0.76^{a}	8.20 ± 0.85^{ns}	8.53 ± 0.73^{a}	8.60 ± 0.72^{a}	8.53 ± 0.78^{a}
4% (w/w) WPI, HPP	8.20 ± 0.85^{a}	8.20 ± 0.89^{ns}	8.03 ± 0.76^{b}	6.37 ± 0.85^{b}	6.53 ± 0.68^{b}
4% (w/w) SPI, Heat	7.33 ± 0.84^{b}	8.10 ± 0.84^{ns}	7.00 ± 0.64^{c}	6.07 ± 0.91^{c}	6.00 ± 0.91^{c}
4% (w/w) WPI, Heat	7.23 ± 0.86^{b}	8.07 ± 0.87^{ns}	3.23 ± 0.50^{d}	2.23 ± 0.57^{d}	2.17 ± 0.59^{d}

All values are means$\pm$sd of three replications.

Means followed by the same letters within each column are significantly different, $P\leq0.05$ (analysed using LSD).

ns, no significant difference ($P>0.05$) for any treatment.

Table 4.18, all sensory attributes evaluated by 36 panelists i.e. acceptability, texture, colour and juiciness of pressure treated grouping are significantly different ($P\leq0.05$) than those of heated grouping, except flavour of all treatment are not significantly different ($P>0.05$). These results also conformed to those of water holding capacity, but less conformed with other instrumentation methods. Moreover the pressurised samples appear glossier and smoother than those heated meat yors from observation. Messens *et al.* (1997) and Apichartsrangkoon *et al.* (1998) pointed out that pressure induced unfolding of protein, and subsequent aggregation leading to the formation of gels, which might ultimately affect the textural quality of food. Pressure-induced gels with wheat gluten differed from those induced by heat, being

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glossier, smoother and softer, and having greater elasticity (Apichartsrangkoon *et al.*, 1999; Ledward, 1995).

It was of interest noting that the pressurised ostrich-meat yor added 4% (w/w) SPI received the highest score of all attributes which might be related to their high water holding capacity with smallest fat droplet size (from previous experiment) suggesting SPI exhibited higher emulsifying properties than other non-meat proteins, (Molina *et al.*, 2001) although the gel strength and rheological properties were the lowest. High pressure of 700 MPa 40°C for holding time 40 min was quite adequate to completely denature meat protein (DSC thermograms, Fig.4.21), but it should be awarded that this set condition was unlikely to completely inactivate bacteria spore or pathogenic organism. Thus subsequent heat treatment might be necessary prior to tasting. Cheftel (1995) stated that in general, gram-positive bacteria (*Listeria monocytogenes*, *Staphylococcus aureus*) are more resistant to pressure than gram-negative (*Pseusomonas*, *Salmonella* spp., *Yersinia enterocolitica*, *Vibrio parahaemolyticus*). Spores at ambient temperature can resist pressures up to 1000 MPa, combined temperatures above 70°C being necessary to obtain a significant level of inactivation. However, it has also been shown that lower pressures (250 MPa) associated with mild temperatures (40°C) can inactivate spores in a two stage process, pressure first inducing germination and then inactivating the bio-sensitive germinated spores. In addition, pressurization can inactivate some parasites such as *Trichinella spiralis* but its efficiency on inactivation of viruses is very limited.

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