CHAPTER 3
MATERIALS AND METHODS

2.0 Materials

- Ostrich-meat trimmings purchased from B.M. Farm, Chiang Mai, Thailand
- Ostrich fat purchased from B.M. Farm, Chiang Mai, Thailand
- Carboxymethylcellulose (CMC) (Nippon Paper Chemicals Co., Ltd., Japan)
- Locust bean gum (LBG) (System Bio-Industries Maroc S.A, Marocco)
- Xanthan gum (CP Kelco U.S., Inc., USA)
- Tapioca starch: Leader price (Thai Better Foods Co., Ltd., Thailand)
- Pure ground pepper: Raitip (Thai Cereals World Co., Ltd., Thailand)
- Garlic (Big C Co., Ltd., Thailand)
- Sugar: Mitr Phol (Mitr Phol Sugar Corp., Ltd., Thailand)
- Plastic sausage casing, 29 mm in diameter (Food EQ Ltd., Thailand)
- DSC Aluminium pans (PerkinElmer Instruments, Shelton, Connecticut, USA)

2.0 Chemicals

- Sodium chloride: Prung Thip (Thai refined salt Co., Ltd., Thailand)
- Sodium tripolyphosphate (Lab P&D, Thailand)
- 40% (w/v) Acrylamide monomer solution containing 5% (w/v) N, N′-methylenebisacrylamide (Amersham Biosciences AB, Sweden)
- Tris [hydroxymethyl] aminomethane (USB Amersham Life Science, USA)
- Sodium Dodecyl Sulfate (USB Corporation, Japan)
- Ammonium persulfate (USB Corporation, Japan)
- Glycerol (Amersham Biosciences AB, Sweden)
- Glycine (USB Corporation, USA)
- Bromophenol blue (USB Amersham Life Science, Austria)
- 2-Mercaptoethanol (Merck, Austria)
- N, N, N′, N′-Tetramethylethylenediamine (TEMED) (USB Corporation, USA)

- Coomassie™ Brilliant Blue G-250 (USB Corporation, UK)
- Full Range Rainbow™ Recombinant Protein Molecular Weight Marker (GE Healthcare UK limited, UK)
- Sodium Thiosulphate 5% (Plus One™ Silver Staining kit, Amersham Biosciences AB, Sweden)
- Silver Nitrate 2.5% (Plus One™ Silver Staining kit, Amersham Biosciences AB, Sweden)
- Protein Formaldehype 37% (Plus One™ Silver Staining kit, Amersham Biosciences AB, Sweden)
- Glutardialdehyde 25% (Plus One™ Silver Staining kit, Amersham Biosciences AB, Sweden)
- EDTA-Na_2 (Plus One™ Silver Staining kit, Amersham Biosciences AB, Sweden)
- Sodium Carbonate (Plus One™ Silver Staining kit, Amersham Biosciences AB, Sweden)
- Sodium Acetate (Plus One™ Silver Staining kit, Amersham Biosciences AB, Sweden)
- Fluorescein Isothiocyanate (FITC) (Sigma-Aldrich, St Louis, MO, USA)
- Nile Red (Sigma-Aldrich, St Louis, MO, USA)
- All chemicals were of analytical grade.

2.0 Instruments

- Meat grinder, model MK-G20N (Matsushita Electric Industrial Co., Ltd, Osaka, Japan)
- Meat chopper, model RS 20 (Meissner GmbH. & Co., Ltd., Bieenkopf-Wallau, Germany)
- Stuffer (Sausagemaking.org, Bolton, Lancashire, UK)
- Differential Scanning Calorimeter (TA Instruments-Waters LLC, New Castle, USA)
- Differential Scanning Calorimeter, model Diamond DSC (PerkinElmer Instruments, Shelton, Connecticut, USA)
- High pressure rig "Food lab" (Stansted Fluid Power, Essex, UK)

- Advance Rheometer AR2000 (TA Instruments-Waters LLC, New Castle, USA)
- Texture Analyser TA-XT Plus (Stable Micro Systems Ltd., Surrey, UK)
- Hot air oven (Binder GmbH Bergstr, Tuttlingen, Germany)
- Scanning Electron Microscope (Jeol JSM-5910 LV, Jeol Ltd., Tokyo, Japan)
- Pelco CPD-2-critical point drier (Ted Palla Co., CA, USA)
- Fine Coat Jeol-JFC-1100 (Jeol Ltd., Akishima, Japan)
- Electrophoresis power supply EPS301 (Amersham Biosciences, Uppsala, Sweden)
- miniVE electrophoresis and electrotransfer unit (Amersham Biosciences, Uppsala, Sweden)
- Leica Scanning Laser Microscope (Leica Microsystems Heidelberg, Heidelberg, Germany)
- Stress Tech Rheometer (Rheological Instruments AB, Lund, Sweden)

2.0 Research designs and methods

2.0.0 Determination of physicochemical properties of ostrich-meat trimmings, carboxymethylcellulose (CMC), locust bean gum (LBG), xanthan gum (XAN) and tapioca starch

2.0.0.0 The chemical quality of raw materials

The chemical composition of ostrich-meat trimmings, carboxymethylcellulose (CMC), locust bean gum (LBG), xanthan gum (XAN) and tapioca starch were analysed by the methods indicated in AOAC (2000) including no. 950.46 for moisture content, no. 991.36 for fat content, no. 920.153 for ash measurement, no. 928.08 for protein and carbohydrate content that is calculated from the rest content of moisture, fat, ash and protein contents for ostrich meat. For hydrocolloids, carbohydrate content are calculated from the rest content of moisture, fat, and protein contents.

2.0.0.0 Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry (TA Instruments-Waters LLC, New Castle, USA) was used to study the thermal phase transition of samples. A heating rate of 5 °C/min was used with temperature increased from 30 to 100 °C.

Samples, 5 ± 1 mg for CMC, LBG, xanthan gum and tapioca starch and 15 ± 1 mg for ostrich-meat trimmings, were weighed accurately and put into DSC aluminium pans and sealed, an empty pan was used as a reference. The DSC-TA instrument was calibrated using indium. The determinations were made in triplicate and all samples were rescanned to assess the reversibility of any changes (Cheah and Ledward, 1996).

2.0.0.0 Avian influenza test

All ostrich-meat trimming samples were checked for avian influenza using the method as recommended by Office International des Epizooties (OIE) chapter 2.1.14 (2004). In brief, a 10% (w/v) suspension of minced ostrich meat in antibiotic solution was centrifuged. Supernatant was collected and inoculated into 2 to 11-day-old embryonated chicken eggs intra-allantoically. The eggs were incubated for 4 days; afterwards allantoic fluid was harvested and tested for hemagglutination (HA) activity. Not Detection diagnostic assays were performed in parallel with avian influenza in order to rule out Not Detection infection. Hemagglutinating agents were subjected to agar gel precipitation test (AGPT) with in influenza type A antiserum. Every type A influenza virus detected would be tested for its virulence be chicken inoculation. Positive samples would also be sent for subtyping at avian influenza reference laboratory. Serological test by AGPT and hemagglutination inhibition (HI) test were also performed by the method as recommended by OIE.

3.4.2 Investigation of the effects of pressure, temperature and holding time on physicochemical properties of ostrich-meat yor

3.4.2.1 Preparation of ostrich-meat yor

Ostrich-meat trimmings and fat were ground separately in a laboratory grinder (MK-G20N, Matsushita Electric Industrial Co., Ltd, Osaka, Japan) through a 5 mm plate, then frozen to -18 °C and stored at -18 °C until used. Before processing, the meat and fat were thawed at 4 °C for approximately 18 h to 0 °C. The ostrich-meat yors were manufactured according to a commercial yor formulation (Supavititpatana and Apichartsrangkoon, 2007). The comminuted ostrich meat was chopped with 2% (w/w) sodium chloride, 0.25% (w/w) sodium tripolyphosphate,

5% (w/w) garlic, 2% (w/w) ground pepper, 1.5% (w/w) sugar and 5% (w/w) tapioca starch. Then 5% (w/w) ostrich fat and 5% (w/w) ice were added to the meat batter to form a homogeneous mass in meat chopper (Meissner GmbH. & Co., Ltd., Bieenkopf-Wallau, Germany) and the final temperature of the meat batter was about 10 °C.

The batter was stuffed into plastic sausage casing, 29 mm in diameter (Food EQ Ltd., Thailand) and hermetically sealed in laminated plastic bags prior to pressure treatment. This experimental plan was subjected to different pressure, temperature and holding time regimes as follows: pressures of 200, 400 and 600 MPa at both 40 and 50 °C for both 40 and 60 min using a "Food lab" high pressure rig (Stansted Fluid Power, Essex, UK). The pressure medium was a mixture of castor oil (Union Science Co., Ltd., Thailand) and ethanol (20:80). After treatment, samples were stored at 4 °C and subject to further subsequent analysis.

The treated samples were subjected to different determinations as described in sections 3.4.2.2-3.4.2.8

2.3.1.1 Viscoelastic characterisation and modeling

The viscoelastic characterisation of heat and high pressure treated samples were investigated by a controlled stress rheometer (Advance Rheometer AR2000, TA Instruments-Waters LLC, New Castle, USA) equipped with a 25 mm parallel plates measuring system. In order to ensure that all mesurements were carried out within the linear viscoelastic regions (LVR), a stress sweep was first done at a frequency of 1 Hz for all samples (a modified method of Apichartsrangkoon and Ledward, 2002) (Figure 3.1 and 3.2). Edges of samples were covered with light silicone oil (Sigma-Aldrich Co. Ltd, Gillingham, UK) to prevent the samples from drying out. All rheological measurements were the mean values determined from assessment of five samples from five individual pressure treatments (n = 5).

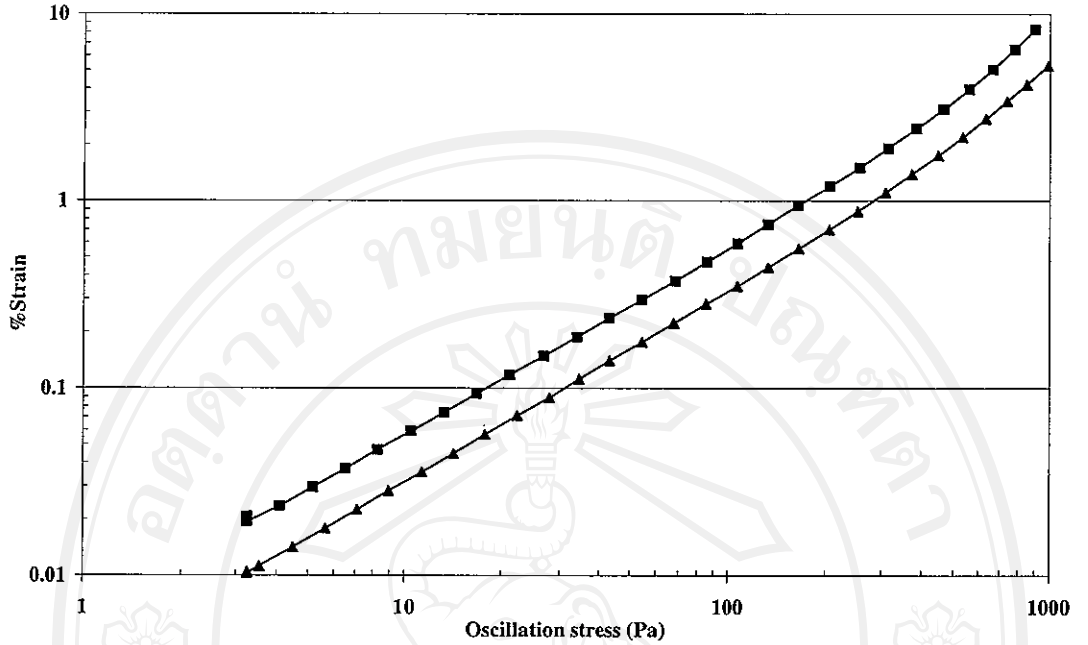

Figure 3.1 Typical oscillatory stress sweep (1-1,500 Pa) at frequency 1 Hz of high-pressure-treated ostrich-meat yor with ■ 200 MPa, 40 °C, and 40 min and, ▲ 600 MPa, 50 °C, and 60 min.

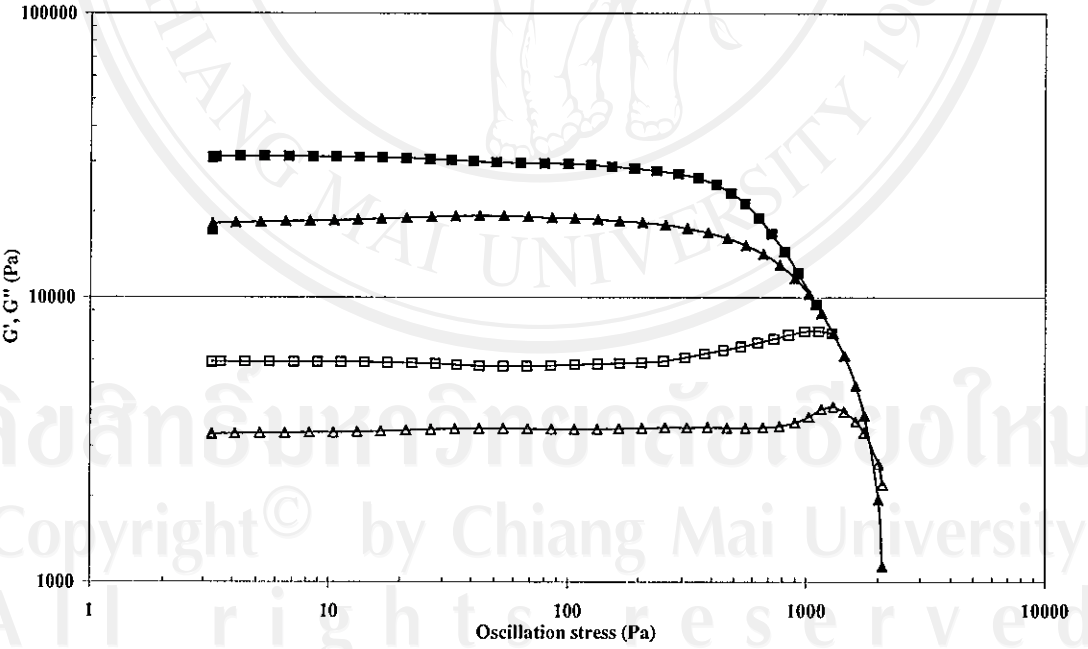

Figure 3.2 Typical stress amplitude sweep (1-1,500 Pa) at frequency 1 Hz of high-pressure-treated ostrich-meat yor with ▲, Δ 200 MPa, 40 °C, and 40 min and ■, □ 600 MPa, 50 °C, and 60 min. The closed symbols = G′ and opened symbols = G″.

3.4.2.2.1 Stress relaxation testing

The treated samples were sliced into 2 mm thick sections prior to stress relaxation analysis. A constant strain was applied at 0.1% within the linear viscoelastic region of the samples (Figure 3.1) and maintained at a constant level for 900 sec, while the stress decay was monitored (a modified method of Rosalina and Bhattacharya, 2001). Mathematical models of these curves were also determined.

3.4.2.2.2 Creep testing

Creep compliance under a constant stress was measured at times for fixed intervals and the recovery was also measured after the stress was instantly removed. Both creep and recovery of the samples were determined (Initial load 30 Pa for 300 sec, unloaded recovery 900 sec). The stress used (30 Pa) was found to be within the linear viscoelastic region (Figure 3.2) (Chattong *et al.*, 2007). The mathematical models of these curves were also determined.

3.4.2.2.3 Dynamic viscoelastic oscillatory measurement

Samples were subjected to a frequency range of 0.01-10 Hz at a controlled stress of 30 Pa (chosen from Figure 3.2) (a modified method of Apichartsrangkoon and Ledward, 2002), subsequently their storage modulus (G′) and loss modulus (G″) were determined.

3.4.2.3 Textural measurements

3.4.2.3.1 Puncture testing

A puncture test was carried out using a Texture Analyser TA-XT Plus (Stable Micro Systems Ltd., Surrey, UK) at ambient temperature. A ball-end plunger with a diameter of 5 mm was used to penetrate 90% of the samples height (29 mm in diameter x 30 mm length) at a speed of 5 mm/sec with a compression load of 5 kg. The first recorded peak corresponded to penetration force (N). Gel strength (N x mm) was calculated by multiplying the penetration force (N) with its penetrated distance. The mean values of five trials were used for replication (a modified method of Tabilo-Munizaga and Barbosa-Cánovas, 2004).

3.4.2.3.2 Compression testing

A uniaxial compression test of treated sample was performed to assess resistance to compression, which was an index of hardness during mastication. The test was performed with Texture Analyser TA-XT Plus (Stable Micro Systems Ltd., Surrey, UK) at ambient temperature. One compression cycle of 75% of the sample height (29 mm in diameter x 30 mm length) was used to measure hardness, defined as the maximum force (N) required to acheive the given deformation of samples. A 35 mm diameter cylinder probe was compressed the samples at a constant speed of 5 mm/sec with at a compression load of 50 kg. The mean values of five trials were used for replication (a modified method of Tabilo-Munizaga and Barbosa-Cánovas, 2004).

2.3.1.1.0 Shear force

Shear force and shear energy of the treated samples were determined using a Texture Analyser TA-XT Plus (Stable Micro Systems Ltd., Surrey, UK) with a 50 kg load cell. The samples were cut into pieces of 29 mm in diameter x 30 mm length and placed on the sample holder. A Warner Bratzler blade was set on the machine and moved perpendicularly to the sample with a cross-head speed of set at 5 mm/sec until 30 mm in depth. The shear strength (N/mm) was calculated as the maximum force recorded, whereas the shear energy (N/mm x sec) was calculated as the area under the force deformation curve from the beginning to the end of the test. The mean values was from five samples (a modified method of Honikel, 1998).

2.3.1.1 Texture Profile Analysis (TPA)

Textural characteristics of treated samples were analysed according to the texture profile analysis (TPA) using Texture Analyser TA-XT Plus (Stable Micro Systems Ltd., Surrey, UK) with a 50 kg load cell. Samples (29 mm in diameter, 30 mm height) were compressed twice to 50% of their original height at a constant cross-head speed of 5 mm/sec, 1 sec apart, with a flat cylinder probe 35 mm in diameter. The TPA parameter, hardness was defined by peak force during the first compression cycle. Cohesiveness was calculated as the ratio of the area under the second curve to the area under the first curve. Springiness was defined as a ratio of

the time recorded between the start of the second area and the second probe reversal to the time recorded between the start of the first area and the first probe reversal. Chewiness was obtained by multiplying hardness, cohesiveness and springiness. Gumminess was obtained by multiplying hardness and cohesiveness (a modified method of Pietrasik and Li-Chan, 2002).

2.3.1.1 Water holding capacity

Water holding capacity (WHC) measured according to a modified method of Funami *et al.* (1998) was expressed as the value of the freely released water plus the water expressible.

2.3.1.1.0 Released water

The percentage of release water of the product was the weight of sample left after blotting water from the surface followed the method as described by Funami *et al.* (1998). Sample with casing was weighed (A) and after removing the casing, the surface water of both product (B) and casing (C) was wiped with filter paper (Whatman No.2, 90 mm in diameter) then reweighed (B, C). The percentage of released water was calculated according to the equation:

$$\text{Released water (\%)} = 100 \times \{(A-B)-C\}/(A-C)$$

2.3.1.1.0 Expressible water

The percentage of expressible water of the product was measured as described by Funami *et al.* (1998). Samples were cut into 15 mm thickness then placed between double layers of filter paper (Whatman No. 2, 90 mm in diameter) and subsequently subjected to compression using a Texture Analyser TA-XT Plus (Stable Micro Systems Ltd., Surrey, UK) with cylindrical aluminium probe (50 mm in diameter). The measurement was performed with a 50 kg load cell at a cross-head speed of 3 mm/sec to 70% strain for 60 sec. The percentage of expressible water was calculated as the ratio of apparent expressible water to the total moisture content measured by the method of AOAC (2000). The percentages of expressible water were calculated according to the equation:

$$\text{Expressible water (\%)} = 100 \times \{(\text{App})/(\text{TM})\}$$

Where App = Apparent expressible water

TM = Total moisture content of meat yor

$$\text{Apparent expressible water (\%)} = 100 \times \{(W_1)-(W_2)/(W_1)\}$$

Where W_1 = Weight before compression

W_2 = Weight after compression

2.3.1.1 Microstructure determination

Microstructure of treated samples were analysed using a scanning electron microscope (SEM), model Jeol JSM-5910 LV (Jeol Ltd., Tokyo, Japan). The sliced samples were fixed with a 2.5% glutaraldehyde in 0.1 M phosphate buffer pH 7.3 as primary fixative at room temperature for 20 min. This primary fixative was conducted 2 times to crosslink protein. The fixed samples were washed with phosphate buffer, and post-fixed with 1% osmium tetraoxide solution in phosphate buffer for 1 h to stabilise unsaturated lipids. The post-fixed samples were then washed with phosphate buffer and dehydrated in a graded "ethanol series" (15%, 30%, 50%, 70%, 80%, 95%, and 100%, 30 min in each solution). Dehydrated samples were then dried with a Pelco CPD-2-critical point drier (Ted Palla Co., CA, USA). Dry sections were then mounted on aluminium SEM stubs and vacuum gold coated with a Fine Coat Jeol-JFC-1100 (Jeol Ltd., Akishima, Japan), using a magnification of 200X and 400X, respectively. The measurement was applied following a method of Ayo *et al.* (2005) .

2.3.1.1 Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimeter (PerkinElmer Instruments, Shelton, USA) was used in this study to follow the thermal denaturation of protein in the pressurised and unpressurised samples. The instrument was calibrated using indium. Samples of 20 ± 3 mg were weighed into aluminium pans and sealed, consequently subjected to DSC with a scanning rate of 10 °C/min over the temperature range of 40-100 °C which have been identified for meat protein by previous worker (Cheah and Ledward, 1996).

At least 3 runs per sample were carried out by using an empty pan as a reference. All samples were rescanned to assess any reversibility, no reversibility was detected.

3.4.2.8 Electrophoretic analysis

Proteins from the pressurised and unpressurised yors were analysed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) in a miniVE electrophoresis and electrotransfer unit (Amersham Biosciences, Uppsala, Sweden). The samples (0.2 g) were dissolved in 1 ml of 0.5 M Tris-HCl (pH 6.8) buffer containing 10% (w/v) SDS, 0.02% (w/v) bromophenol blue and 20% (v/v) glycerol and 5 μg of extracted samples were applied to each well. Broad Range standard (Full Range Rainbow Protein Molecular Weight Marker, GE Healthcare UK limited, UK) was used for comparison of molecular weight. The bands of protein were identified using 7.5% polyacrylamide "running" gels. The gels were stained for 4 h in 0.25% (w/v) Coomassie Blue G-250 (USB Corporation, UK), 40% methanol and 7% acetic acid. Excessive stain was removed by immersion in destain solution I (40% methanol, 7% acetic acid) and solution II (5% methanol, 7% acetic acid), respectively. For reduced samples, the samples were reduced in 5% (v/v) 2-mercaptoethanol for 15 min prior to loading. To visualised the protein bands two staining methods were applied, either silver or Coomassie Blue staining (Apichartsrangkoon and Ledward, 2002; Ulmanen *et al.*, 2005) to confirm the effect of treatment condition on the meat yor product.

3.4.2.9 Statistical analysis

The main experimental design in this study was 3 x 2 x 2 factorial experiment in a completely randomized design with varying pressures of 200, 400 and 600 MPa, with temperatures of 40 and 50 °C and holding times of 40 and 60 min. A confidence level of 5% was used to compare means ($P \leq 0.05$) between treatments. The mean values were compared using Duncan's New Multiple Range Test (DNMRT) procedure. All data were analysed by analysis of variance using SPSS 10.0.1 software (SPSS Inc., Chicago, USA).

3.4.3 Investigation of the effects of carboxymethylcellulose (CMC), locust bean gum (LBG) and xanthan gum (XAN) on the physicochemical properties of pressurised ostrich-meat yor

2.3.2.0 Preparation of ostrich-meat yor

The best combined condition of pressure, temperature and holding time obtained from section 3.4.2 was used to produce samples of yor containing different concentrations of carboxymethylcellulose (CMC) (Nippon Paper Chemicals co., Ltd., Japan), locust bean gum (LBG) (System Bio-Industries Maroc S.A, Marocco) and xanthan gum (XAN) (CP Kelco U.S., Inc., USA). The ostrich-meat yors were manufactured according to the same formulation as described in section 3.4.2.1. Either single or combined gums were added to the batter within the range of 0-1% (w/w) during the chopping stage, a control treatment (no gum added sample) was also produced, the formulations of ostrich-meat yor with various amount of gums was as shown in Table 3.1:

Table 3.1 Formulations of ostrich-meat yor with different levels of gums.

Treatment	CMC	LBG	Xanthan
1	1.00	0	0
2	0	1.00	0
3	0	0	1.00
4	0.50	0.50	0
5	0.50	0	0.50
6	0	0.50	0.50
7	0.33	0.33	0.33
8	0	0	0

The samples were subjected to testing as described in sections 3.4.3.2-3.4.3.9

2.3.2.0 Viscoelastic characterisation and modeling

All samples were initially subjected to a stress sweep at a frequency of 1 Hz to determine the linear viscoelastic region (a modified method of Apichartsrangkoon and Ledward, 2002). Figure 3.3 and 3.4 show only curves of the strongest and the weakest samples examined. The measurement was performed following a method as described in section 3.4.2.2.

2.3.2.0.0 Stress relaxation testing

A 0.2% constant strain was chosen (Figure 3.3) for stress relaxation test applied at 900 sec. The measurement was performed following a method as described in section *3.4.2.2.1.*

2.3.2.0.0 Creep testing

The creep tests were undertaken using load stress of 50 Pa (chosen from Figure 3.2) for 300 sec and unloaded recovery time of 900 sec. The measurement was performed following a method as described in section *3.4.2.2.2.*

2.3.2.0.0 Dynamic viscoelastic oscillatory measurement

Samples were subjected to a frequency ranging from 0.01 to 10 Hz at a controlled stress of 50 Pa (chosen from Figure 3.2). The measurement was performed following a method as described in section *3.4.2.2.3.*

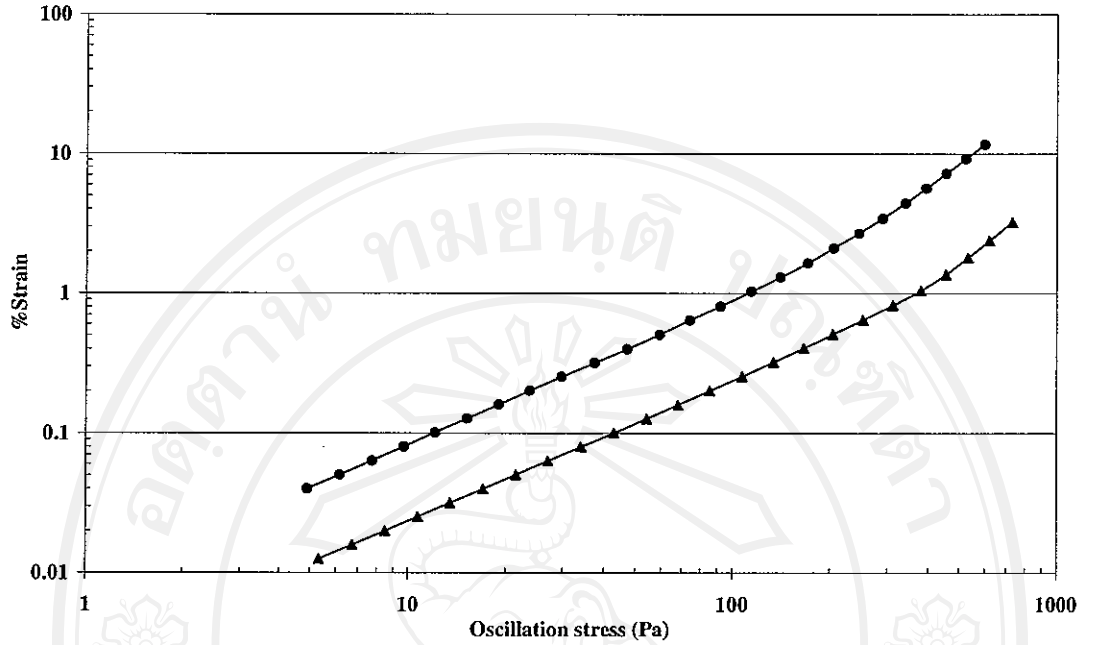

Figure 3.3 Oscillatory stress sweep (1-1,000 Pa) at frequency 1 Hz of pressurised ostrich-meat yor with ● added 0.5% (w/w) CMC with 0.5% (w/w) xanthan gum (Treatment 5) and ▲ added 1.0% (w/w) LBG (Treatment 2).

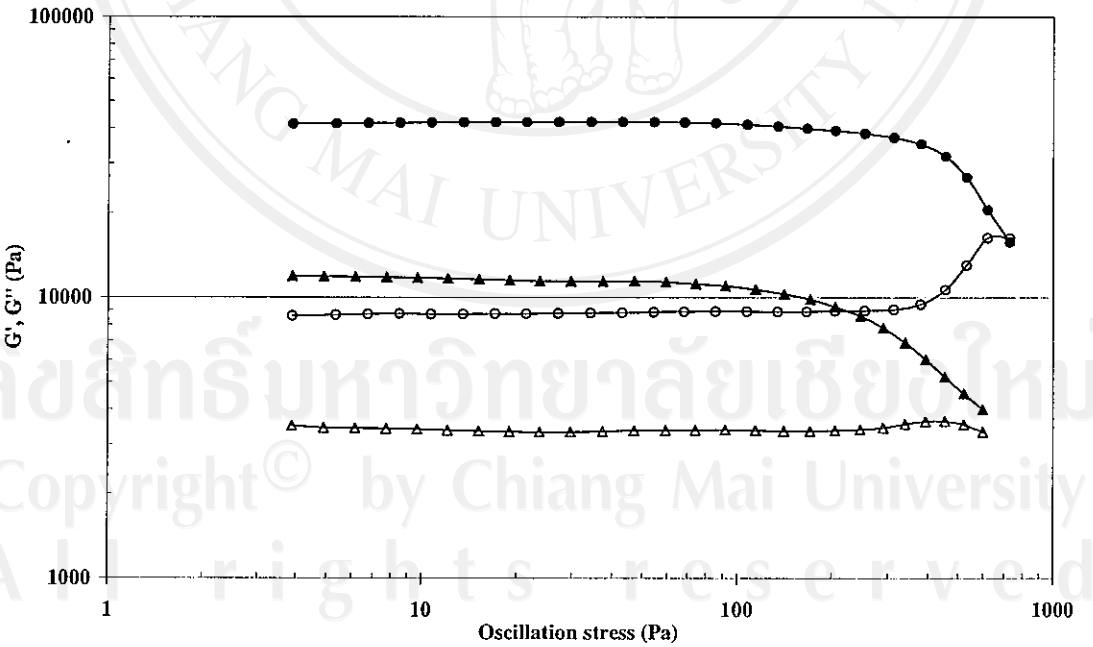

Figure 3.4 Stress amplitude sweep (1-1,000 Pa) at frequency 1 Hz of pressurised ostrich-meat yor, storage modulus (G′; closed symbols) and loss modulus (G″; opened symbols), ▲, Δ added 1.0% (w/w) LBG (Treatment 2), ▲, Δ added 0.5% (w/w) CMC with 0.5% (w/w) xanthan gum (Treatment 5).

3.4.2.4 Textural measurements

3.4.2.4.1 Puncture testing

Method as described in section *3.4.2.3.1.*

3.4.2.4.2 Compression testing

Method as described in section *3.4.2.3.2.*

3.4.2.4.3 Shear force

Method as described in section *3.4.2.3.3.*

3.4.2.5 Texture Profile Analysis (TPA) and sensory evaluation

3.4.2.5.1 Texture Profile Analysis (TPA)

Method as described in section 3.4.2.4

3.4.2.5.2 Sensory evaluation

Sensory evaluation were performed by student panelists in the Department of Food Science and Technology of Chiang Mai University to assess the physical attributes of the various products including colour, flovour, juiciness, texture and overall acceptability. Fifty-untrained panelists evaluated the yor samples on a "9-point" hedonic scale (9 = like extremely; 1 = dislike extremely). Samples consisted of 1 cm thick slices which were identified by a 3-digit random number which were placed on a plastic plate and served at room temperature (about 25 °C). Panelists were advised to taste along with unsalted crackers and to rinse their mouths out with bottled water after finishing each trial (a modified method of Barbut and Mittal, 1996). The main experimental design in this study was a Randomized Complete Block Design (RCBD) and the mean values were compared using Duncan's New Multiple Range Test (DNMRT) procedure. All data were analysed by analysis of variance using SPSS 10.0.1 software (SPSS Inc., Chicago, USA).

3.4.2.6 Water holding capacity

Method as described in section 3.4.2.5.

3.4.2.7 Microstructure determination

Confocal Scanning Laser Microscopy (CSLM) was used to examined the structure of ostrich-meat yor. Minced ostrich meat purchased from Alternatived Meats Ltd., Shropshire, UK was used to manufacture the ostrich yors in this section of the investigation. The samples were prepare by the same procedure as for the samples for viscoelastic measurement in section 3.4.3.1 but the ostrich fat was replaced by linseed oil (because ostrich fat was unavailable in Reading, UK).

The samples (1-2 mm thick) were stained with a mixture of Fluorescein Isothiocyanate (FITC, 0.02% w/v in water/ethanol) with the ratio of 1:1 and Nile Red (0.02% w/v in ethanol) for colouring the protein and the fat, respectively (Greenspan *et al.*, 1985; Ljunglöf and Hjorth, 1996). The staining solution was dropped directly to the samples (3-5 drops) for 30 min and then placed on a microscope slice. A Leica Scanning Laser Microscopy (Leica Microsystems Heidelberg, Germany) equipped with a Helium/Neon laser was used for the fluorescence excitation of the samples (500-530 nm for FITC and 505-586 nm for Nile Red). Pictures of selected representative areas from each sample were taken using a 10X magnification objective (Trespalacios and Pla, 2007). ImageJ software was used for image processing and analysis. The digital image files (*.TIF) were converted to 8-bit greyscale for analysis of the particle size/material distribution.

2.3.2.0 Differential Scanning Calorimetry (DSC)

Method as described in section 3.4.2.7.

2.3.2.0 Electrophoretic analysis

Method similar to that described in section 3.4.2.8. however the gels were not subject to silver staining.

2.3.2.0 Microbiology determination

The raw and pressurised ostrich-meat yors were examined for *Salmonella* spp., *Staphylococcus aureus*, *Clostridium perfringens*, *Escherichia coli* and Total count of the viable bacteria, according to Thai Industrial Standards for yor sausage and were determined by the method of AOAC (2000).

2.3.2.0 Statistical analysis

All data were analysed using an analysis of variance using a Mixture Experiment in Simplex Centroid Design, using regression analysis and a special cubic model (Montgomery, 2001) are as follow:

$$Y_i = \beta_{1i} X_1 + \beta_{2i} X_2 + \beta_{3i} X_3 + \beta_{12i} X_1X_2 + \beta_{13i} X_1X_3 + \beta_{23i} X_2X_3 + \beta_{123i} X_1X_2 X_3$$

Where Y = a predicted dependent variable, β_{1i}, β_{2i}, β_{3i}, β_{12i}, β_{13i}, β_{23i}, β_{123i} = corresponding parameter estimates for each linear, quadratic and special cubic term for i sets of treatments, X_1 = Carboxymethylcellulose (CMC), X_2 = Locust bean gum (LBG) and X_3 = Xanthan gum (XAN).

The data of sensory evaluation and TPA parameters were analysed by an analysis of variance using SPSS 10.0.1 software (SPSS Inc., Chicago, USA). Duncan's New Multiple Range Test (DNMRT) was used to determine differences among formulations and a correlation procedure was performed to evaluate the correlation of instrumental vesus sensory methods (Grigelmo-Miguel *et al.*, 1999).

2.3.2 Determination of the effect of the concentration of xanthan gum on viscoelastic characteristics and microstructure of pressurised ostrich-meat yor

2.3.2.0 Preparation of ostrich-meat yor

The samples were prepared by the same procedure as for those prepared for microstructural analysis in section 3.4.3.6 but modified by varying, at four different concentration levels, the xanthan gum, 0.50%, 0.75%, 1.00% and 1.25% (w/w). After pressure treatment, the products were assessed by transient testing for both creep compliance and oscillatory testing, to determine both the storage (G') and loss (G'') moduli.

2.3.2.0 Viscoelastic measurement and modeling

The oscillation stress sweep was performed over the range of 0.5-500 Pa at a frequency of 1 Hz to find out the optimal linear viscoelastic region. A Stress Tech Rheometer (Rheological Instruments AB, Lund, Sweden) was used to assess the viscoelastic characteristics of six samples from six individual pressure treatments (n = 6).

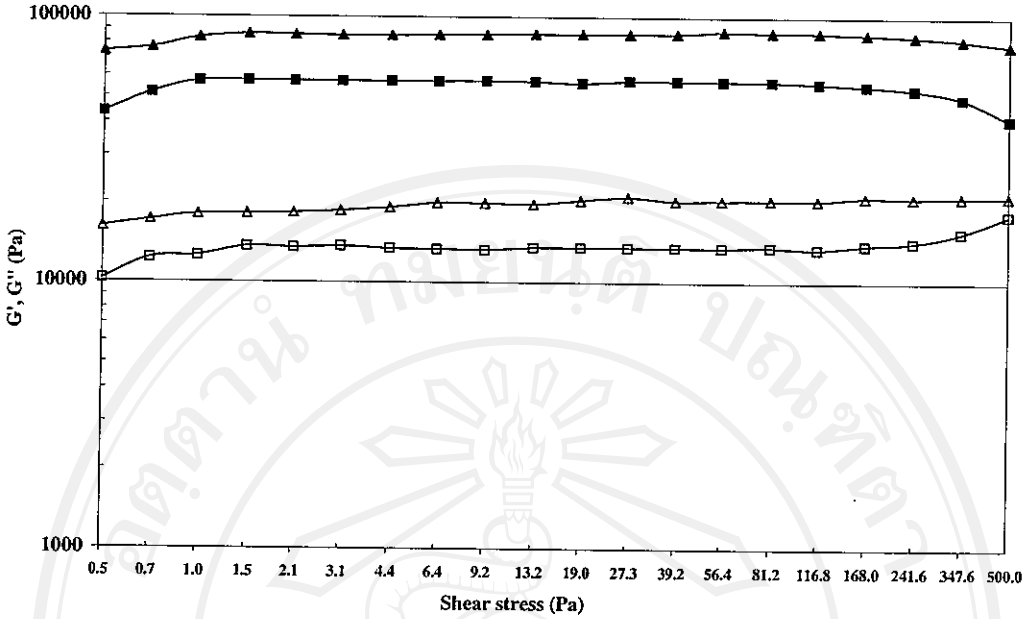

Figure 3.5 Stress amplitude sweep (0.5-500 Pa) at frequency 1 Hz of pressurised meat yors, storage modulus (G′; closed symbols) and loss modulus (G″; opened symbols), ▲, Δ no added xanthan gum, ■, □ added xanthan gum 1% (w/w).

2.3.2.0.0 Dynamic viscoelastic oscillatory measurement

Samples were subjected to a frequency range 0.01-10 Hz at a constant stress of 5 Pa (Figure 3.5).

2.3.2.0.0 Creep testing

The creep tests were undertaken using constant stress of 50 Pa (chosen from Figure 3.5) for a load time of 300 sec followed by an unloaded recovery time of 900 sec.

2.3.2.0 Microstructure determination

Method as described in section 3.4.3.6.

2.3.2.0 Statistical analysis

All data were analysed by analysis of variance using SPSS 10.0.1 software (SPSS Inc., Chicago, USA). Duncan's New Multiple Range Test (DNMRT) was used to determined any significant differences ($P \leq 0.05$) between the treatment means.