

# TIME DEPENDENT PROPERTIES OF THERMOPLASTIC PROTEIN PRODUCED FROM BLOODMEAL WITH SODIUM SULPHITE AS AN ANTI-CROSSLINKING AGENT

J. M. Bier<sup>1</sup>, D. van der Merwe, C. J. R. Verbeek<sup>1</sup> and M. C. Lay<sup>1</sup>

<sup>1</sup>School of Engineering  
University of Waikato:  
Private Bag 3105  
Hamilton 3240  
Jmb101@waikato.ac.nz

## ABSTRACT

The aim of this research was to investigate how the time dependent mechanical behaviour of bloodmeal-based thermoplastic was affected by varying sodium sulphite content at two injection moulding temperatures (120°C at exit or 140°C at exit). Thermoplastic protein was prepared by extrusion with 2, 3 or 4g sodium sulphite (SS), 3g sodium dodecyl sulphate, 10 g urea, 20 g triethylene glycol and 40 g water per 100 g bloodmeal, then injection moulded into test specimens. Pull to break, creep and stress relaxation tests were performed on conditioned samples and glass transition temperature ( $T_g$ ) was determined by dynamic mechanical analysis.

Ultimate tensile strength was 7.9, 7.6 and 5.6 MPa for samples moulded at 120°C and 7.6, 6.3 and 5.7 MPa when moulded at 140°C with 2, 3 and 4 g SS respectively. Experimental creep data was modelled with a 4 element model, consisting of a spring and dashpot in parallel, in series with an additional spring and dashpot. Plotting creep compliance versus time showed increasing chain mobility as SS content increased. Relaxation was modelled with the Struik equation for short-time experiments. Relaxation times were 530, 360 and 250 s with 2, 3 and 4 g SS respectively when moulded at the lower temperature. At 140°C, relaxation times were 440, 430 and 190 s for these SS contents.  $T_g$  was in the range 57-65°C (1 Hz peak in  $\tan\delta$ ) for all samples, but was lowest for samples with 4 g SS.

These results show that both increased sodium sulphite and the higher moulding temperature increased chain mobility in the processed plastic.

## INTRODUCTION

Environmental concerns regarding conventional plastics are motivating research into bio-based and biodegradable alternatives. Thermoplastic protein is one such alternative, which can be produced from agricultural by-products, such as bloodmeal, which do not compete with human food supply.

Proteins are much more complex than synthetic homo-polymers and have a wide range of inter-molecular interactions that reduce molecular mobility and increase the softening point to around their degradation temperature [1]. Plasticisers, such as water or other low molecular weight compounds, are necessary to lower the softening point for processing without excessive degradation [1]. In addition to plasticisation, thermoplastic processing of bloodmeal requires additional additives such as sodium sulphite (SS) to break covalent cross links along with sodium dodecyl sulphate (SDS) and urea to further disrupt protein-protein interactions [2].

While the combined effect of SS and urea reduces crosslinking (either physical or chemical), increasing chain mobility [3], high processing temperatures and specific mechanical energy input can cause excessive degradation and crosslinking in proteins [1]. For example, degradation at injection moulding temperatures above 130°C negatively impacts on the mechanical properties of soy protein/starch blends [4].

Crosslinking reduces chain mobility, which will slow the rate at which chains can rearrange to dissipate forces at constant strain (relaxation) or slide past each other in response to constant stress (creep). Studying these time dependent viscoelastic phenomena should reveal information about the effect of sodium sulphite or injection moulding temperature on the strength of chain interactions.

Viscoelastic behaviour is typically modelled as a combination of Hookean springs (where stress is proportional to strain) and Newtonian dashpots (where stress is proportional to strain rate) in series or parallel (Fig. 1) [5]. The Maxwell model of a spring and dashpot in series provides a theoretical treatment for stress relaxation, while the Kelvin/Voigt model of a spring and dashpot in parallel provides a theoretical treatment of creep behaviour. Both are ideal models, but introducing additional elements can come closer to modelling experimental behaviour of real polymers, such as the time dependence of creep compliance (Eq. 1) [5]. Alternatively, stretched exponentials such as the Struik equation, for short time experiments (Eq. 2), also describe the experimentally observed creep and relaxation behaviour of polymers [5].

The purpose of this research was to investigate the time dependent mechanical behaviour of bloodmeal-based thermoplastics as affected by varying sodium sulphite content at two injection moulding temperatures (120°C at exit or 140°C at exit).

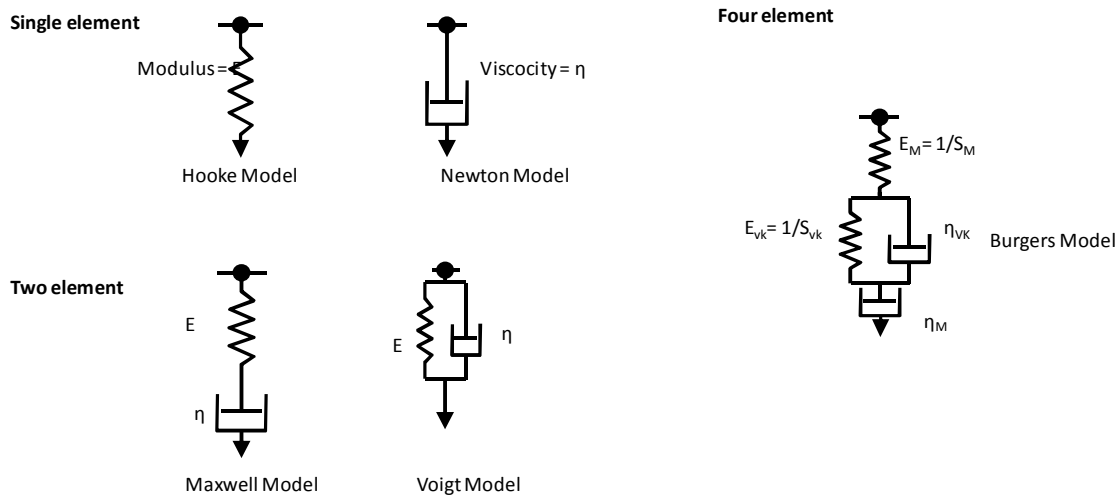


Fig. 1: Models of viscoelastic behaviour [5].

$$S(t) = \frac{\varepsilon(t)}{\sigma_0} = S_{VK} \left[ 1 - e^{\left(\frac{-t}{\tau_{ret}}\right)} \right] + S_M + \frac{t}{\eta_M}$$

Eq. 1

Where:

$S(t)$  = Creep compliance

$S_{VK}$  = Voigt-Kelvin compliance

$\tau_{ret}$  = Retardation time ( $\eta_{VK}S_{VK}$  in terms of the elements shown in Fig. 1)

$S_M$  = Maxwell compliance

$\eta_M$  = Maxwell viscosity

$$E(t) = \frac{\sigma(t)}{\varepsilon_0} = E_0 e^{\left(\frac{-t}{\tau_0}\right)^m}$$

Eq. 2

Where:

$E(t)$  = Relaxation modulus at given time

$\varepsilon_0$  = Initial strain

$E_0$  = Initial relaxation modulus ( $\sigma/\varepsilon$  at  $t=0$ )

$m$  = Constant (usually around 1/3)

$\tau_0$  = Characteristic relaxation time for material (dependant on temperature and aging)

## METHODS

### Materials and sample preparation

Thermoplastic protein (NTP) was prepared by adding a plasticiser solution to dry bloodmeal. The solution contained 40 g water, 20g triethylene glycol, 10 g urea, 3 g sodium dodecyl sulfate and 2, 3 or 4 g sodium sulphite per 100 g bloodmeal ( $p_{\text{pHBM}}$ ). The resulting mixture was extruded at 150 RPM in a Thermoprism TSE-16-TC twin screw extruder (temperature profile: 70, 100, 100, 100 and 120 °C from feed zone to die), granulated, then injection moulded into Type 1 tensile test specimens [6] using a BOY - 35A injection moulder with one of two temperature profiles (Tab. 1). Test pieces were conditioned at 50% RH and 23°C for 7 days. Formulations used are shown in Tab. 1.

Tab. 1: Sample formulations used and their abbreviations

Sample Name	Sample formulation (g)						Injection moulding temperature (From feed zone to die)
	Blood meal	Water	Urea	SS	SDS	TEG	
2-120	100	40	10	<b>2</b>	3	20	100, 115, 120, 120, <b>120</b> °C
3-120	100	40	10	<b>3</b>	3	20	100, 115, 120, 120, <b>120</b> °C
4-120	100	40	10	<b>4</b>	3	20	100, 115, 120, 120, <b>120</b> °C
2-140	100	40	10	<b>2</b>	3	20	100, 120, 135, 140, <b>140</b> °C
3-140	100	40	10	<b>3</b>	3	20	100, 120, 135, 140, <b>140</b> °C
4-140	100	40	10	<b>4</b>	3	20	100, 120, 135, 140, <b>140</b> °C

### Analysis

Three kinds of tests were performed in tension using an Instron model 33R4204 fitted with an extensometer (gauge length of 50 mm):

- Pull to break: samples were subjected to constant strain rate of 5 mm/min until fracture to determine ultimate tensile strength (UTS) and strain to break. A secant modulus was calculated between 0.0005 and 0.0025 strain.
- Creep: samples were pulled to a strain of 1% at 50 mm/min then held at the corresponding load for 20 minutes while strain was measured.
- Stress relaxation: samples were pulled to a strain of 1% at 50 mm/min then held at constant strain for 20 minutes while load was measured.

The glass transition temperature ( $T_g$ ) was determined using a DMA8000 (Perkin Elmer) fitted with a high temperature furnace, controlled with DMA software version 14306 and cooled with liquid nitrogen. Samples were cut from larger injection moulded pieces with approximate geometry of 3.5 x 6.5 x 30 mm and tested in single cantilever bending mode with a free length of 12.5 mm, dynamic displacement of 0.03 mm and frequency of 1 Hz. Experiments were performed between -100 and 120°C at a programmed heating rate of 2°C/min.  $T_g$  was determined from peak in  $\tan\delta$ .

All samples were conditioned for 7 days at 23°C and 50% relative humidity and tested in a climate controlled room.

## Modelling

The time dependent relaxation modulus from stress relaxation experiments was modelled with the Struik equation (Eq. 2) [5]. Initial estimates of  $\tau_0$  were calculated with Eq.3 and of  $m$  were  $m = 0.33$ . These values were then varied to obtain the best fit to experimental data using Microsoft Excel, using the least-sum-of-errors-squared method.

$$\tau_{0(est)} = t, at \sigma(t) = \frac{\sigma_0}{e} \quad \text{Eq. 3}$$

Where:

$\tau_{0(est)}$  = Initial estimate of Struik relaxation time

$\sigma(t)$  = Stress at time,  $t$

$\sigma_0$  = Initial stress at 1% strain

$e$  = Euler's number

Time dependent creep compliance was modelled using the Burgers four-element model (Fig. 1 and Eq. 1).  $S_M$  was determined from the instantaneous stress at strain = 0.01.  $\eta_M$  was determined from the slope of the compliance vs. time curve towards the end of the test.  $S_{VK}$  and  $\tau_{ret}$  were varied to obtain the best fit to experimental data.

## RESULTS

### Pull to break

Typical stress-strain behaviour for thermoplastics was observed in pull to break tensile tests (Fig. 2). Ultimate tensile strength (UTS) and strain at break are shown in Fig. 3. Smaller UTS values were observed in 4-120 and 4-140 than in the other four formulations, indicating a reduction in the strength of intermolecular interactions at higher SS content. Similarly, a smaller strain-at-break was observed for samples 2-120 and 2-140 than for higher SS contents showing SS decreased ductility.

Rather than inducing thermal crosslinking (which would restrict movements causing higher stiffness and lower strain at break), higher processing temperature increased ductility in the finished product. Samples moulded at 140°C with 2 or 3 pph<sub>BM</sub> SS showed increased ductility over those moulded at 120°C at the same SS content. Increased mobility could either be due to degradation of protein chains, with the breaking of peptide linkages, or due to a reduction in covalent crosslinking between chains. At 3 pph<sub>BM</sub> SS, increased temperature also reduced strength, however, the difference in strength between moulding temperature was less pronounced at 2 pph<sub>BM</sub> SS. Similarly, at 4 pph<sub>BM</sub> SS, little difference was observed between the mechanical properties at each moulding temperature.

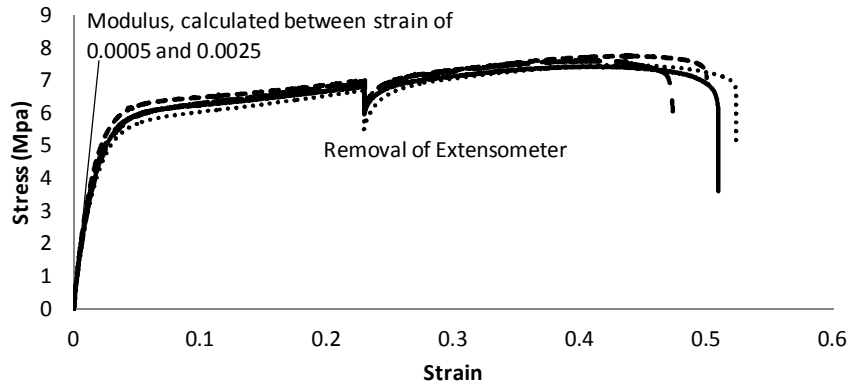


Fig. 2: Typical stress strain diagram for NTP. Displayed are five repeats for 3-120°C.

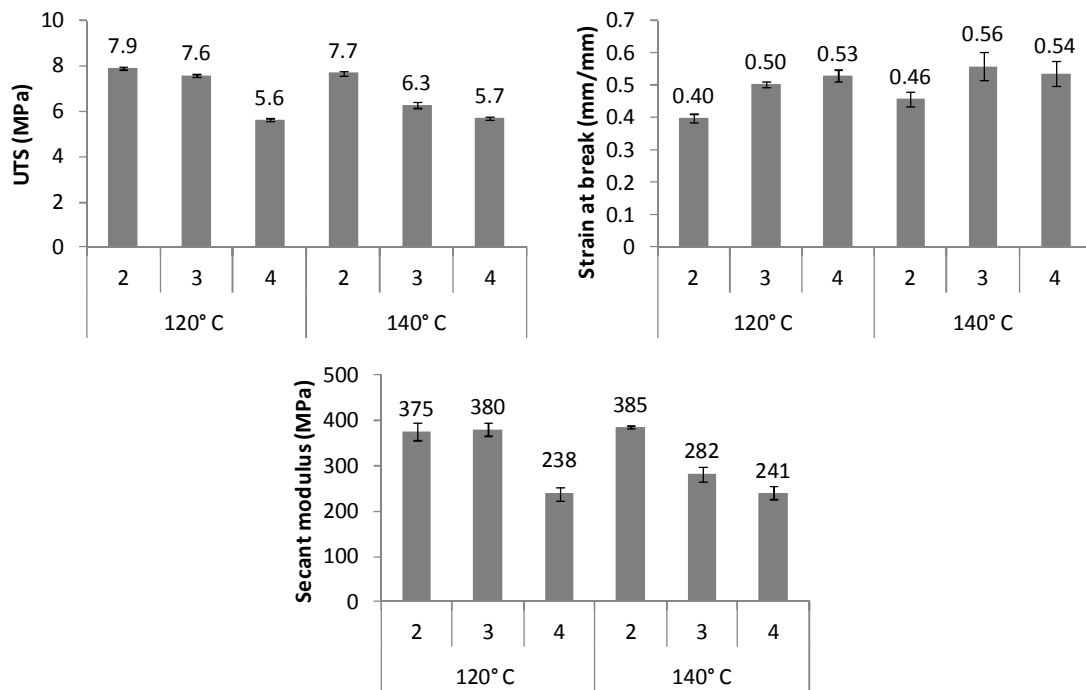


Fig. 3: Mechanical properties determined from pull to break tensile tests at constant strain rate. Average from five samples, error bars denote standard error of the mean. X-axes show sample SS content (in pph<sub>BM</sub>) and highest temperature from injection moulding profile, per Tab.1.

### Stress /relaxation

NTP held at constant strain showed considerable drop in stress with only 26-32% of the initial stress remaining after 20 minutes (Tab. 2). This structural relaxation provides evidence that chain rearrangements occur on a time scale comparable to the experimental time at room temperature. Amorphous polymers below their  $T_g$  are meta-stable and chain rearrangements still occur, albeit more slowly than in a polymer melt. The ratio of relaxation time to experimental observation time is called the Deborah number [7]. At low Deborah number (observation time  $\gg$  relaxation time) apparent elastic behaviour is observed, while at high Deborah numbers (relaxation time  $\gg$  observation time), apparently viscous behaviour is observed. If the observation time and relaxation time are

similar, (that is, a Deborah number of  $\approx 1$ ) viscoelastic phenomena are apparent, such as the relaxation seen here. This confirms an experimental length of 20 minutes is adequate for exploring these chain rearrangements, whereas the frequencies needed to detect such motions at room temperature in an oscillatory experiment, such as dynamic mechanical analysis would need to be very low (eg  $< 0.01$  Hz). The conditioning period of seven days is significantly longer than the timescale of observed structural relaxation, so it is possible that polymer aging may have occurred during conditioning. An additional implication is that in applications where the material is exposed to similar stresses to this experiment for a similar time, the material will flow. This was demonstrated in the creep experiments discussed later in this paper.

Modelling of the experimentally collected data with the Struik-equation (Eq. 2) enabled an average relaxation curve to be calculated for each formulation, based on the experimental repetitions (Fig. 4). Considerable variation between relaxation times (Tab. 2) was seen at lower SS contents for both moulding temperatures, leading to high standard deviation. This may be due to a smaller reduction of disulphide linkages during processing resulting in a less homogenous melt and hence less uniformity between moulded samples. Alternatively, small errors in measured properties may propagate to larger differences at longer times. Higher SS content showed greater reproducibility, as well as a shorter mean relaxation time. The high variability at lower SS contents made comparison between the two moulding temperatures difficult; however, relaxation time for 4-140 was shorter than that seen for 3-140, suggesting that higher moulding temperature also contributed to increased chain mobility.

The best fit to the Struik equation for stress relaxation was found with  $m \approx 0.16-0.19$ . Relaxation behaviour predicted by the Maxwell model fits to a simple exponential, without an “m” exponent. To describe real polymer behaviour, a number of Maxwell models would need to be superimposed with different relaxation times to describe experimentally observed polymer behaviour [5]. Alternatively, the Struik equation for short time experiments takes the form of a stretched exponential (or Kohlrausch-Williams-Watts (KWW)) function. Stretched exponentials describe relaxation processes in the presence of complexity [8]. A stretching exponent of 1 simplifies to simple exponential decay, whilst values closer to zero indicate greater stretching. Although typically  $\approx 0.33$  for a synthetic homopolymer [5], it is sensible to expect that NTP would have a greater stretch to its relaxation function than as in proteins there are a wider variety of molecular interactions.

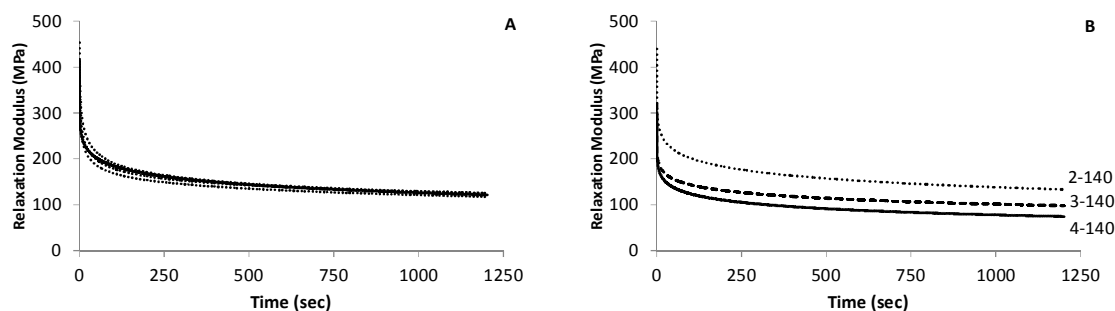


Fig. 4: Decay of relaxation modulus at 1% strain. A) Actual data (dashed lines) and Struik equation using average parameters from each (solid line) for 3-120. B) Struik

equation using average parameters from 3 repeats of each of the different SS contents moulded at 140°C

Tab. 2: Relaxation parameters. Average from three samples. Standard deviation is shown in brackets.

g SS/100g BM	Fraction of strain remaining after 20 minutes		Fitted Struik relaxation time (s)		Fitted Struik “m” parameter	
	120°C	140°C	120°C	140°C	120°C	140°C
	2	0.32 (0.02)	0.31 (0.03)	532 (206)	438 (162)	0.17 (0.02)
3	0.30 (0.02)	0.31 (0.01)	356 (87)	426 (89)	0.17 (0.01)	0.16 (0.00)
4	0.27 (0.01)	0.26 (0.00)	254 (31)	190 (7)	0.19 (0.01)	0.18 (0.00)

### Creep

NTP held at a constant stress showed appreciable creep on the experimental time scale of 20 minutes. The fitted Burgers model is made up of Maxwell element in series with a Voigt Kelvin element (Fig. 1). The compliance at  $t = 0$  is equivalent to  $S_M$ , the compliance of the Maxwell element. As time increases, the compliance curve tends to become linear, the slope of which is  $1/\eta_M$ . The shape of the curve between these extremes is dependent on the Voigt/Kelvin element, described in terms of the retardation time and  $S_{VK}$ . Fitted retardation times were in the range of 100 - 180 s, but with considerable variation (Tab. 3).  $S_{VK}$  values, however, were appreciably higher with 4 pph<sub>BM</sub> SS than at lower levels, and for each level were higher in samples moulded at 140°C than those moulded at 120°C. Although samples moulded at 120°C had similar  $S_M$ , increased  $S_M$  with increased SS was seen for samples moulded at 140°C. As with the retardation time, large standard deviations were seen in  $\eta_M$ . Tab. 3 also shows total creep at the end of an experiment. For samples moulded at 120°C, it is apparent that as sodium sulphite content increased, the total creep experienced in the 20 minute experiment also increased. At 140°C, all three sodium sulphite contents showed similar total creep in the 20 minute duration. This does not imply equal mobility, as creep rate also depends on starting stress which differed in each sample due to differing sample stiffness. It is for this reason that compliance has been modelled rather than strain.

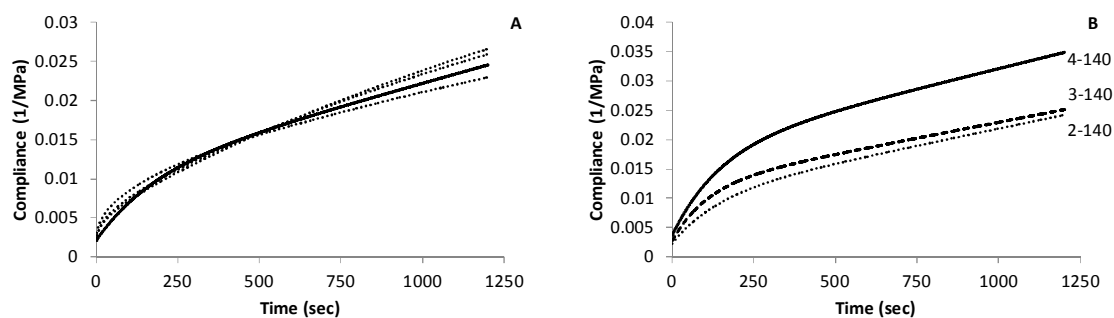


Fig. 5: Increase in compliance at constant stress. A) Actual data (dashed lines) and Burgers model fit using average parameters from each (solid line) for 3-120. B) Burgers model fit using average parameters from 3 repeats of each of the different SS contents moulded at 140°C

Tab. 3: Fitted Burgers model parameters for creep experiments. Average from three samples. Standard deviation is shown in brackets.

	SS content (p <sub>ph<sub>BM</sub></sub> )	Moulding Temperature	
		120°C	140°C
<b>Voigt/Kelvin Element</b>			
S <sub>VK</sub> (1/MPa)	2	0.0073 (0.0011)	0.0080 (0.0020)
	3	0.0084 (0.0006)	0.0094 (0.0011)
	4	0.0137(0.0016)	0.0147 (0.0009)
Retardation time (s)	2	134 (42)	140 (37)
	3	177 (33)	107 (6)
	4	149 (41)	145 (43)
<b>Maxwell Element</b>			
S <sub>M</sub> (1/MPa)	2	0.0024 (0.0001)	0.0023 (0.0002)
	3	0.0022 (0.0002)	0.0028 (0.0001)
	4	0.0023 (0.0003)	0.0037 (0.0003)
η <sub>M</sub> (MPa.S)	2	95000 (17000)	86000 (9400)
	3	86000 (18000)	93000 (31000)
	4	71000 (37000)	73000 (8500)
<b>Total creep</b> (mm/mm)	2	0.09 (0.02)	0.11 (0.01)
	3	0.12 (0.02)	0.10 (0.02)
	4	0.16 (0.05)	0.10 (0.01)

### Dynamic mechanical analysis

Dynamic mechanical analysis revealed a glass transition in the range of 58-65°C for all samples (Tab. 4), showing that all materials were >30°C below T<sub>g</sub> at the test temperature



of 23°C. Although differences between the formulations were minor, at both moulding temperatures the samples containing 4 pph<sub>BM</sub> had the lowest T<sub>g</sub>.

Tab. 4 T<sub>g</sub> determined from peak in tanδ, averaged from two samples. Standard deviation is shown in brackets.

SS content (pph <sub>BM</sub> )	Moulding Temperature	
	120°C	140°C
2	62 (1)	63 (3)
3	65 (1)	63 (2)
4	60 (1)	58 (0)

T<sub>g</sub> is normally assumed to be an indication of chain mobility. In this work large differences in the T<sub>g</sub> were not observed, but clear differences were observed when evaluating the time-dependant properties. This technique is therefore useful to evaluate subtle differences between chain mobility. It was evident from the work that between 2 and 3 pph<sub>BM</sub> SS there is little difference, but 4 pph<sub>BM</sub> did make a difference. However, the overall goal is not to increase chain mobility too much, as strength drops accordingly. This too was evident from pull to break results.

## CONCLUSIONS

Six varieties of thermoplastic protein were produced from bloodmeal by varying sodium sulphite content and injection moulding temperature to study the effect of these parameters on time dependent viscoelastic behaviour. Glass transition, determined from the peak in tanδ was in the range of 57-65°C for all samples, more than 30°C above the test temperature, and lowest for samples with the most sodium sulphite.

Ultimate tensile strength and strain at break results from pull to break tests at constant strain rate indicated that both increased sodium sulphite content and the higher injection moulding temperature increased chain mobility.

Samples held at a constant strain of 1% exhibited stress relaxation which was modelled using a stretched exponential. High variability between samples was seen at lower SS contents, however the trend of increased SS increasing mobility (characterised by a shorter relaxation time) was supported. At 4 pph<sub>BM</sub> SS, less variability was observed and higher moulding temperature showed shorter relaxation time.

Samples held at constant stress exhibited creep behaviour, which was modelled with the four-element Burgers model. Increased SS content increased the compliance of both the Maxwell and Voigt/Kelvin elements of the model.

Together, these results confirmed that increased sodium sulphite increased chain mobility. Additionally, it was found that injection moulding at 140°C instead of 120°C did not cause excessive thermal crosslinking, but rather increased chain mobility at some sodium sulphite contents. Whilst increased chain mobility in the conditioned plastic comes at the expense of strength, and is not always desirable, this widens the range of acceptable injection moulding parameters which may be useful in adjusting injection settings for appropriate filling of different moulds. Additionally, depending on the stresses that will be encountered when using this material, creep may need to be considered.

## REFERENCES

1. Verbeek, C.J.R. and L.E. van den Berg, *Extrusion Processing and Properties of Protein-Based Thermoplastics*. Macromolecular Materials and Engineering., 2010. **295**(1): p. 10-21.
2. Verbeek, C.J.R. and L.E. van den Berg, *Development of Proteinous Bioplastics Using Bloodmeal*. Journal of Polymers and the Environment., 2010: p. 1-10.
3. Verbeek, C.J.R. and L.E. van den Berg, *Structural changes as a result of processing in thermoplastic bloodmeal*. Journal of Applied Polymer Science, 2012: p. n/a-n/a.
4. Huang, H.C., T.C. Chang, and J. Jane, *Mechanical and physical properties of protein-starch based plastics produced by extrusion and injection molding*. Journal of the American Oil Chemists Society, 1999. **76**(9): p. 1101-1108.
5. van Krevelen, D.W. and K.t. Nijenhuis, *Properties of polymers :their correlation with chemical structure : their numerical estimation and prediction from additive group contributions*. 4th, completely rev. / ed. 2009, Amsterdam ;Boston: Elsevier. xxvi, 1004.
6. ASTM International, *D638 -03 Standard Test Method for Tensile Properties of Plastics*. 2004, ASTM International,: West Conshohocken, PA 19428-2959, United States.
7. Menard, K.P., *Dynamic mechanical analysis :a practical introduction*. 2nd ed. 2008, Boca Raton, FL: CRC Press. xix, 218.
8. Rajagopal, A.K., K.L. Ngai, and S. Teitler, *Effective temperature and relaxation in complex-systems*. Journal of Chemical Physics, 1990. **92**(1): p. 243-246.