Shear work induced changes in the viscoelastic properties of model Mozzarella cheese

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Abstract

We investigated the effect of shear work on the viscoelastic properties of Mozzarella type cheeses. Three model cheeses (full-fat, non-fat and full-fat with added tri-sodium citrate) were prepared by working cheese components together at 70 ^{o}C in a twin screw Blentech cooker. G' at 70 ^{o}C increased with shear work input suggesting work thickening. At lower shear work inputs (<30 kJ kg^{-1}), cheese behaved like a viscoelastic liquid exhibiting typical entangled polymer melt behaviour with moderate frequency dependence. A definite critical point for structural and viscoelastic transition was identified at higher shear work levels (~ 58 kJ kg^{-1} at 150 rpm). Excessive shear work levels (>70 kJ kg^{-1}) resulted in a viscoelastic solid material exhibiting low frequency dependence. Similar viscoelastic property changes occurred in non-fat cheese suggesting that major changes were taking place in the protein matrix during working. Good correlation was found between oscillatory rheological properties such as G' and LT_{max} and the melting properties of test cheeses.

1. Introduction

Pasta-filata type cheeses such as Mozzarella are known for their fibrous macroscopic and microscopic structure (McMahon, Fife & Oberg, 1999). The fibrous structure means that they are anisotropic in both microstructure and mechanical properties (Bast et al., 2015). The cooking and stretching steps during cheese manufacture promote the formation of fibrous structure through kneading action. This not only creates the desirable texture but also helps in the distribution of fat and serum channels within the cheese matrix (McMahon et al., 1999). The heterogeneous distribution of these channels is required to facilitate melting during baking of a pizza because they allow migration of fat and moisture to the cheese surface, preventing the surface from drying and thus facilitating flow of the molten cheese on the pizza (Rudan & Barbano, 1998). The energy supplied as shear work during the working of molten cheese is used for formation of new bonds and breakage of some bonds. The dynamics of these two reactions governs the melt and stretch characteristics of the cheese. In order for the cheese to flow on a pizza the bonds between protein molecules should be flexible and transient so that they break temporarily and are subsequently reformed with different protein molecules in the structure. Pasta-filata cheeses are also required to stretch. The stretching characteristics are governed by the relaxation and reformation of bonds between adjacent protein molecules during deformation (Lucey, Johnson & Horne, 2003). These melt and stretch properties of pasta-filata type cheeses are related to the proportion of calcium associated with proteins (Lucey & Fox, 1993; Joshi, Muthukumarappan, & Dave, 2002).

Oscillatory rheology has been widely used for characterisation of the melting behaviour of cheese because the methods are relatively straightforward (Tunick et al., 1993; Hsieh, Yun, & Rao, 1993; Ak & Gunasekaran, 1996; Subramanian & Gunasekaran, 1997; Guinee, Feeney, Auty & Fox, 2002; Venugopal & Muthukumarappan, 2003; Karoui, Laguet & Dufour, 2003; Joshi, Muthukumarappan & Dave, 2004; Rock et al., 2005; Udyarajan, Horne & Lucey, 2007; Hussain, Grandison & Bell, 2012; Ma, Balaban, Zhang, Emanuelsson-Patterson & James, 2014). Measurement of storage modulus (G'), loss modulus (G") and loss tangent (LT or δ) with respect to strain amplitude, frequency and temperature are common ways of performing experiments. G' is an index of stiffness or elasticity of a material and is also a measure of the energy stored and released in one oscillation cycle. G" indicates the energy lost per oscillation cycle through viscous dissipation (Lucey et al., 2003). LT, a ratio of viscous to elastic properties, is related to the relaxation of bonds in the cheese matrix (Lucey, 2002) and can be used as an indicator of cheese meltability or flowablity (Lucey et al., 2003). LT can also be used as a material function to describe viscoelastic behaviour (Steffe, 1996).

Strain amplitude sweeps are usually conducted to determine the linear viscoelastic limit of a material. Frequency sweeps are useful to characterise the state of a material during processing. They have been widely used in characterising the viscoelastic behaviour of polymer melts. Entangled polymeric networks demonstrate significant frequency dependence whereas viscoelastic solids show very little frequency dependence. Temperature sweeps are important to understand the melting behaviour of a material. Decreases in dynamic moduli with increase in temperature reflect softening of the cheese matrix upon heating. A crossover temperature for G'-G" on a temperature sweep indicates the gel-sol transition point (Schenkel, Samudrala & Hinrichs, 2013). The maximum value of LT on a temperature sweep (LT_{max}) is considered as an indicator of melt (Mounsey & O'Riordan, 1999) and/or flow (Guinee, Auty & Mullins, 1999). Oscillatory rheology has been successfully used to distinguish between the following aspects of cheeses - different cheese types, range of fat levels, effect of storage, processing conditions, compositional differences (Mounsey &

O'Riordan, 1999). We have used each of the above methods to explore changes in the properties of model Mozzarella cheeses during working.

Before our work only two studies had investigated the effect of working of Mozzarella cheese on rheology and functionality (Mulvaney, Rong, Barbano & Yun, 1997; Yu & Gunasekaran, 2005). Both studies used thermo-mechanical energy to create pasta-filata structures and both concluded that screw speed and temperature could be used as process control variables to obtain the desired functionality. Both used a narrow shear work range (2-6.5 kJ kg^{-1}) and studied the combined effect of thermal and mechanical energy. Our recent work reported changes in steady shear rheology during the mechanical working of cheese in a Blentech twin-screw cooker (Sharma et al., 2016). Rheology and melt functionality were strongly dependent on total shear work input. Apparent viscosity at 0.01 s^{-1} increased exponentially with shear work input increasing 198 fold over the shear work range of 2.8 to 185 kJ kg^{-1}, indicating strong work thickening behaviour. Good negative correlation (R^2=0.90) was found between apparent viscosity and melt score. Our objective in this study is to explore the effect of shear work input on the oscillatory properties of three model Mozzarella cheeses. Our main focus was to study changes in full-fat cheese. Non-fat cheese and cheese with tri-sodium citrate added were also prepared to study shear-induced changes in the absence of fat and with minerals chelated. A broad range of shear work (2-125 kJ kg^{-1}) was used to exaggerate any work thickening effects and changes in structure.

2. Materials and methods

2.1 Materials

Renneted and acidified protein gel manufactured from skim milk was obtained at -20 ^{o}C from Fonterra Research and Development Centre (FRDC) pilot plant, Palmerston North, NZ. The proximate composition of the protein gel was typically about 50% moisture and 46% protein.

The frozen blocks were thawed for 1 d at 11 ^{o}C and ground in a Rietz grinder (Rietz Manufacturing, Santa Rosa, CA, USA) with 6 mm grind size. Cream was obtained from FRDC as a fresh lot on each trial day. Cheese salt was obtained from Dominion Salt (Mount Maunganui, New Zealand). Tri-sodium citrate (TSC) was obtained from Jungbunzlauer (Basel, Switzerland).

2.2 Manufacture of model mozzarella cheeses

Model Mozzarella cheese was made at FRDC by mixing, cooking and working protein gel, cream, water and salt in a counter rotating twin-screw cooker (Blentech, model CC-0045, Blentech Corporation, Rohnert Park, CA, USA). The batch size and working volume of the cooker were 25 kg and 29.45 L respectively. Three types of model Mozzarella cheese were made - full-fat, non-fat and full-fat with 0.5 % TSC as a chelating agent. The target composition of full-fat cheese was 23% fat, 21 % protein, 53% moisture and 1.4 % salt. All results are for full-fat cheese unless otherwise noted. Non-fat cheese used the same protein/moisture and protein/salt ratios as full-fat cheese. Sharma et al. (2016) give further details of the processing methods, sampling times, sample storage conditions and final compositions. Screw speeds of 50, 150 and 250 rpm were used for shear work treatment. Each run was repeated on a different day at least one month after the first run to ensure that similar results were obtained with raw materials obtained from different lots but with similar composition.

Shear work input was calculated by numerical integration of the torque-time curve with respect to time (Sharma et al., 2016). The meltability of Mozzarella cheese was measured by the modified Schreiber test (Muthukumarappan, Wang & Gunasekaran,1999) with some variations (Sharma et al., 2016).

2.3 Dynamic rheological measurements

The dynamic rheological properties of the cheeses were studied on an Anton Paar MCR 301 rheometer (Anton Paar, Graz, Austria) with a 20 mm diameter serrated plate geometry (PP20/P2) and a Peltier temperature hood (H-PTD 200) (Sharma et al., 2015, 2016). Disc-shaped cheese samples of 20 mm diameter and ~2 mm thickness were prepared and equilibrated for 2 min at test temperature as previously except that a 1 N normal force was used to define the measurement gap at 20 ^{o}C (Sharma et al., 2015). A ring of soybean oil was placed around the sample periphery to avoid moisture loss during rheological measurements.

Strain amplitude sweeps ranging from 0.01-100 % were conducted at 0.1, 1 and 10 Hz and at 70 ^{o}C to determine the linear viscoelastic (LVE) limit of the cheeses. In temperature sweeps, amplitude and frequency were 0.2 % and 1 Hz respectively and temperature was increased from 20^{o}C to 90^{o}C. To ensure nearly isothermal conditions during temperature sweeps, the rate of temperature rise of the Peltier heating system was maintained at 1.8^{o}C per min. Preliminary experiments placing a thermocouple in the thermal centre of the specimen and monitoring temperature rise at different heating rates had shown that this slow heating rate was necessary. Frequency sweeps were conducted by applying frequencies in descending order from 100 Hz to 0.01 Hz at 70^{o}C using 0.2% strain amplitude. The frequency dependence of G' and G'' for the molten cheeses was fitted to the following equations (Steffe, 1996; Tunick, 2011).

$$G' = k_{elastic}\omega^{n} \quad (1)$$

$$G'' = k_{viscous}\, \omega^{n} \quad (2)$$

where n, $k_{elastic}$ and $k_{viscous}$ are constants, and n is the degree of frequency dependence. All rheological measurements were conducted at least in duplicate. All data points are the means of the two or more replicates.

2.4 Statistical analysis

Descriptive statistics, non-linear regression and correlation analysis were conducted on the data using SPSS software (version 20). Non-linear regression analysis was performed using curve estimation functions and the best curve was selected based on goodness of fit (R^2). Pearson's correlation coefficient was used to test for significance with a two-tailed t- test at $P<0.01$. For comparison of the two methods for obtaining critical shear work values paired t-tests were used at 5% level of significance.

3. Results

3.1 Linear viscoelastic limit

Strain amplitude sweeps for cheese samples having shear work in the range 4.9-185 kJ kg^{-1} indicated that the limit of the LVE range was about 10 % strain for all samples (Fig. 1). G' at low strains increased from ~194 Pa at shear work 4.9 kJ kg^{-1} to 3890 Pa at shear work 185 kJ kg^{-1}, indicating considerable stiffening of the cheese with prolonged working. LVE limits were also tested at different temperatures in preliminary experiments. A strain amplitude of 0.2% was selected for frequency and temperature sweeps to be well within the linear viscoelastic range.

3.2 Frequency dependence of viscoelastic properties

Frequency sweeps on model Mozzarella cheeses (Fig. 2) demonstrate how viscous and elastic properties change with rate of application of strain or with timescale of deformation. G' and G" increased with increasing frequency for all cheeses but the rate of increase was affected by shear work input. The degree of frequency dependence is given by n in equations 1 and 2. At a low shear work input (8.8 kJ kg^{-1}), both moduli increased at relatively similar rates (n=0.722 for G' and n=0.8132 for G'') and G" was always higher than G' throughout the practical range of frequency (0.1-10 Hz). This cheese therefore behaved like a viscoelastic liquid with moderate frequency dependence. Frequency dependence of G' was highest (of the four cheeses tested) (n=0.8861) with a slightly higher shear work input (26.3 kJ kg^{-1}, Fig. 2b). G' increased at a faster rate than G" resulting in a cross over (LT =1) at ~6.4 Hz. Such frequency dependent behaviour and the presence of a G'-G" crossover on the frequency sweep is demonstrated by soft gels formed by entangled polymer networks or physical gels with weak bond strengths (Stading & Hermansson 1990; Tunick, 2011).

At a shear work input of 58.2 kJ kg^{-1}, the frequency dependence of G' was much lower (n=0.54). Coincidently, G' and G" followed almost the same path with respect to frequency dependence for this cheese (Fig. 2c). If both G' and G" exhibit power-law behaviour with a similar exponent, the loss tangent should become independent of frequency (Fatimi, Tassin, Quillard, Axelos & Weiss, 2008). As the loss tangent is about 1 and independent of frequency, this cheese meets the Winter-Chambon criteria of a gel transition point (Winter & Chambon, 1986). Clearly the cheese is undergoing a major phase change or viscoelastic transition at this level of shear work during shearing at 70^{o}C with transition from a predominantly liquid-like behaviour to a predominantly solid-like behaviour. We consider this cheese to be at a critical point in the shear induced structure formation and work thickening process.

Excessively worked samples (73. 7 kJ kg^{-1}, Fig. 2d) showed a very low frequency dependence of G' (n = 0.26), behaviour typical of strong gels, e.g. cross linked gels involving permanent covalent bond formation (Stading & Hermansson 1990; Tunick, 2011). G' was greater than G'' throughput the frequency range indicating the dominance of elastic behaviour, typical for a viscoelastic solid. After prolonged shear therefore, cheese has transformed from a viscoelastic liquid into a viscoelastic solid.

Non-fat cheese also demonstrated a decrease in frequency dependence (from n=0.96 to 0.78) with increase in shear work input (6-128 kJ kg^{-1}) (data not shown). Non-fat cheese had higher frequency dependence than full-fat cheese. Cheese with added TSC exhibited typical entangled polymer type behaviour with moderate frequency dependence (n=0.85) (data not shown). Shear work input had no significant effect on the frequency dependence of full-fat cheese with TSC added, suggesting that shearing caused no significant changes in the cheese structure. TSC chelates calcium strongly and so diminishes the role of calcium in holding the casein gel network together. Chelation of calcium weakens protein-protein interactions and results in breakdown and opening of the gel network and solubilisation of proteins (Brickley et al., 2008; Mizuno & Lucey, 2005). This modified structure therefore is insensitive to work thickening as the protein chains no longer participate in polymerization or cross linking of adjacent chains to strengthen the network upon shearing.

3.3 Temperature dependence of viscoelastic properties

Viscoelastic properties during the melting of model Mozzarella cheese were studied by conducting temperature sweeps in the range 20-90^{o}C. Repeated temperature sweeps on one sample having 5.0 kJ kg^{-1} of shear work input were conducted first (Fig. 3). Both G' and G" decreased with temperature rise throughout the test temperature range. LT continuously increased during heating, reached a peak at about 79^{o}C and then decreased with further increase in temperature. G' and G" reflect the total number and strength of protein-protein bonds in the cheese, so a decrease in these values is evidence of a weakening of protein-protein interactions (Lucey et al., 2003). The rate of decrease for both G' and G" appeared relatively faster in two temperature ranges, 20-35^{o}C and 50-65^{o}C, the first attributed to melting of the fat phase and the second to softening of the protein matrix. For non-fat cheese the faster melting region at 20-35 ^{o}C was missing due to absence of fat. In the temperature range 20-50^{o}C, the cheese behaved like a viscoelastic solid as G'>G" and LT<1. For the temperatures above 52^{o}C, it acted like a viscoelastic liquid as G">G' and LT>1. The crossover temperature where G" = G' was at ~51.4^{o}C (Fig. 3) and indicates initiation of softening of the protein matrix. Elastic properties dominate below the crossover temperature whereas viscous properties dominate above the crossover temperature. After a slow increase at <40^{o}C, LT increased sharply until it reached LT_{max} of 2.64 at ~79 ^{o}C. LT_{max} is often regarded as an indicator of flowability or melt functionality. These changes in G', G" and LT indicate increased mobility of the cheese matrix with increasing temperature. The standard deviations for G', G'' and LT all increased with increasing temperature and were highest at 75 – 90 ^{o}C.

The effect of shear work on viscoelastic properties is clearly evident (Fig. 4) particularly in the temperature range 30-80 ^{o}C. G' and G" were higher at a given temperature with increased shear work input. However, LT_{max} generally decreased with increased shear work input. The biggest differences in G' and LT_{max} after the given shear work treatments occurred in the temperature range 65-75^{o}C. Cheeses with lower shear work inputs, 3.3-26.3 kJ kg^{-1}, did not differ much in G' and G" at ~70^{o}C, suggesting minor changes to macroscopic structure in this shear work range. Higher shear work inputs (>50 kJ kg^{-1}) showed much higher values of both G' and G" at temperatures of 60^{o}C and above. Cheese with a shear work input of 58.2 kJ kg^{-1} showed overlap of G' and G" values in the range 60-75^{o}C (Fig. 5a). This corresponds well with the behaviour exhibited in the frequency sweep at 70^{o}C (Fig. 2c), supporting the hypothesis that the cheese is in transition at this level of shear work input. The cheese is undergoing a transition from a viscoelastic liquid to a viscoelastic solid because of enhanced attractive protein-protein interactions at 70 ^{o}C in the high shear environment of the Blentech. At a still higher level of shear work input (73.7 kJ kg^{-1}), G' values were significantly higher than at lower shear work levels at all temperatures (Fig. 4). G' was also higher than G" at all temperatures and no crossover temperature was observed (Fig. 5b). Typically viscoelastic solids exhibit such behaviour. LT_{max} was 0.74 indicating that elastic behaviour was dominant. With this large amount of shear work input, the cheese was transformed into a viscoelastic solid.

G'-G" crossover temperature and LT_{max} values for the replicate runs at 150 rpm were plotted against time of working and shear work input (Fig. 6). Slightly more consistent curves were found when both parameters were plotted against shear work. Steady shear rheology and melt functionality data also showed better reproducibility when plotted against shear work (Sharma et al., 2016). Results versus shear work obtained at different screw speeds can also be plotted together. Therefore, further plots were done as a function of shear work as in Sharma et al. (2016).

LT_{max} and crossover temperature data for all 32 samples of full-fat cheese without TSC were plotted versus shear work (Figs. 7, 8). LT_{max} decreased with shear work input, indicating less tendency to flow upon melting after high shear work inputs. Crossover temperature increased from ~50 to ~60^{o}C with shear work increase from 3.3 to ~60 kJ kg^{-1}. Crossover temperature is an indicator of the softening point of the cheese matrix (Gunasekaran & Ak, 2003).

Sharma et al. (2016) showed that during manufacturing of model Mozzarella cheeses at 150 and 250 rpm screw speeds torque increased steadily to a maximum and then declined quite rapidly. Changes after the torque maximum included macroscopic failure of the typical pasta filata structure, loss of stretch and expulsion of some serum fluid. These effects indicated a transition of the cheese into a new state that was completely different from the initial one. These changes could occur after a critical amount of shear work. In an attempt to gain further insight into this transition, G' and G" at 70^{o}C were plotted against shear work for the day 2 runs at 150 rpm (Fig. 9). With progressive shear work input G' increased faster than G'' including a clear transition from viscoelastic liquid to viscoelastic solid at a critical point or crossover point where G'=G" (72.4 kJ kg^{-1}). The maximum in the torque-time curve occurred at 66.3 kJ kg^{-1} for this run. These two shear work values were quite close. The two shear work values were therefore compared for the 4 d of experiments using 150 and 250 rpm screw speeds (Table 1). There is reasonably good agreement between the two values for shear work input for all 4 d. The estimated shear work at the transition point for day 1 at150 rpm screw speed was in the range 54-60 kJ kg^{-1}. This matches very well with the transition behaviour observed in the frequency sweeps at 58.2 kJ kg^{-1} shear work input (Fig. 2c) for this day. Data at 50 rpm were not included in this comparison because the accumulated shear work inputs were a maximum of 14.5 kJ kg^{-1}, well below the shear work needed for the viscoelastic transition. At 250 rpm higher values of shear work were obtained at the structural transition point than at 150 rpm. Shear work input at 250 rpm appears to be less damaging to the structure than at 150 rpm (Sharma et al., 2016).

Increasing shear work input increased crossover temperature and decreased LT_{max} with the size of changes in the order full-fat cheese > non-fat cheese > cheese with added TSC (Fig. 10). For non-fat cheese crossover temperature increased from 50.1 to 55.7^{o}C with increase in shear work from 4.4 to 128.1 kJ kg^{-1}. Similarly, a ~50% decrease (2.2 to 1.1) in LT_{max} was recorded for non-fat cheese upon prolonged working. For non-fat cheese the type of G', G'' crossover shown in Fig. 9 did not occur till 128 kJ kg^{-1} (Data not shown). These pronounced changes in the viscoelastic properties of non-fat cheese suggest that the absence of fat did not prevent changes occurring to the structure on prolonged shear work input. The protein phase is clearly very important to the changes in viscoelastic properties of the full-fat cheese. Full-fat cheese with added TSC was relatively insensitive to increasing shear work input. No definite trend was observed for either LT_{max}, crossover temperature (Fig. 10), G' or G'' in the shear work input range 2-80 kJ kg^{-1}. Interrupting calcium-mediated casein-casein interactions by adding a calcium chelating salt (TSC) also yielded different process characteristics such as the absence of the typical pasta-filata fibrous structure and the occurrence of a more flowable mass even after high shear work inputs (80 kJ kg^{-1}) (Sharma et al., 2016).

3.4 Relationship between melt functionality and oscillatory rheology

Both LT_{max} and crossover temperature correlated quite well with melt score with reasonable goodness of fit (Fig. 11a). LT_{max} correlated positively (R^2=0.87, P<0.01) and crossover temperature correlated negatively (R^2=0.90, P<0.01) with melt score. The low probability values suggest that the models explain the data well. LT_{max} changed about 7 fold over the shear work range whereas crossover temperature was constrained within a limited range (50-60^{o}C), i.e. crossover temperature was less shear work sensitive. However, LT_{max} was much more variable than crossover temperature because it is determined in the temperature range where temperature sweeps had a much higher standard deviation (Fig. 3). Mounsey and O'Riordan (1999) reported a good correlation between melting behaviour and LT_{max} and recommended LT_{max} as a useful indicator for predicting melting behaviour of cheese. G' at 70 ^{o}C was also found to correlate negatively (R^2 = 0.89) with melt score (Fig. 11b).

4. Discussion

G' and G" indicate the strength and extent of bonding in the cheese network (Lucey et al., 2003). Increase in these values upon working suggests creation of stronger bonds (Fig. 1, 2, 9). During working at 70 ^{o}C, hydrated proteins appear to interact strongly to form an increasingly elastic network giving rise to large increases in G' with shear work. The large increases in G' and G'' with increasing shear work are strong evidence of work thickening.

With increasing shear work input there is a transition from viscoelastic liquid behaviour at 70 ^{o}C to viscoelastic solid behaviour. This transition is shown in the dramatic changes in frequency dependence of G' and G" over the experimental frequency range 0.1-10 Hz (Fig. 2). There is a point of critical shear work input for this transition where G' = G''. This critical point is shown in three different sets of data – the overlap of the frequency sweep curves for G' and G'' (Fig. 2c), the overlap of the G' and G" curves from 55 to 75^{o}C in temperature sweeps at 58.2 kJ kg^{-1} (Fig. 5a) and the crossover of G' and G'' curves as a function of shear work (Fig. 9). The shear work values at the peak in Blentech torque-time curves (Sharma et al., 2016) correspond reasonably well to the shear work values at this critical point for structural transition (Table 1).

Shear power intensity or power input per unit volume had a significant impact on the extent of viscoelastic changes with shear work. Working molten curd at 50 rpm didn't change the viscoelastic properties much (Fig. 7, 8). The relatively slow rate of deformation at 50 rpm must have allowed enough time for relaxation of the acting stresses giving minimal changes in structure. On the other hand, at the higher screw speeds major changes in rheology and structure of the model cheese were observed. It is suggested that to attain structural and rheological transition, a threshold screw speed or shear power intensity is required in addition to critical shear work levels. Higher values of critical shear work were obtained at 250 rpm than at 150 rpm (Table 1). The most likely explanation for this behaviour is the viscoelastic nature of the material (Sharma et al., 2016). Mulvaney et al. (1997) note that screw speed is proportional to shear rate and therefore screw speed effects can be compared with frequency effects in oscillatory linear viscoelastic measurements. G' increases faster than G'' with frequency below the critical transition (Fig. 2a, b) indicating the dominance of the elastic nature at higher frequencies or screw speeds. Therefore, at 250 rpm a higher proportion of elastic or recoverable energy would be expected and this recoverable energy does not cause changes in cheese structure.

G’ and G” at 70 ^{o}C changed significantly with shear work in the low shear work regime (2-10 kJ kg^{-1}) (Fig. 9). However, other rheological properties such as K, n, apparent viscosity at 0.01 s^{-1}, melt score (Sharma et al., 2016) and G’-G’’ crossover temperature (Fig. 8) don’t change very much in this shear work range. This suggests that G’ and G” could be useful parameters to monitor minor structural changes in the cheese. LT_{max} has been reported by various researchers as a useful parameter to determine melt and flow characteristics, but we found LT_{max} to be quite variable particularly at high melt score (Fig. 11). This variability may be arising from the inherent nature of cheese at higher temperatures. Crossover temperature correlated better with melt properties than LT_{max} (Fig. 11).

The results indicate that protein-protein interactions strengthen as the cheese is sheared at 70 ^{o}C. The role of calcium in these interactions is shown as cheese with added TSC, a strong calcium chelating agent, did not strengthen. Several studies have shown the role of calcium in the formation of protein fibres in Mozzarella type cheeses and their role in functionality (Mizuno & Lucey, 2005; McMahon, Paulson & Oberg 2005; Guinee et al., 2002; Joshi, Muthukumararappan & Dave, 2003a, b, 2004). Protein-protein interactions can be strongly enhanced by calcium, either through neutralizing charge repulsion between caseins or by bridging or cross linking between proteins due to its divalent nature (Pastorino, Ricks, Hansen & McMahon, 2003). These strengthened protein-protein interactions would lead to a more rigid cheese structure with increased hardness, decreased melt, and syneresis as observed by Sharma et al. (2016) and McMahon et al. (2005). Manski, van der Zalm, van der Goot and Boom (2008) also reported strengthening (higher values of G’) of a fibrous protein-fat matrix upon shear in the presence of transglutaminase as a cross linking agent. Absence of fat did not prevent formation of the typical fibrous protein structure and did not result in any substantial decrease in viscoelastic properties (Fig. 10).

A schematic model is proposed in Fig. 12 to describe structural changes in the model cheese as it is progressively sheared. Three structures are proposed to depict the structural and viscoelastic changes taking place – at low shear work, i.e. viscoelastic liquid, at the critical transition and then at high shear work, i.e. viscoelastic solid. At a moderate level of shear work (e.g. ~26 kJ kg^{-1}, Fig. 2b), the frequency behaviour indicates that the shear induced protein structures appear to interact with each other through physical entanglements (Fig. 12a). These entanglements are not permanent bonds. There may also be some disentanglement of protein strands if the timescale of deformation is faster than the relaxation of molecular interactions (Sharma et al., 2015). Shear work imparted at this stage slightly increases the probability of encounter with other protein strands. Therefore, only weak physical interactions are expected that lead to slow structure development. Behaviour typical of entangled polymers is exhibited (Fig. 2b) with a viscoelastic liquid nature at low frequencies and viscoelastic solid at higher frequencies and the presence of a G'-G'' crossover point.

At higher shear work levels (>50 kJ kg^{-1}) near the transition point stronger bonds with a relatively longer lifetime are proposed. These bonds may be a combination of weak physical entangled polymer interactions and stronger interactions such as covalent, ionic or hydrophobic interactions. These stronger bonds in the structure near the transition point are depicted by parallel chains of protein polymer (Fig. 12b). Excessive shear work levels (>70 kJ kg^{-1}) eventually transform the material into an entirely different structure. The material becomes more elastic (LT <1; G'>G''), stronger (G' = 3.9 kPa), less frequency dependent (n = 0.26) and exhibits no G'-G'' crossover in a temperature sweep, all typical rheological behaviour of strong gels or networks. Macroscopically the structure looks non-cohesive, crumbly and brittle and exhibits almost no stretch. The structure we propose has highly aggregated, dense protein structures but these structures do not bind tightly to one another

(Fig. 12c). In summary, shear work appears to alter the interaction behaviour between two or more adjacent polymeric chains made of protein strands either with entanglements or cross-links or both. The changes in fat morphology and particle size depicted in Fig. 12 will be reported in a later paper.

5. Conclusions

Viscoelastic properties of model Mozzarella cheese were greatly affected by shear work input. A transition from viscoelastic liquid to viscoelastic solid was observed with increasing shear work. A critical point for structural transition of cheese melt was clearly evident at a shear work level that depended on screw speed. Frequency sweeps indicated a decrease in frequency dependence of G' with increasing shear work. A schematic model is proposed where cheese is transformed from an entangled polymer network structure to a strongly cross-linked network state after high shear work input. It is proposed that the structural and rheological changes occurring during the working of model Mozzarella cheese are caused by stronger protein-protein interactions that are enhanced by calcium bridging. The rheology of non-fat cheese also changed very significantly with shear work input suggesting that absence of fat did not halt the changes in viscoelastic properties. It is therefore concluded that the major changes in the viscoelastic properties of model Mozzarella cheese were governed by changes in the protein matrix.

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References

Ak, M. M., & Gunasekaran, S. (1996). Dynamic rheological properties of mozzarella cheese during refrigerated storage. *Journal of Food Science, 61*, 566-568.

Bast, R., Sharma, P., Easton, H.K.B., Dessev, T.T., Lad, M., & Munro, P.A. (2015). Tensile testing to quantitate the anisotropy and strain hardening of Mozzarella cheese. *International Dairy Journal, 44*, 6-14.

Brickley, C. A., Govindasamy-Lucey, S., Jaeggi, J.J., Johnson, M.E., McSweeney, P.L.H., & Lucey, J.A. (2008). Influence of emulsifying salts on the textural properties of non-fat process cheese made from direct acid cheese bases. *Journal of Dairy Science, 91*, 39-48.

Fatimi, A., Tassin, J. F., Quillard, S., Axelos, M. A. V., & Weiss, P. (2008). The rheological properties of silated hydroxypropylmethylcellulose tissue engineering matrices. *Biomaterials, 29*, 533-543.

Guinee, T. P., Auty, M. A. E., & Mullins, C. (1999). Observations on the microstructure and heat-induced changes in the viscoelasticity of commercial cheeses. *Australian Journal of Dairy Technology, 54*, 84-89.

Guinee, T. P., Feeney, E. P., Auty, M. A. E., & Fox, P. F. (2002). Effect of pH and calcium concentration on some textural and functional properties of Mozzarella cheese. *Journal of Dairy Science, 85*, 1655-1669.

Gunasekaran, S., & Ak, M. M. (2003). *Cheese rheology and texture.* Boca Raton, FL, USA : CRC Press LLC.

Hsieh, Y.L., Yun, J.J. & Rao, M.A. (1993). Rheological properties of mozzarella cheese filled with dairy, egg, soy proteins, and gelatin. *Journal of Food Science, 58*, 1001-1004.

Hussain, I., Grandison, A.S., & Bell, A.E. (2012). Effects of gelation temperature on mozzarella-type curd made from buffalo and cows' milk. 1: Rheology and microstructure. *Food Chemistry, 134,* 1500-1508.

Joshi, N. S., Muthukumarappan, K., & Dave, R. I. (2002). Role of soluble and colloidal calcium contents on functionality of salted and unsalted part-skim mozzarella cheese. *Australian Journal of Dairy Technology, 57,* 203-210.

Joshi, N. S., Muthukumarappan, K., & Dave, R. I. (2003a). Effect of calcium on physicochemical properties of fat-free mozzarella cheese. *Journal of Food Science, 68*, 2289-2294.

Joshi, N. S., Muthukumarappan, K., & Dave, R. I. (2003b). Understanding the role of calcium in functionality of part skim Mozzarella cheese. *Journal of Dairy Science, 86*, 1918-1926.

Joshi, N. S., Muthukumarappan, K., & Dave, R. I. (2004). Viscoelastic properties of part skim mozzarella cheese: Effect of calcium, storage, and test temperature. *International Journal of Food Properties, 7*, 239-252.

Karoui, R., Laguet, A. & Dufour, E. (2003). Fluorescence spectroscopy: A tool for the investigation of cheese melting - Correlation with rheological characteristics. *Lait, 83,* 251-264.

Lucey, J. A. (2002). Formation and physical properties of milk protein gels. *Journal of Dairy Science, 85*, 281-294.

Lucey, J. A., & Fox, P. F. (1993). Importance of calcium and phosphate in cheese manufacture: A review. *Journal of Dairy Science, 76,* 1714–1724.

Lucey, J. A., Johnson, M. E., & Horne, D. S. (2003). Invited review: Perspectives on the basis of the rheology and texture properties of cheese. *Journal of Dairy Science, 86,* 2725–2743.

Ma, X., Balaban, M. O., Zhang, L., Emanuelsson-Patterson, E. A. C. & James, B. (2014). Quantification of pizza baking properties of different cheeses, and their correlation with cheese functionality. *Journal of Food Science*, 79, E1528-E1534.

Manski, J.M., van der Zalm, E.E.J., van der Goot, A.J., & Boom, R.M. (2008). Influence of process parameters on formation of fibrous materials from dense calcium caseinate dispersions and fat. *Food Hydrocolloids, 22,* 587-600.

McMahon, D. J., Fife, R.L., & Oberg, C. J. (1999). Water partitioning in Mozzarella cheese and its relationship to cheese meltability. *Journal of Dairy Science*, *82*, 1361-1369.

McMahon, D. J., Paulson, B., & Oberg, C. J. (2005). Influence of calcium, pH, and moisture on protein matrix structure and functionality in direct-acidified non-fat Mozzarella cheese. *Journal of Dairy Science, 88*, 3754-3763.

Mizuno, R., & Lucey, J. A. (2005). Effects of emulsifying salts on the turbidity and calcium-phosphate-protein interactions in casein micelles. *Journal of Dairy Science, 88,* 3070–3078.

Mounsey, J. S., & O'Riordan, E. D. (1999). Empirical and dynamic rheological data correlation to characterize melt characteristics of imitation cheese. *Journal of Food Science, 64*, 701-703.

Mulvaney, S., Rong, S., Barbano, D. M., & Yun, J. J. (1997). Systems analysis of the plasticization and extrusion processing of Mozzarella cheese. *Journal of Dairy Science, 80,* 3030-3039.

Muthukumarappan, K., Wang, Y.-C., & Gunasekaran, S. (1999). Modified Schreiber test for evaluation of Mozzarella cheese meltability. *Journal of Dairy Science, 82,* 1068–1071.

Pastorino, A. J., Ricks, N. P., Hansen, C. L., & McMahon, D. J. (2003). Effect of calcium and water injection on structure-function relationships of cheese. *Journal of Dairy Science, 86,* 105-113.

Rock, S., Stenz, B., Hahn, A., Sedlmeyer, F., Fischer, U. & Kulozik U. (2005). Rheological characterisation of the meltability of cheese products: influence of gap width, the measuring mode and geometry. *Milchwissenschaft, 60,* 154–158.

Rudan, M.A., & Barbano, D.M. (1998). A model of Mozzarella cheese melting and browning during pizza baking. *Journal of Dairy Science, 81,* 2312-2319.

Schenkel, P., Samudrala, R., & Hinrichs, J. (2013). Thermo-physical properties of semi-hard cheese made with different fat fractions: Influence of melting point and fat globule size. *International Dairy Journal, 30*, 79-87.

Sharma, P., Dessev, T.T., Munro, P.A., Wiles, P.G., Gillies, G., Golding, M., James, B. & Janssen, P. (2015). Measurement techniques for steady shear viscosity of Mozzarella-type cheeses at high shear rates and high temperature. *International Dairy Journal, 47*, 102-108.

Sharma, P., Munro, P.A., Dessev, T.T., Wiles, P.G., & Buwalda, R.J. (2016). Effect of shear work input on steady shear rheology and melt functionality of model Mozzarella cheeses. *Food Hydrocolloids*, *54,* 266-277.

Stading, M., & Hermansson, A. M. (1990). Viscoelastic behaviour of beta-lactoglobulin gel structures. *Food Hydrocolloids, 4,* 121-135.

Steffe, J. F. (1996). *Rheological methods in food process engineering* (2nd ed.). East Lansing, MI, USA: Freeman Press.

Subramanian, R. & Gunasekaran, S. (1997). Small amplitude oscillatory shear studies on mozzarella cheese. Part I. Region of linear viscoelasticity. *Journal of Texture Studies, 28,* 633-642.

Subramanian, R., Muthukumarappan, K., & Gunasekaran, S. (2003). Effect of methocel as a water binder on the linear viscoelastic properties of mozzarella cheese during early stages of maturation. *Journal of Texture Studies, 34,* 361-380.

Tunick, M. H. (2011). Small-strain dynamic rheology of food protein networks. *Journal of Agricultural and Food Chemistry, 59,* 1481-1486.

Tunick, M. H., Mackey, K. L., Shieh, J. J., Smith, P. W., Cooke, P. & Malin, E. L. (1993). Rheology and microstructure of low-fat mozzarella cheese. *International Dairy Journal, 3*, 649–662.

Udyarajan, C. T., Horne, D. S., & Lucey, J. A. (2007). Use of time-temperature superposition to study the rheological properties of cheese during heating and cooling. *International Journal of Food Science and Technology, 42*, 686-698.

Venugopal, V. & Muthukumarappan, K. (2003). Rheological properties of cheddar cheese during heating and cooling. *International Journal of Food Properties, 6,* 99-114.

Winter, H. H., & Chambon, F. (1986). Analysis of linear viscoelasticity of a cross-linking polymer at the gel point. *Journal of Rheology, 30,* 367-382.

Yu, C., & Gunasekaran, S. (2005). A systems analysis of pasta filata process during Mozzarella cheese making. *Journal of Food Engineering*, *69*, 399-408.

Figure Captions

Fig. 1. Strain sweeps of model Mozzarella cheeses subjected to different amounts of shear work; 4.9 (♦), 62 (■),185 (×) kJ kg^{-1}. Experiments were conducted at a frequency of 1 Hz and 70 °C. Model Mozzarella cheeses were manufactured in the Blentech cooker at 250 rpm screw speed (Day 2) and 70 °C.

Fig. 2. Frequency sweeps at 70°C on model Mozzarella cheeses subjected to varied amounts of shear work; a. 8.8, b. 26.3, c. 58.2, d. 73.7 kJ kg^{-1}. Model Mozzarella cheeses were manufactured in the Blentech cooker at 150 rpm screw speed (Day 1) and 70 °C. G' (♦), G"(■) and Loss tangent (▲).

Fig. 3. Temperature sweeps on model Mozzarella cheeses subjected to 5.0 kJ kg^{-1} of shear work. Model Mozzarella cheeses were manufactured in the Blentech cooker at 50 rpm screw speed (Day 1) for 780 s and 70°C. Error bars represent one standard deviation (3 samples). G' (♦), G"(■) and Loss tangent (▲).

Fig.4. Temperature sweeps of model Mozzarella cheeses subjected to varied amounts of shear work ; 3.3 (♦), 4.3 (■), 8.8 (▲), 26.3 (×), 58.2 (✱) and 73.7 (●) kJ kg^{-1} corresponding to shearing times 375, 395, 635, 1515, 3035 and 3950 s. a. G'; b. G"; c. Loss Tangent. Model Mozzarella cheeses were manufactured in the Blentech cooker at 150 rpm screw speed (Day 1) and 70°C.

Fig. 5. Temperature sweeps on model Mozzarella cheeses subjected to a. 58.2 b.73.7 kJ kg^{-1} shear work. Model Mozzarella cheeses were manufactured in the Blentech cooker at 150 rpm screw speed (Day 1) and 70°C. G' (♦) and G"(■).

Fig. 6. G'-G" crossover temperature (a,b) and LT_{max} (c,d) of model mozzarella cheeses versus time (a,c) and shear work (b,d). Model Mozzarella cheeses were manufactured in the Blentech cooker at 150 rpm screw speed and 70°C. Day 1 (♦), Day2 (■).

Fig. 7. Effect of shear work on LT_{max} of model Mozzarella cheeses. Model Mozzarella cheeses were manufactured in the Blentech cooker at 50, 150 and 250 rpm screw speeds and 70°C. Each trial was repeated twice (day 1 and day 2) on a different day at least one month interval. 50 rpm, day 1 (■) and day 2 (♦), 150 rpm, day 1 (▲) and day 2 (Δ), 250 rpm, day 1 (o) and day 2 (●).

Fig. 8. Effect of shear work on G'-G" crossover temperature of model Mozzarella cheeses. Model Mozzarella cheeses were manufactured in the Blentech cooker at 50, 150 and 250 rpm screw speeds and 70°C. Each trial was repeated on a different day at least one month after the first trial. 50 rpm, day 1 (■) and day 2 (♦), 150 rpm, day 1 (▲) and day 2 (Δ), 250 rpm, day 1 (o) and day 2 (●).

Fig. 9. Effect of shear work on G' and G" at 70°C for model Mozzarella cheeses. Model Mozzarella cheeses were manufactured in the Blentech cooker at 150 rpm screw speed (Day 2) and 70°C. Dashed arrow indicates shear work at the transition state. G' (♦) and G"(■).

Fig. 10. Effect of shear work on (a) G'-G" crossover temperature and (b) LT_{max} of model Mozzarella cheeses - full fat, nonfat and with TSC added. Model Mozzarella cheeses were manufactured in the Blentech cooker at 150 rpm screw speed and 70°C. Full fat (♦), nonfat (■) and TSC added (▲) cheeses. Data from both trial days are included.

Fig. 11. Correlation of LT_{max} and G'-G" crossover temperature (a) and G' at 70 °C (b) with melt score for the model Mozzarella cheeses. Model Mozzarella cheeses were manufactured in the Blentech cooker at 50, 150 and 250 rpm screw speeds and 70 °C. Data from both trial days are included.

Fig. 12. Schematic model proposed for shear induced structural changes during working of model Mozzarella cheese at 70 °C in a twin screw Blentech cooker; a. entangled polymer type structure; b. structure at critical point c. structure after excessive working. Yellow indicates fat particles. (Not to scale.)

Table

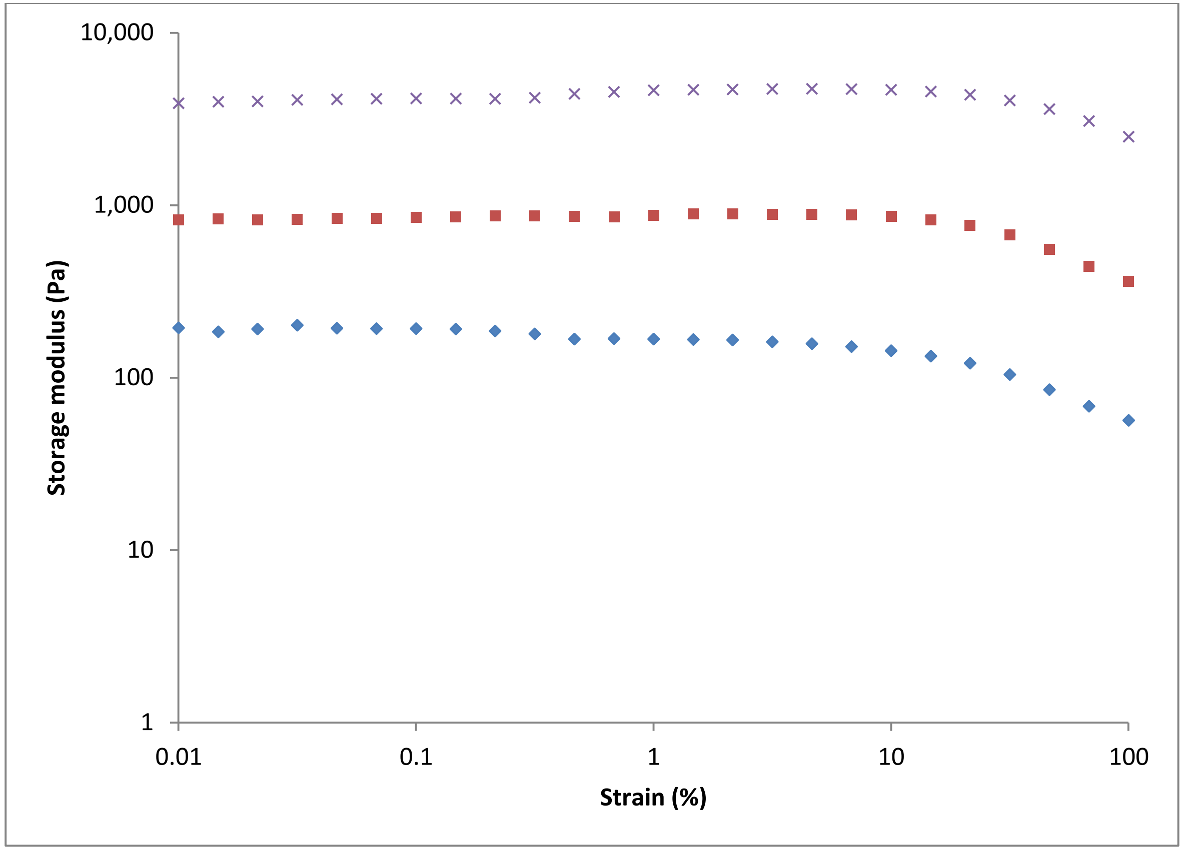
Table 1

Shear work input at crossover of G’ and G” and at peak torque.

RPM	Day	Crossover G’-G”* kJ kg^{-1}	Peak Torque* kJ kg^{-1}
150	1	59.2	54.6
150	2	72.4	66.3
250	1	124.3	126.0
250	2	114.7	108.6

* No significant difference (P>0.05) between shear work values in each row.

Figure



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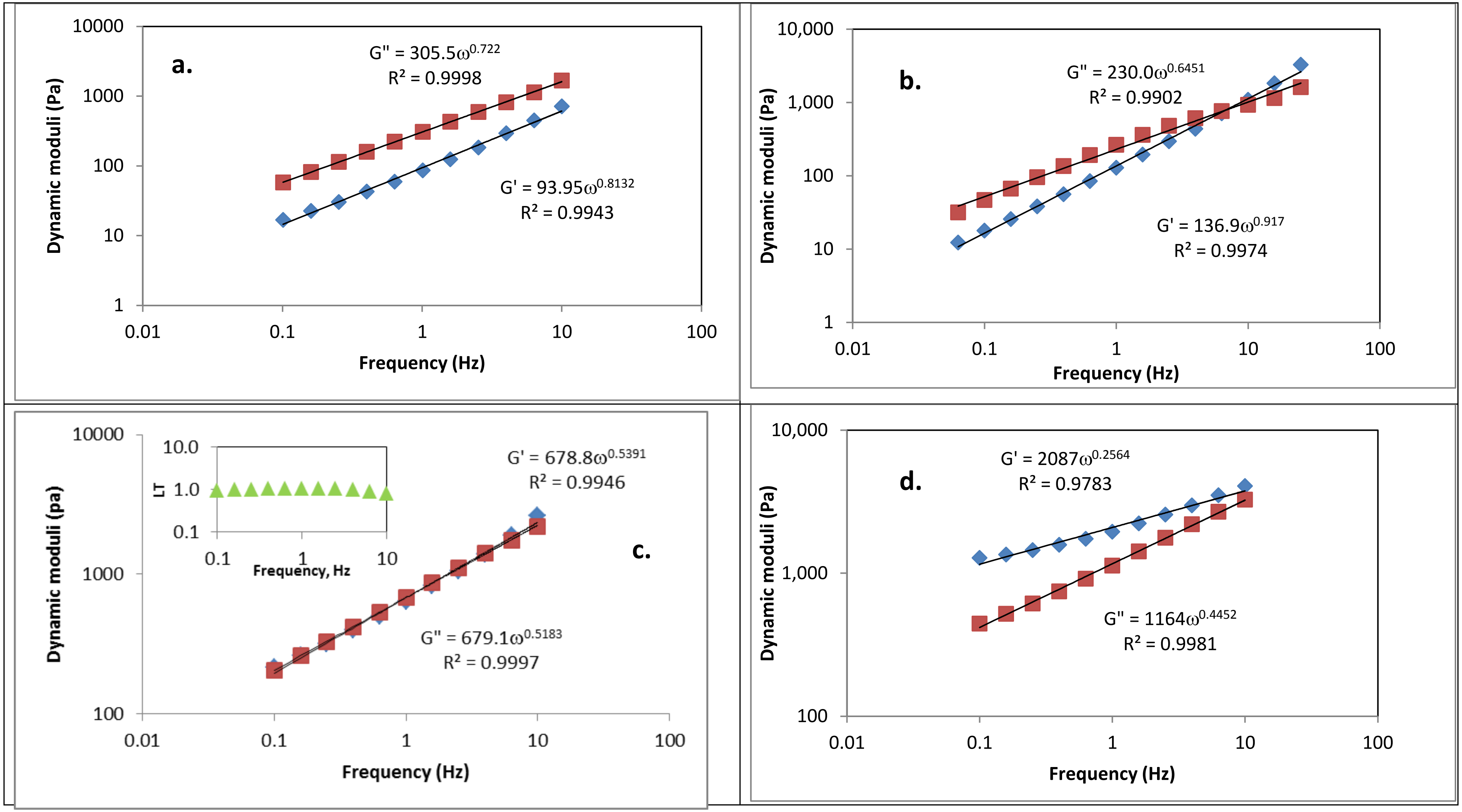

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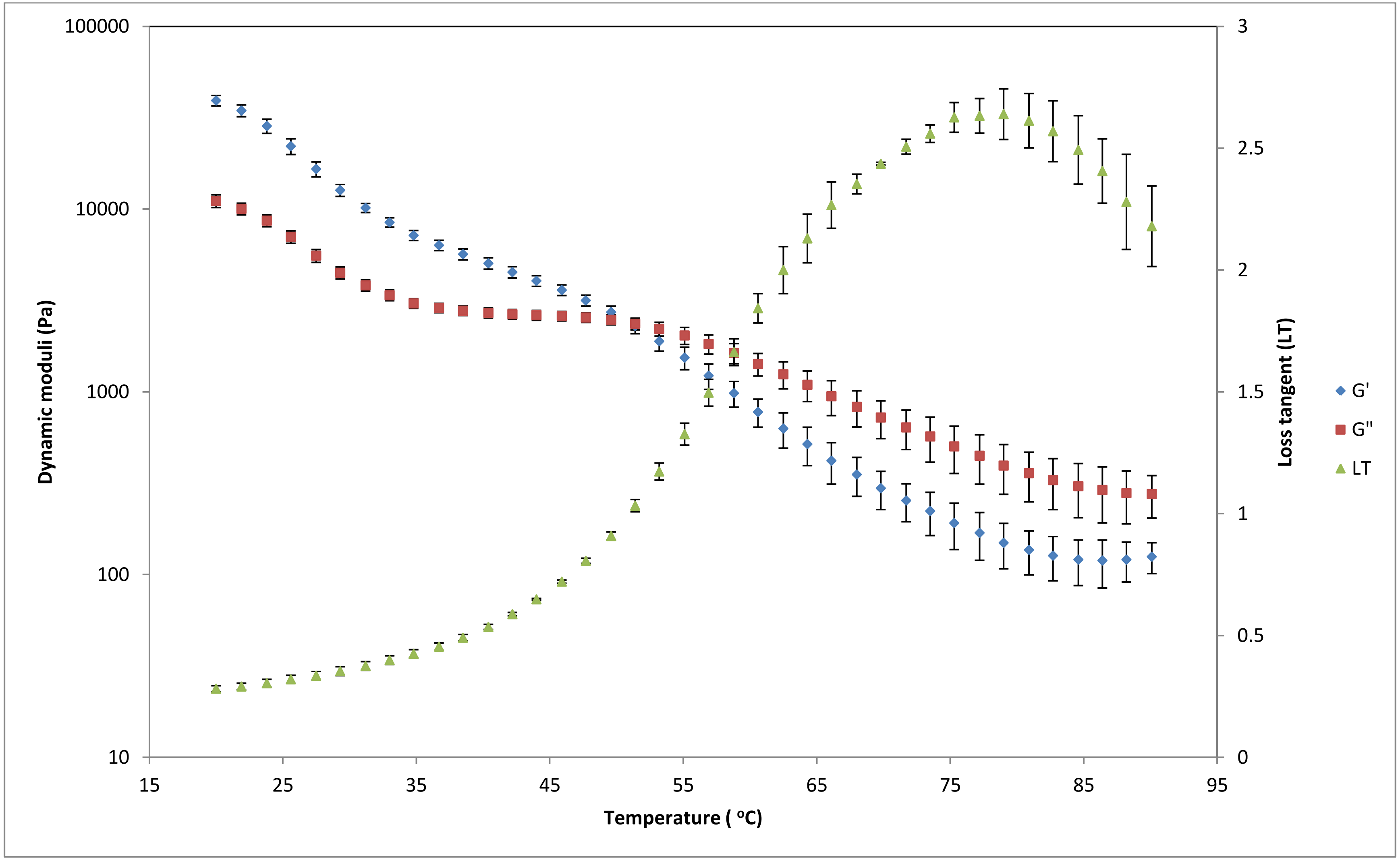

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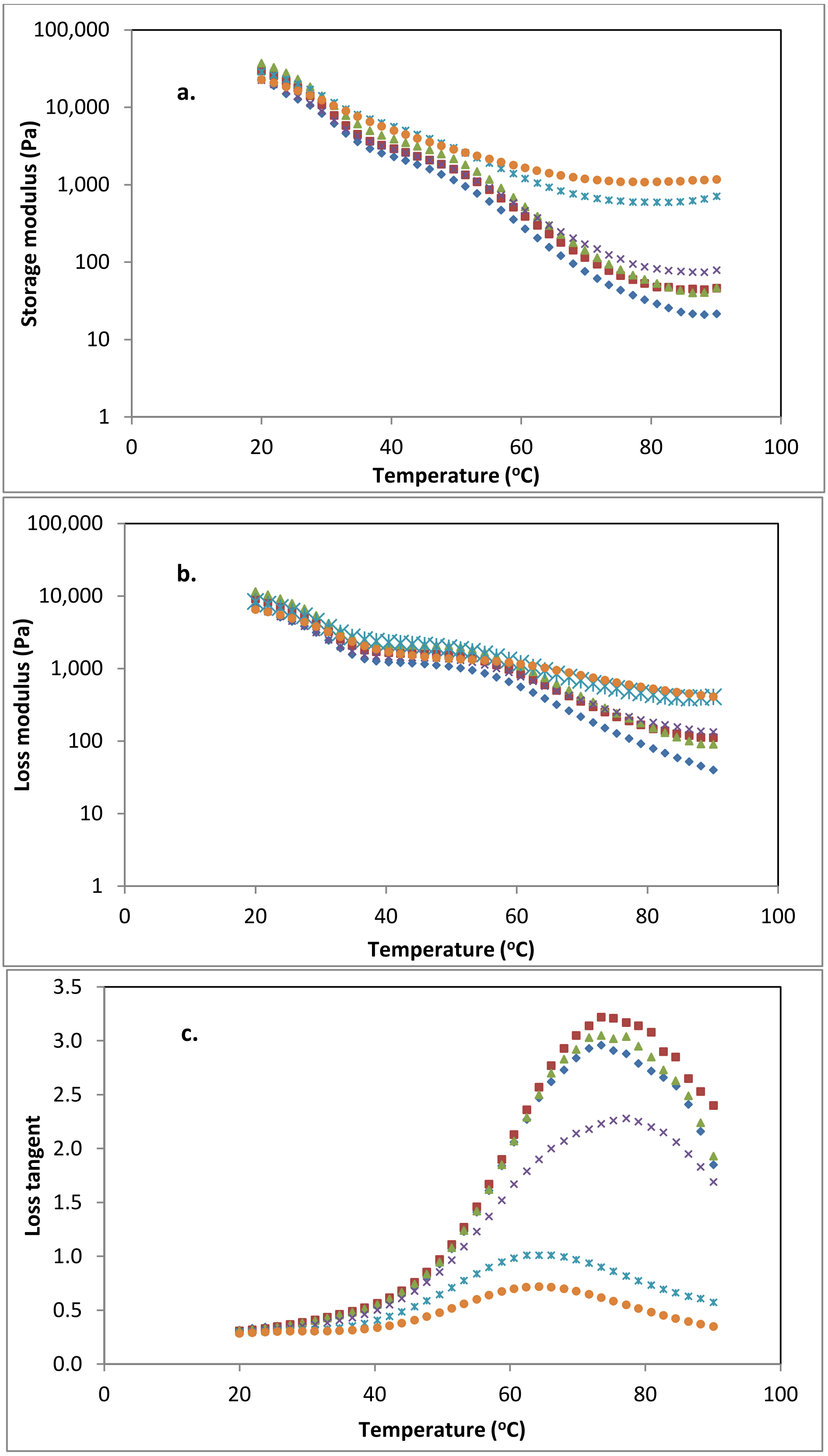

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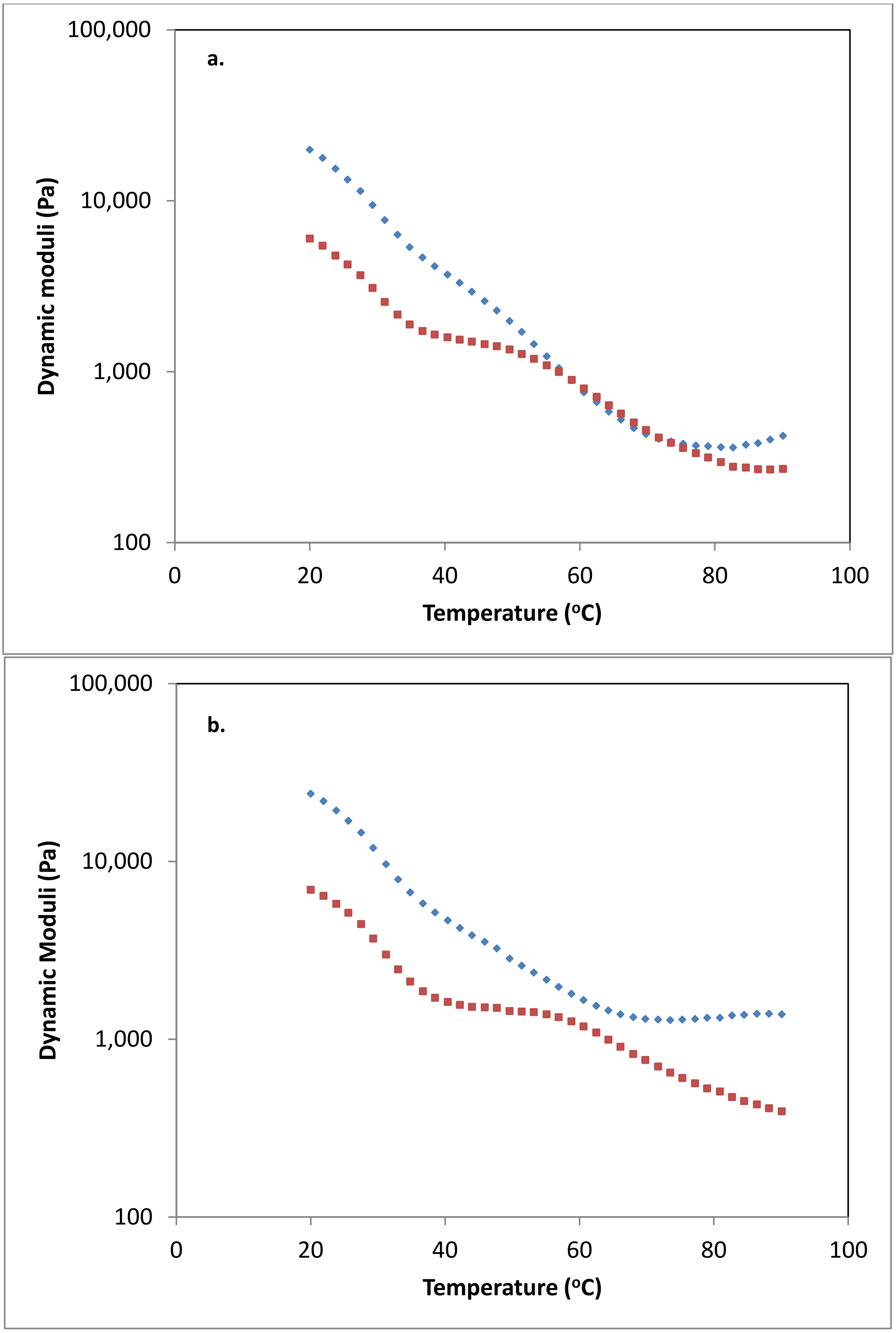

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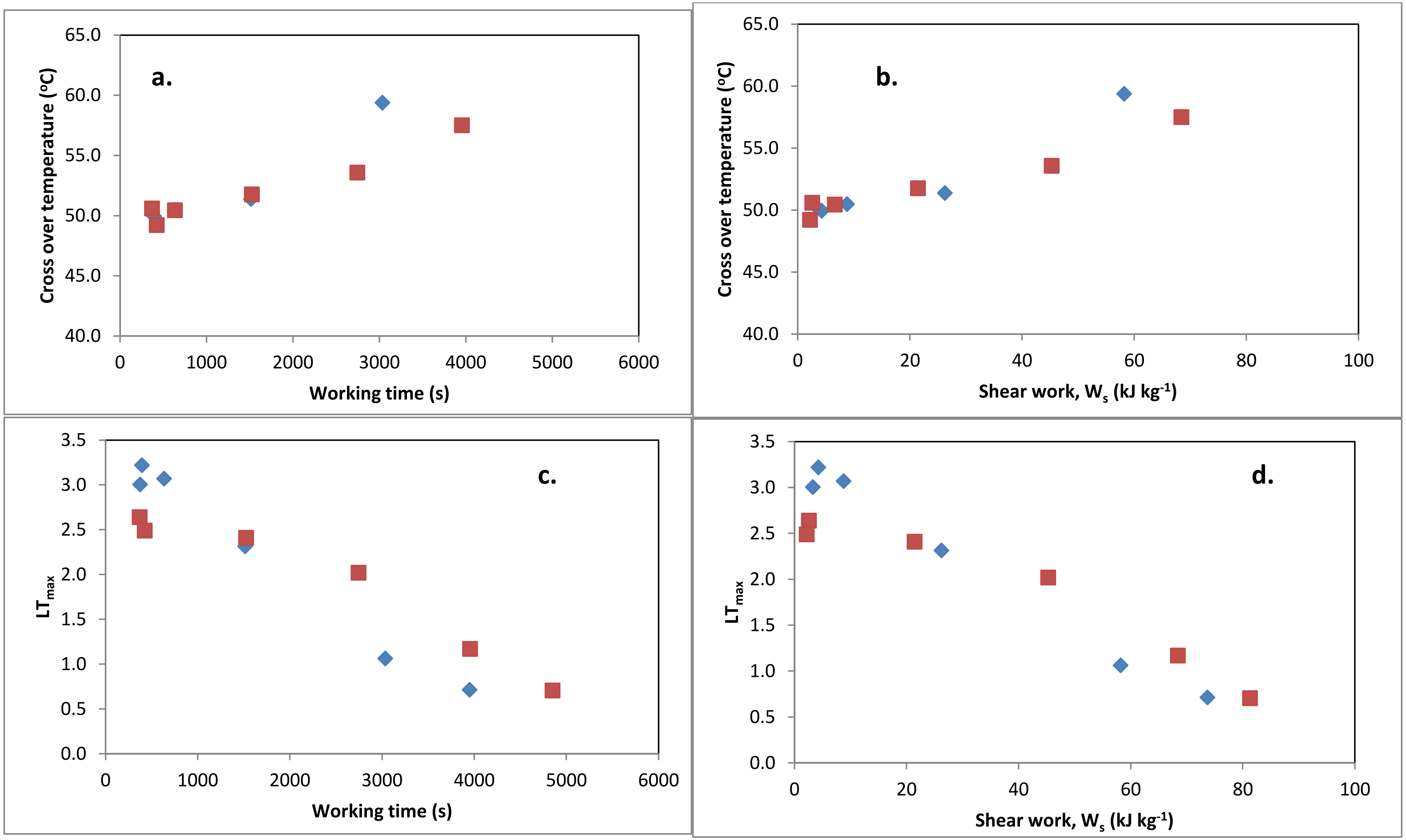

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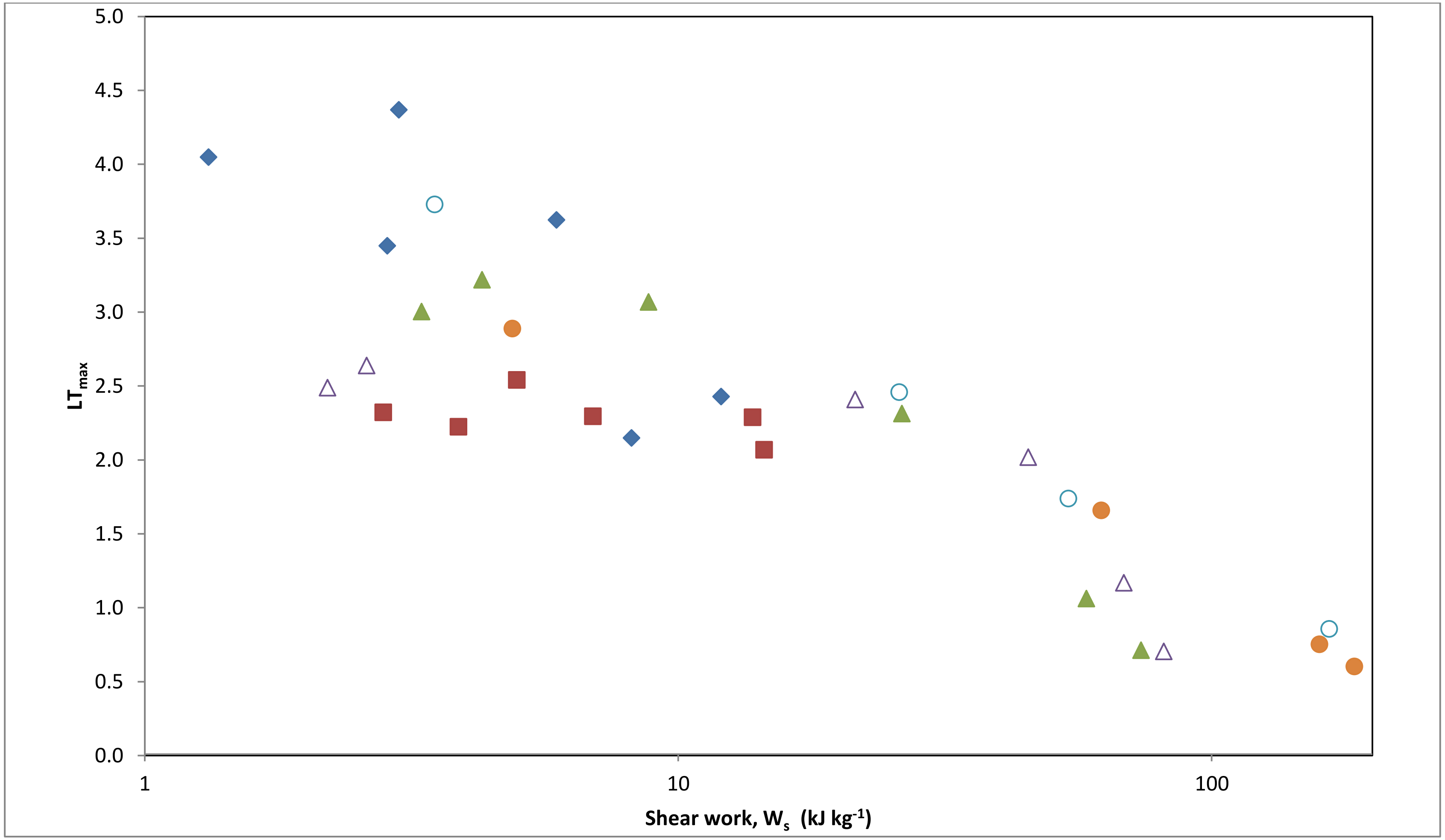

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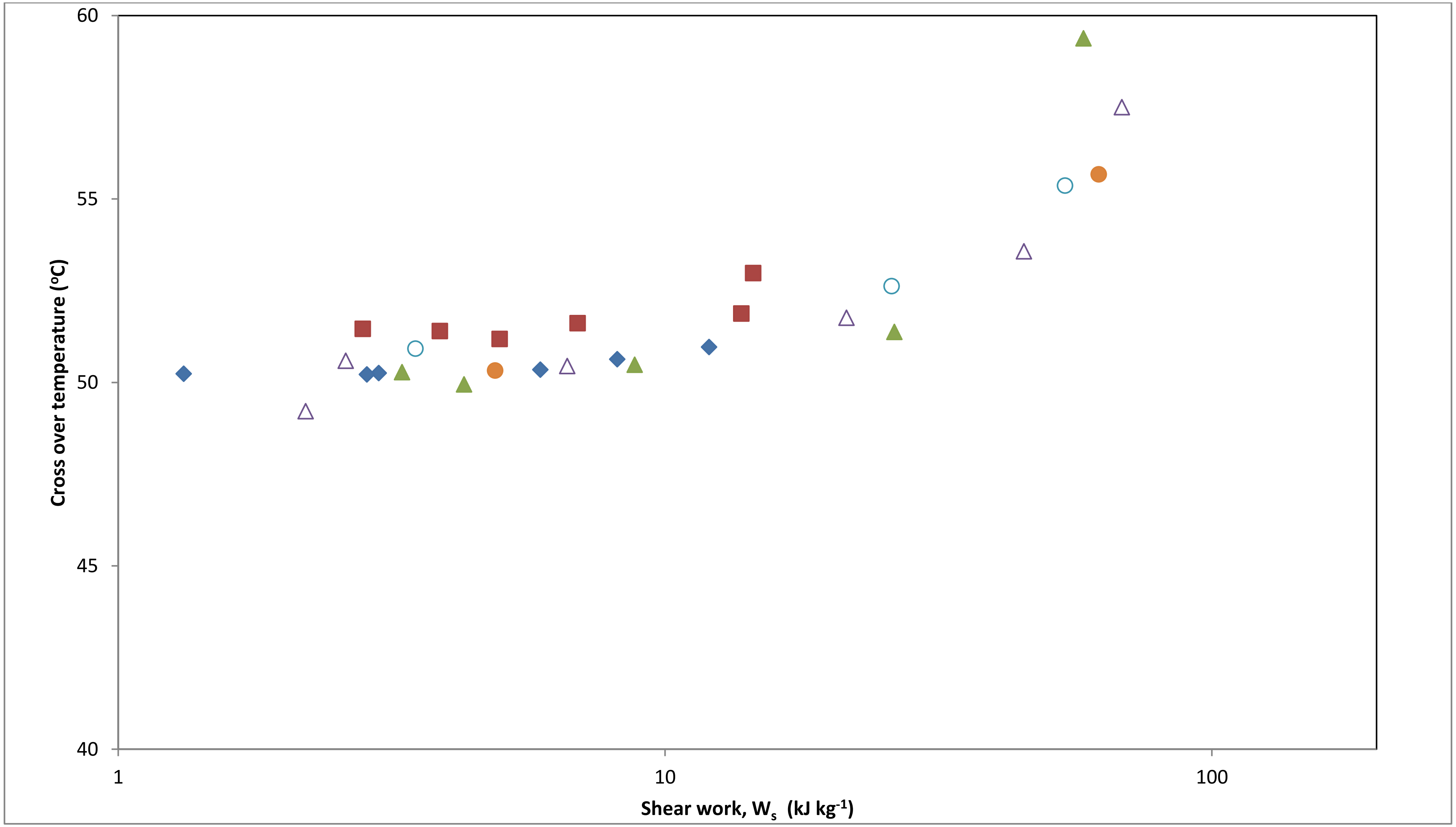

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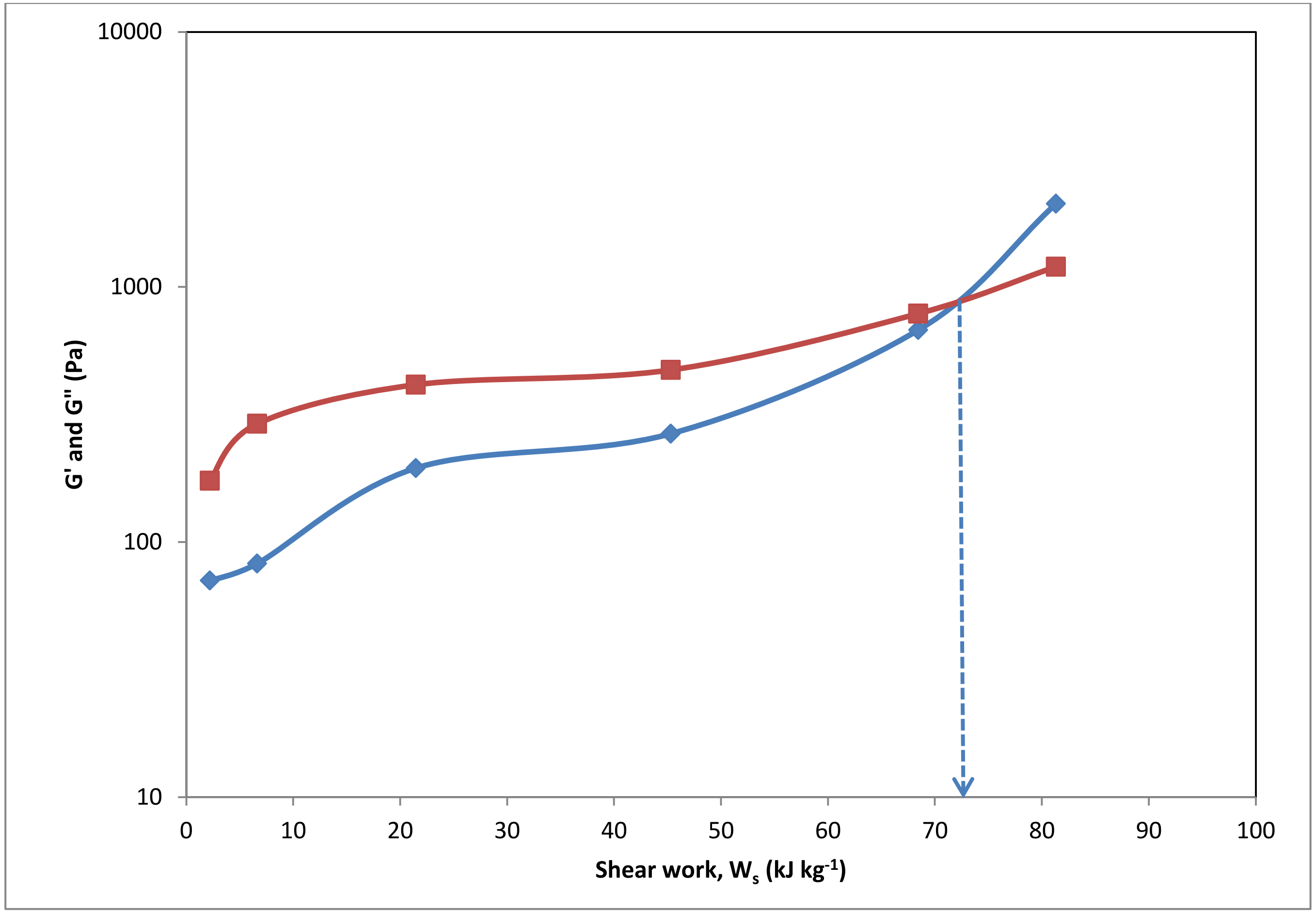

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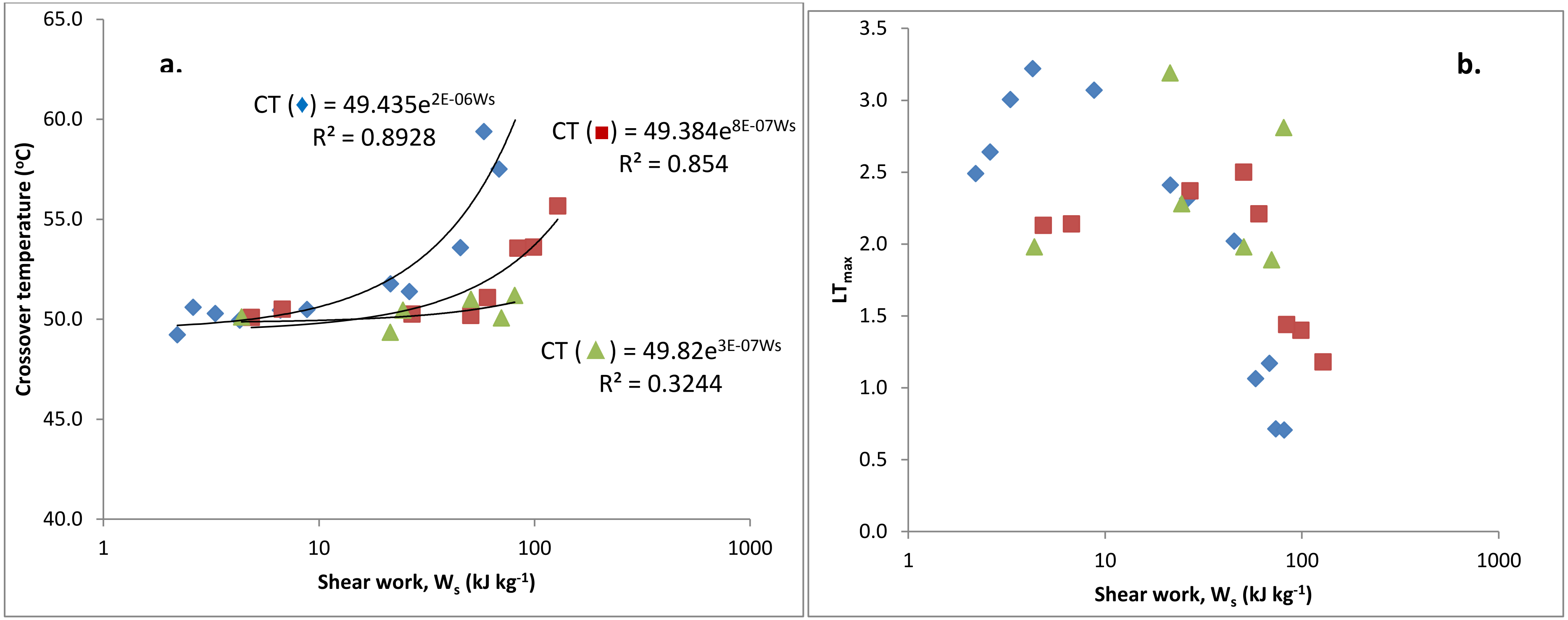

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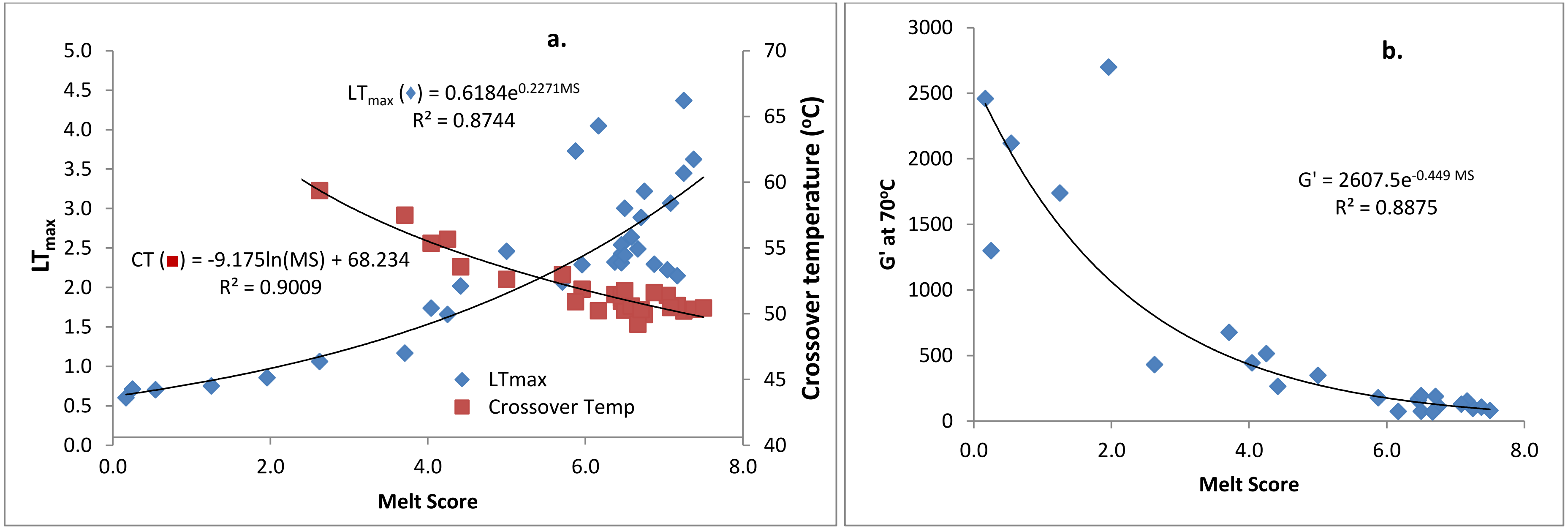

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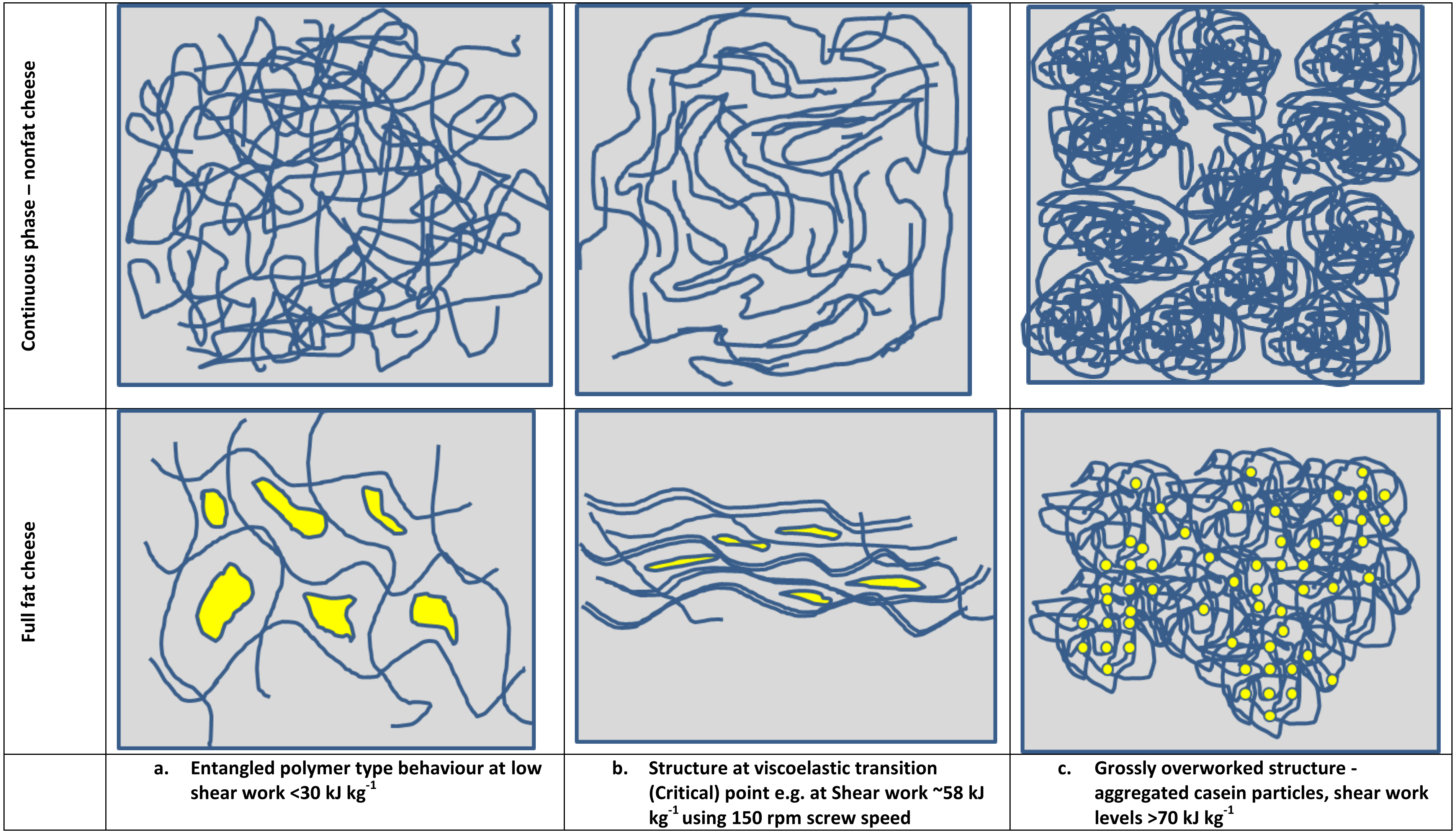

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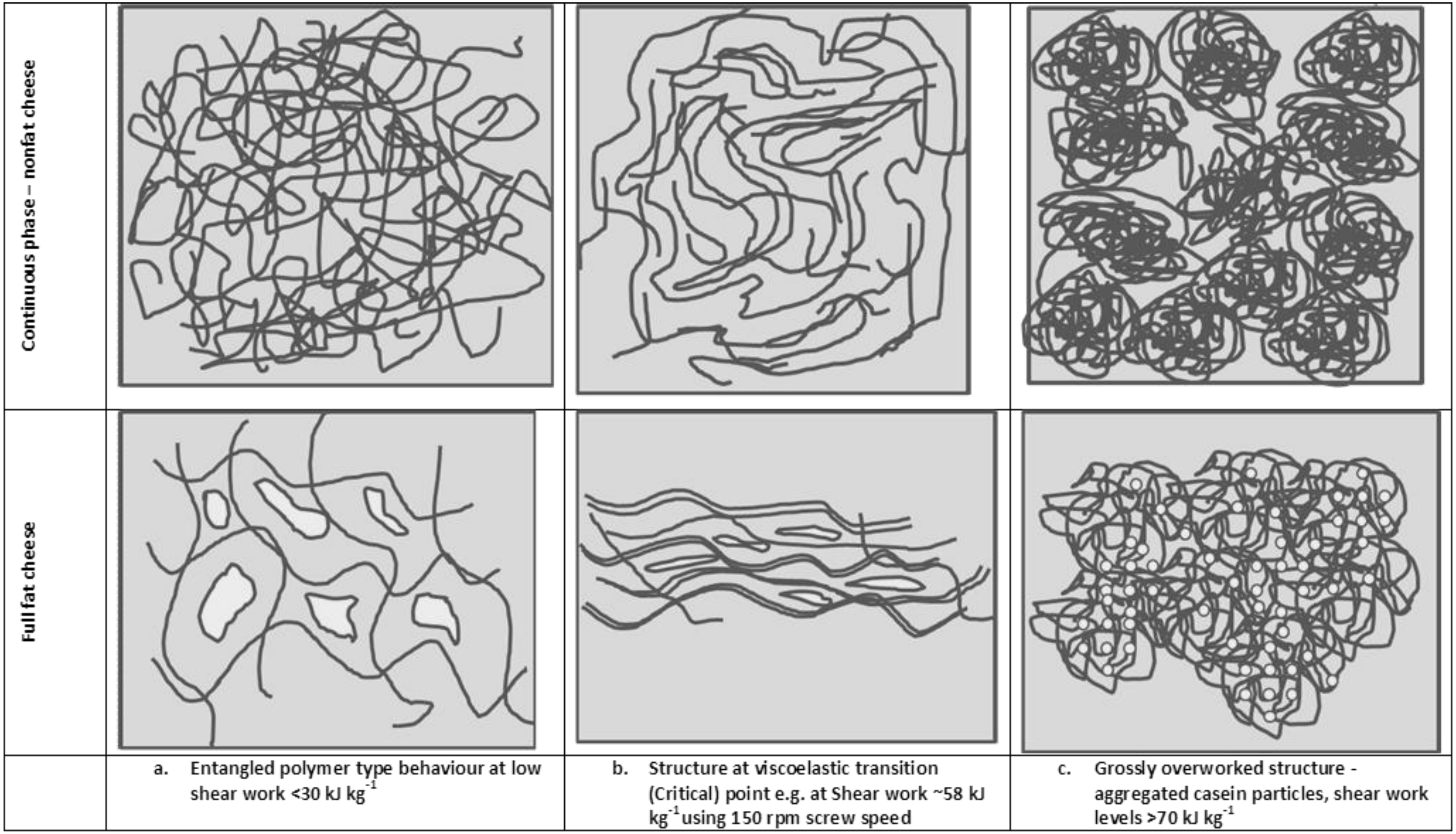
Black and white version of Fig. 12.



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