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Shear work induced changes in the rheology of model Mozzarella cheeses

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With the blessings of

Grandparents

Late Smt. Dharmo Devi and Late Sh. Mool Chand Sharma, &

Parents

Smt. Leelwati Sharma and Sh. Suresh Chandra Sharma

I dedicate this thesis to

My beloved wife **Bharti Sharma,**

Son Master **Pratham Sharma, &**

the departed souls of my dear ones.

- Prateek Sharma

Abstract

Mozzarella cheese is a pasta filata type of cheese. Its manufacture includes a kneading – stretching step that creates a fibrous protein network and distributes fat-serum channels to attain desirable melt functionality on a pizza. During processing and manufacturing of pasta-filata cheese, large deformations take place. For appropriate characterization of a food material, rheological evaluation should be conducted in similar operating conditions, length scales and time scales to those taking place in the actual process. Development of the typical fibrous pasta-filata structure of mozzarella cheese depends on composition and process variables. Critical process variables in the development of cheese structure are time, temperature and shear. In this study we studied the effect of shear work on rheology, structure and melt functionality of model Mozzarella cheese.

Three types of model cheeses (full-fat, non-fat and full-fat with added tri-sodium citrate) were prepared by working cheese components together at 70 °C in a twin screw Blentech cooker. Varied amounts of shear work input (2.8-185 kJ/kg) were given to the cheese samples using 50, 150 and 250 rpm screw speeds. Samples were subjected to a range of rheological tests, confocal laser scanning microscopy, fat particle size measurements (DLS) and melt functionality evaluation.

While measuring steady shear viscosity of Mozzarella-type cheeses in a rotational rheometer at 70°C, three main difficulties were encountered; wall slip, structural failure during measurement and viscoelastic time dependent effects. A flow curve method was successfully devised to measure steady shear rheology by using serrated plates as surface modification to avoid wall slip, giving enough measurement duration at low shear rate to avoid viscoelastic effects and selecting limited shear steps to cause minimum structural changes. These techniques enabled successful measurement of steady shear viscosity of molten Mozzarella-type cheeses at 70°C at shear rates up to 250 s⁻¹.

Strong work thickening was observed for full fat Mozzarella cheese from steady shear rheology, oscillatory rheology, creep, elongational viscosity and tensile testing data. Steady shear rheology and melt functionality were found to be strongly dependent on total shear work input. An exponential increase in consistency coefficient (K from power law model) was observed with increasing amounts of accumulated shear work, indicating work thickening behaviour. An exponential work thickening equation is

proposed to describe this behaviour. Excessively worked cheese samples exhibited liquid exudation, poor melting and poor stretch. Nonfat cheese exhibited similar but smaller changes after excessive shear work input.

At lower shear work inputs (<30 kJ/kg), cheese behaved like a viscoelastic liquid exhibiting typical entangled polymer melt behaviour with moderate frequency dependence and at excessive shear work levels (>70 kJ/kg) it behaved like a viscoelastic solid with low frequency dependence. A definite critical point for structural and viscoelastic transition was identified at a medium shear work level (~ 58 kJ/kg at 150 rpm). Similar viscoelastic property changes occurred in non-fat cheese suggesting that major changes were taking place in the protein matrix during working.

Confocal microstructures plus macroscopic observations showed systematic changes in structure with increased shear work inputs with unmixed buttery liquid observed at <5 kJ/kg, typical Mozzarella type microstructures (elongated fat-serum channels) at 6-15 kJ/kg and homogeneously distributed, small size fat droplets at >58 kJ/kg. At very high shear work inputs, > 75 kJ/kg, striations or anisotropy in the microstructures had disappeared and small micro-cracks were evident. Volume-weighted mean fat particle size decreased with shear work input and particle size distributions also changed. To account for the short and long term relaxation response behaviour, a 4-element Burger's model was found adequate for fitting the creep data of model cheese at 70 °C but a 6-element model was required at 20 °C. As shear work input increased, retarded compliance decreased and zero shear viscosity increased indicating the more elastic behavior of the cheeses with higher shear work input.

Fracture stress and strain for longitudinal samples from elongated full fat cheese did not vary significantly with shear work input up to 26.3 kJ/kg then decreased dramatically at 58.2 kJ/kg. Longitudinal samples with shear work input <30 kJ/kg, demonstrated significant strain hardening. At shear work inputs <30 kJ/kg strong anisotropy was observed in both fracture stress and strain. After a shear work input of 58.2 kJ/kg anisotropy and strain hardening were absent. Perpendicular samples did not show strain hardening at any level of shear work input.

A good correlation was found between the steady shear, oscillatory shear and transient rheological properties and the melting properties of the cheeses. The order for the rheological properties in terms of their sensitivity towards both shear work input and

melt functionality is $\eta_{app} > G' > \text{elongational viscosity} > \text{consistency coefficient, } K$. It was concluded that the dominant contributor to the changes in rheology, structure and melt properties with increased shear work was shear induced structural changes to the protein matrix. An increase in calcium induced protein-protein interactions after high shear work at 70 °C.

In summary, this thesis provides useful insights to shear work induced changes in material properties. It proposes useful linkages between the manufacturing process and the application of model Mozzarella cheese using appropriate rheological methods. Since the linkages were validated for only one composition and in only one processing environment, it is proposed that they should be tested in other conditions. In order to build a more complete picture, a molecular level study is proposed for future work to elucidate chemical changes during working and find appropriate linkages with physical and functional characteristics.

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List of Symbols

$\gamma(t)$	Shear strain at time t
$\dot{\gamma}_A$	Apparent shear rate, s^{-1}
$\dot{\gamma}$	Shear rate, s^{-1}
γ_{end}	Shear strain at the end of the recovery phase
γ_r	Relative recovery, %
δ	Phase angle shift, degrees
ϵ_b	Biaxial strain
ϵ_f	Tensile fracture strain
ϵ_H	Hencky tensile strain
ϵ_0, γ_{max}	Maximum strain attained during creep phase
$\dot{\epsilon}_b$	Biaxial strain rate, s^{-1}
$\dot{\epsilon}$	Strain rate in extension, s^{-1}
η	Shear viscosity, Pa.s
η^*	Complex viscosity, Pa.s
η_B^*	Biaxial extensional viscosity, Pa.s
η_0	Zero shear viscosity or viscosity before shear thickening, Pa.s
η_E	Tensile or extensional viscosity, Pa.s
η_T	Coefficient of viscous traction or extensional viscosity, Pa.s
η_∞	Infinite shear viscosity, Pa.s
η_{app}	Apparent viscosity, Pa.s
η_1 & η_2	Shear viscosity in viscoelastic region, Pa.s
η_b, η_B	Biaxial transient elongational viscosity, Pa.s
η_v	Newtonian viscosity, Pa.s
λ	Stress relaxation time or retardation time, s; Stretching of a fluid element
λ_1 & λ_2	retardation times of two retarded elements = $\frac{\eta_1}{G_1}$ and $\frac{\eta_2}{G_2}$, s
σ_b	Biaxial extensional stress, Pa
σ_f	Tensile fracture stress, Pa
τ	Shear stress, Pa
τ_0	Yield shear stress, Applied shear stress, Pa

τ_w	Wall shear stress, Pa
τ_1, τ_2 and T_{ret}	Retardation time, s
Λ	Lyapunov exponent of the flow
ω	Angular frequency, rad.s^{-1}
a_T	Shift factor in TTS
ΔP	Pressure drop, Pa
a,b	Work thickening constants
A_0	Initial cross sectional area of the specimen, m^2
$B(t)$	Time dependent compliance in bulk creep, Pa^{-1}
C	Constants in Table 2.1
$D(t)$	Time dependent compliance in tension or compression creep, Pa^{-1}
$E(t)$	Time dependent modulus in tension, Pa
E_a	Activation energy, J.mole^{-1}
$F(t)$	squeezing force at time t, N
F, F_N	Squeezing force, N
F_0	Baseline force in Blentech, N
$G(t)$	Time dependent modulus in shear, Pa
G^*	Complex modulus, Pa
G'	Storage/elastic modulus, Pa
G''	Viscous/loss/shear modulus, Pa
G_0	Initial modulus in stress relaxation, Pa
G'_{70}	Storage modulus at 70 °C
G_1 & G_2	Viscoelastic moduli of two retarded elements, Pa
$H(t)$	Height at time t, m
H_0	Height at zero time, m
J_0	Instantaneous shear compliance, Pa^{-1}
$J(t)$	Time dependent compliance in shear creep, Pa^{-1}
J_1 and J_2	Retarded compliance, Pa^{-1}
K	Consistency coefficient, Pa.s^n
$K(t)$	Time dependent modulus in bulk compression, Pa
K_E, K_s, K_m	Constants in Table 2.1
$k_{elastic}$	Power index for frequency dependence of G'
K_{SH}	Strength coefficient, Pa

k_{viscous}	Power index for frequency dependence of G''
L	Capillary length, Distance of the force sensor from the rotating shaft axis, m
l_n	Length at time t , m
l_0	Initial length, m
m	Mass, kg
$M(t)$	Torque at time t , N.m
n	Flow behaviour index, degree of frequency dependence
n_{SH}	Strain hardening index (SHI)
Q	flow rate in the capillary, $\text{m}^3 \cdot \text{s}^{-1}$
R	Capillary radius, m; Radius of circular disc cheese sample used in LSF, m; Universal gas constant, $\text{J} \cdot \text{mole}^{-1} \cdot \text{K}^{-1}$
t	Time, s
T	Temperature, K
T_0, T_{ref}	Reference temperature, K
T_R	Trouton ratio, dimensionless
V_z	Constant deformation rate, s^{-1}
W_s	Shear work, kJ/kg

List of Abbreviations

ASH	Apparent strain hardening
CSLM	Confocal scanning laser microscopy
DLS	Dynamic light scattering
FRDC	Fonterra Research and Development Centre
HBC	Hydrodynamic boundary condition
HDPE	High Density Polyethylene
LAOS	Large amplitude oscillatory shear
LLDPE	Linear low density polyethylene
LSF	Lubricated squeezing flow
LT	Loss tangent
LVDT	Linear variable differential transformer
LVE	Linear visco-elastic limit/range
MMC	Model Mozzarella cheese
MS	Melt score
RP	Rheological property
SAOS	Small amplitude oscillatory shear measurement
SER	Sentmanat Extensional Rheometer
SHI	Strain hardening Index
SHR	Strain hardening ratio
SIS	Shear induced structuring
SME	Specific mechanical energy, J/kg
SPR	Sliding plate rheometer
TSC	Tri-sodium citrate
TTS	Time-temperature superposition
VI	Viscoelasticity index
WLF	William-Landel-Ferry equation

List of Publications from PhD work

1. **Sharma, P.**, Munro, P.A., Dessev, T.T. & Wiles, P.G. (2016). Shear work induced changes in the viscoelastic properties of model Mozzarella cheese. *International Dairy Journal*, 56, 108-118.
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3. **Sharma P**, Dessev, T, Munro, PA, Gillies, G, Wiles, P, 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.
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List of Conference Presentations from PhD work

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3. **Sharma P.**, Munro, P.A., Dessev, T., Wiles, P., (2015). Effect of shear work on viscoelastic properties, structure and melt functionality of a mozzarella-type cheese. *IFT Annual Meeting and Food Expo*, Chicago, IL, USA, 11-14 July (Oral and Poster) (Appendix 3).
4. **Sharma P.**, Munro, P.A., Dessev, T., Wiles, P., (2015). Effect of accumulated shear work on the rheology, structure and melt functionality of a mozzarella-type cheese. *7th International Symposium of Food Rheology and Structure*, ETH, Zurich, Switzerland, 7-11 June (Oral).
5. **Sharma P.**, Dessev, T., Munro, P.A., Gillies, G., Wiles, P., Golding, M., James, B. and Janssen, P. (2014). Techniques to successfully measure steady shear viscosity of Mozzarella cheese. *IFT Annual Meeting and Food Expo*, New Orleans, Louisiana, USA, 21-25 June (Oral and Poster) (Appendix 3).
6. Munro, P.A., Bast, R., Dessev, T., and **Sharma, P.** (2013). Food structure design for optimum functionality. *NZ Conference of Chemical and Materials Engineering (NZCCME)*, Auckland, New Zealand, 25–26 November (Oral).

1.0 Introduction

Cheese is a popular food commodity consumed globally and a great proposition for return on investment for manufacturers. Fonterra is supplier of Mozzarella-type cheeses and has a competitive advantage with large scale manufacturing operations. This project aimed to develop further scientific understanding on a model Mozzarella type cheese.

Mozzarella cheese is a pasta filata variety of cheese which has a typical fibrous structure with a plastic appearance. This typical structure is created by kneading and stretching steps (also known as working) traditionally performed by hand stretching in hot water (60-80 °C). This process causes protein molecules to coalesce with each other to form a macroscopic fibrous network consisting of protein strands oriented in the stretching direction. Fat and serum channels are also formed within the cheese matrix during this step and are important to attain optimum melt functionality in pizza applications. The traditional manual process is now replaced by modern mechanised cheese cookers with various configurations e.g. single screw, twin screw or scraped surface heat exchangers. Development of suitable equipment and processes requires good characterization of the processing material. For selecting suitable scale up criteria, real material behaviour is required. For optimal process development, equipment design and scale-up to attain the desired functionality in cheese melt, needs fundamental rheological data close to the real operating conditions (high shear and temperature).

The energy imparted during working (Shear work) is used for the creation of a typical pasta filata cheese structure. This leads to the creation of new bonds and break up of some bonds between protein molecules. The work history and dynamics of these interactions and the time scale of operation, control the final structure of the material. Therefore, accumulated shear work and shear power intensity could be very useful parameters to control the structure of the material, and also the rheology and melt functionality. Only two studies (Mulvaney et al., 1997; Yu & Gunasekaran, 2005) have been reported studying the effect of thermomechanical treatment on rheology and melt functionality of Mozzarella cheese. Both studies suggested that the desired functionality of Mozzarella cheese can be attained by manipulating the temperature and specific mechanical energy variables. However, they used a very small range of shear work (2-6.5 kJ/kg) and this led to a narrow range of melt functionality. Studying the impact of

shear work (energy) independently and exaggerating it by extending the shear work range would lead to better understanding of the material behaviour during working.

Mozzarella cheese rheologically is a complex material. Its rheology depends on multiple factors e.g. Steady shear viscosity measurement on molten Mozzarella cheese are difficult to perform particularly at the conditions relevant to processing and finished product applications, as cheese exhibits wall slip, fat separation, and time dependency. Moisture loss and fat oozing/leakage at the surface at elevated temperatures are other problems. The non-Newtonian nature and structural anisotropy further complicate measurements. Overcoming these problems would be a promising way to obtain rheological data at the desired conditions. Various measures have been tried to eliminate these problems but the available measurements are still well below the actual processing conditions. On the other hand, conducting oscillatory shear experiments on these types of cheeses is much easier but the extent and type of deformation in these techniques are less relevant to real application conditions.

Anecdotal experience with Mozzarella cheese suggests that it work thickens and strain hardens. Work thickening is an increase in mechanical strength of a material upon working, while strain hardening is a nonlinear increase in stress with respect to strain during large strain mechanical testing. Work thickening could arise from changes in structure. This could lead to the changes in melt functionality upon working. Therefore, there should be a link between work thickening and melt functionality. Appropriate models need to be developed to suggest the dependence of viscosity on process variables. This will enable us to find appropriate links with end user application requirements. However, there is no systematic study to provide these links. The core hypothesis for this study was that Mozzarella cheese work thickens and the main sub-hypothesis was that unmelt on pizza is because of excessive working. The overall goal of the project was to use appropriate rheological methods to establish and validate links between the manufacturing process and the application of model Mozzarella cheese (MMC). Specific objectives of the study were:

1. To develop a detailed understanding of work-thickening – how shear work changes product rheology and functional properties
2. To find the best rheological property that changes most with processing and correlates well with melt functionality.

3. To establish appropriate links between structure, rheology and melt functionality of MMC.

The overall approach for this work was producing samples with a broad range of melt, conducting a series of rheological tests on these samples and then finding the best rheological property that changes most with melt score, and finally finding appropriate links between structure-rheology-melt functionality. Based on the core hypothesis and sub hypothesis that Mozzarella cheese work thickens and therefore, would cause unmelt upon excessive working, samples with a range of melt were produced by giving a range of shear work input.

This thesis is divided into 9 chapters including 6 experimental chapters. It starts with a thorough review on rheology of Mozzarella type cheeses including steady shear, oscillatory shear, transient and extensional rheology; measurement related problems, work thickening and strain hardening (Chapter 2). Subsequently chapters (3-8) are divided according to the type of rheological testing. Chapter 3 is focussed on development of a suitable method for the measurement of steady shear viscosity of molten Mozzarella type cheeses at higher shear rates. This chapter highlights a method which overcomes wall slip, structure failure and transient viscoelastic effects to obtain smooth flow curves. The effect of shear work on steady shear rheology and melt functionality of MMC is discussed in Chapter 4 which also includes a model of work thickening. Oscillatory rheology and its relationship with melt functionality as influenced by shear work treatment are given in Chapter 5. Structural characteristics of sheared cheese are given in Chapter 6 with Creep-recovery, fat particle size and confocal data presented. Chapter 7 focuses on large strain rheological testing of sheared MMC. Strain hardening, anisotropy and fracture properties are studied. Elongational viscosity using lubricated squeezing flow and time-temperature superposition are presented in Chapter 8. Chapter 9 binds all previous chapters together in the form of an overall discussion and conclusion. Chapter 10 proposes some further work in continuation to this project.

2.0 Rheological properties of Mozzarella like cheese- Literature review

Chapter Summary

This chapter reviews literature on rheology of Mozzarella type cheeses. Mozzarella cheese and its variants are rheologically complex materials as they demonstrate both viscous and elastic behaviour depending on temperature and time scale of deformation. The viscoelastic nature of the material results in start-up transient effects and fat melting causing wall slip and shear banding during rheological measurement. These phenomena are well studied with polymers but limited studies have been conducted on food materials. During processing and manufacturing of pasta-filata cheese large deformations take place. For appropriate characterization of a food material, rheological evaluation should be conducted in similar operating conditions, length scales and time scales to those taking place in the actual process. The use of literature from polymer melt rheology may help in understanding some of the rheological phenomena in alternate make cheese. Development of the typical fibrous pasta-filata structure of Mozzarella cheese depends on composition and process variables. Critical process variables in development of cheese structure are time, temperature and shear. An analogy can be taken from dough rheology in optimal use of these variables in order to attain desired functionality in the finished product.

2.1. Introduction

Mozzarella like cheeses are complex in terms of their rheological response under various conditions. They behave like viscoelastic solids at lower temperatures but like viscoelastic liquids at higher temperatures. This transformation is important for process control, structure formation and end user functionality. Processing of Mozzarella cheese involves a step of working of the molten curd mass to produce a pasta-filata fibrous structure. In order to design processing equipment for cheese manufacture and to scale it up, optimise the process and to ensure end user functionality through process control, it is important to study process rheology. Appropriate links have to be established between process rheology, structure formation and end user functionality. Higher shear rates and higher temperatures make it difficult to measure the rheology of Mozzarella like cheese because of changes in samples during measurement, wall slip effects from fat melting, strong temperature dependence and viscoelastic instabilities.

The rheological properties of food materials can be classified into three main groups i.e. viscous (viscometric), elastic and viscoelastic. When an external force is applied, food responds in a different manner from ideal materials in terms of flow and deformation. Food materials existing in liquid or liquid like states exhibit mainly shear flow deformation while solid foods or gels demonstrate mainly elastic deformation plus some shear deformation. Mozzarella cheese, a solid at low temperature, acts as an elastic material. However, above its gel-sol transition temperature the cheese starts flowing and is thus characterized as a viscoelastic fluid. The combination of viscous and elastic elements to the rheological models of Mozzarella cheese makes the measurements more complex. Mozzarella cheese differs from other varieties of cheeses because of its unique oriented pasta filata fibrous structure which arises due to kneading, i.e. working and stretching of the molten cheese curd, at high temperature i.e. 60-70°C using open channel, single screw or twin screw augers (Oberg et al., 1993; Ak and Gunasekaran, 1997). Rheology of Mozzarella cheese is important for two main reasons. First, fundamental rheological understanding is required for optimal process development, equipment design and scale-up to attain desired functionality in the final cheese including cheese melt. Second, the key requirement from any MMC process is a macroscopically homogenous flow of molten cheese with the required cheese functional properties - thus the link between relevant rheological aspects and functionality of cheese should be studied in detail. This section deals with various rheological tests which could potentially be applied for understanding the rheology of MMC.

2.2. Flow properties

Flowability is the second most important functional property after meltability for Mozzarella meant for pizza applications. Flow behaviour of food materials is generally characterised by their viscosities. Various flow models for rheological characterization of materials have been proposed by various workers (Table 2.1).

Flow characteristics of molten cheese mass are also of great importance for design and construction of cooker-stretcher and conveying systems during Mozzarella manufacture. Shear deformation prevails during flow of a liquid material. Rheological measurement under shear flow can be done either with flow rheometers using various geometries, e.g. concentric cylinder, parallel plates, cone and plate, and sliding plate, where shear is generated between a fixed surface and a moving surface, or with pressure driven

rheometers like the capillary wherein shear is generated by a pressure difference between two regions (Macosko, 1994). The selection of type of rheometer is generally based on many considerations including range of shear rate, uniformity in shear flow, ease of use and operating conditions.

Several researchers used a T-bar spindle on a Brookfield viscometer with helical motion for steady shear viscosity measurements on cheese samples (Lee et al. 1978; Guinee and O' Callaghan 1997). Lee et al. (1978) obtained a characteristic viscosity curve as a function of temperature. Formation of strands during rotation and upward movement of the T-bar spindle could be an issue for Mozzarella cheese. Patarin, Galliard, Magnin & Goldschmidt (2014) used vane geometry for studying large-strain shear rheology.

Table 2.1 Two or three parameter rheological flow models for describing the shear rate ($\dot{\gamma}$) versus shear stress (τ) relationship

$\tau = \eta \dot{\gamma}$	Newtonian Model
$\tau = \frac{\dot{\gamma}}{\frac{1}{\eta_0} + K_E (\tau)^{\frac{1}{\eta_E}} - 1}$	Ellis model for low shear rate data containing η_0 zero shear viscosity
$\tau = \eta_{\infty} \dot{\gamma} + K_S \dot{\gamma}^{\eta_s}$	Sisko model for high-shear rate data containing η_{∞} infinite shear viscosity
$\tau = K \dot{\gamma}^n$	Power law model used extensively in handling applications, K consistency coefficient, n flow behaviour index
$\tau = \tau_o + K \dot{\gamma}^n$	Herschel-Bulkley model with yield stress (τ_o)
$\sqrt{\tau} = \sqrt{\tau_o} + \sqrt{K} \sqrt{\dot{\gamma}}$	Casson model used especially for chocolates
$\frac{\eta(\dot{\gamma}) - \eta_{\infty}}{\eta_0 - \eta_{\infty}} = \frac{1}{1 + (c \cdot \dot{\gamma})^p}$	Cross model for data over a wide range of shear rates
$\tau = \frac{\eta_0 \cdot \dot{\gamma}}{(1 + \frac{\dot{\gamma}}{\dot{\gamma}_c})^c}$	Carreau model for data over a wide range of shear rates
$\tau = \tau_o + K \dot{\gamma}$	Bingham model
$\tau = \tau_o + (\tau_1 - \tau_o) \cdot [1 - \exp(-\dot{\gamma}/\dot{\gamma}^*)]$	Windhab (2001) model used to evaluate chocolate melt
$\sqrt{\tau} = \tau_o + K_M \dot{\gamma}^{n_m}$	Mizrahi and Berk (1972) – a the modification of the Casson model

(Rao, 2007 and Mezger, 2011)

Steady shear viscometry measurement can encounter problems with cheese samples especially at higher temperature where the fat is melted and oozes out to the surface of

the cheese mass in contact with the rotating geometry, leading to wall slip and thus unreliable measurement of the viscosity. Another problem encountered is the Weissenberg effect where the elastic component of the stress tensor leads to normal forces acting on the sample in an upward direction (orthogonal). This sometimes leads to climbing of cheese melt on the inner cylinder in the concentric cylinder geometry or to a strand/thread of cheese coming out of the gap of the parallel plate geometry (Ruegg et al., 1991).

Highly viscous and viscoelastic materials like Mozzarella cheese also exhibit transient (time dependent) behaviour especially at low shear rates ($<1 \text{ s}^{-1}$). In steady state viscosity measurement this transient behaviour leads to transient viscosity peaks known as start-up effects at low shear conditions. To avoid these effects, longer measurement times in the low shear rate range are needed to accurately measure the steady shear viscosity. According to Mezger (2011), the measuring point duration should be at least as long as the reciprocal shear rate ($1/\dot{\gamma}$).

A recent study on Mozzarella cheese obtained flow characteristics at shear rates ($0.0001\text{-}0.01 \text{ s}^{-1}$) and temperatures ($40\text{-}80 \text{ }^\circ\text{C}$) relevant to pizza baking and applied a mathematical model to predict viscosity as a function of shear rate (Zhu, Brown, Gillies, Watkinson and Bronlund, 2015).

Capillary rheometry is another potential method to measure shear viscosity at relatively high shear rates. The cheese melt mass is pushed through a capillary extrusion die and the pressure drop measured and used for the calculation of wall shear stress. The flow rate is used to calculate shear rate.

$$\tau_w = \frac{\Delta PR}{2L} \quad (2.1)$$

$$\dot{\gamma} = \frac{4Q}{\pi R^3} \quad (2.2)$$

Where, τ_w wall shear stress, $\dot{\gamma}$ wall shear rate ΔP pressure drop, R and L capillary radius and length respectively, Q flow rate in the capillary.

Viscosity measurements using capillary rheometers are based on absolute and well defined parameters such as shear stress and shear rate, thus enabling reliable data generation even at higher shear rates suitable for engineering design. Capillary rheometers have been applied for various milk products in the past e.g. butter (Shukla

and Rizvi 1995); ice-cream (Martin et al. 2008) and Mozzarella cheese (Smith et al. 1980; Cavella et al. 1992; Taneya et al. 1992; Muliawan and Hatzikiriakos 2008; Bahler and Hinrichs, 2013, Bahler, Back & Hinrichs, 2015). Cortes-Martines et al. (2005) used a capillary extrusion method to produce mozzarella like string cheese. Flow instabilities, melt fracture and wall slip could be of great concern because they will affect experimental results and may give rise to processing difficulties. These phenomena could arise due to the viscoelastic nature of the material, edge and entry effects. These phenomena have been observed to cause phase separation in extruded molten cheese, i.e. fat and serum exuding from the cheese (Smith 1980, Bahler and Hinrichs, 2013).

2.3. Visco-elastic properties of cheeses using small amplitude oscillatory shear (SAOS) measurements

Measurement of dynamic mechanical and rheological properties of food is now a familiar technique and has had many applications in cheese. The technique offers continuous measurement of both elastic modulus and viscous modulus at various frequencies and temperatures within a short time without causing structural and physical damage to the food material. At extremely small strains (typically less than 1%) the relationship between stress and strain is almost linear which offers better modelling and interpretation of data as the Boltzmann superimposition principle works well under such conditions (Konstance and Holsinger 1992; Dealy 1982; Muliawan, 2008). The region where linear dependency of the stress and strain is observed is known as the linear viscoelastic (LVE) range (Fig. 2.1a). Within the linear range, viscoelastic characteristics are independent of extent of force and deformation rate (Ferry 1980). The LVE limit for Mozzarella and other varieties of cheeses reported by various workers is in the range of 0.1-1% strain (Nolan et al., 1989; Steffe 1992; Rao and Steffe 1992; Ak and Gunasekaran, 1996; Subramanian and Gunasekaran 1997a; Muliawan and Hatzikiriakos, 2007).

Small amplitude (strain) oscillatory shear (SAOS) measurement is a technique to determine dynamic moduli (G^* , G' , G'') of the food material that involves putting the sample between rotating and stationary geometries and applying continuous sinusoidal deformation (or stress) on the specimen and measuring the response stress (or strain) continuously. For ideal elastic materials the strain and stress are in phase (phase angle δ 0°) while for ideal viscous materials they are completely out of phase (phase angle δ

90°). On the other hand for viscoelastic materials the phase angle is between 0 and 90°. The tangent of phase angle ($\tan \delta$), also known as loss tangent or loss factor, is the ratio of viscous modulus (G'') to elastic modulus (G') and it represents the extent of loss of the energy.

It is now well-accepted that cheese is a viscoelastic material which means it exhibits both elastic and viscous behaviour during stress loading or unloading. The elastic component is known for storage of the energy while the viscous portion is tagged with loss of the energy to the food material i.e. permanent deformation. Viscoelastic material can be subjected to small or large deformations during rheological measurement.

Studies on variation of the LVE range of Mozzarella cheese with temperature and age indicated a decrease in LVE range (smallest range 0.05% strain amplitude) with increasing heating temperature (10-70°C) and age (12 weeks at refrigerated temperature) because of the fact that cheese becomes softer with higher temperature (thermal softening) and with age (proteolysis during maturation). Both phenomena result in a decrease in both elasticity and viscosity (Creamer et al., 1982; Subramanian and Gunasekaran 1997a; Muliawan and Hatzikiriakos, 2007). Thus, selection of the correct LVE range for a variety of cheeses at a certain temperature and age is most critical before proceeding to dynamic rheological measurements. A smaller strain or stress than the LVE limit should be used to obtain measurements (Muliawan and Hatzikiriakos, 2007).

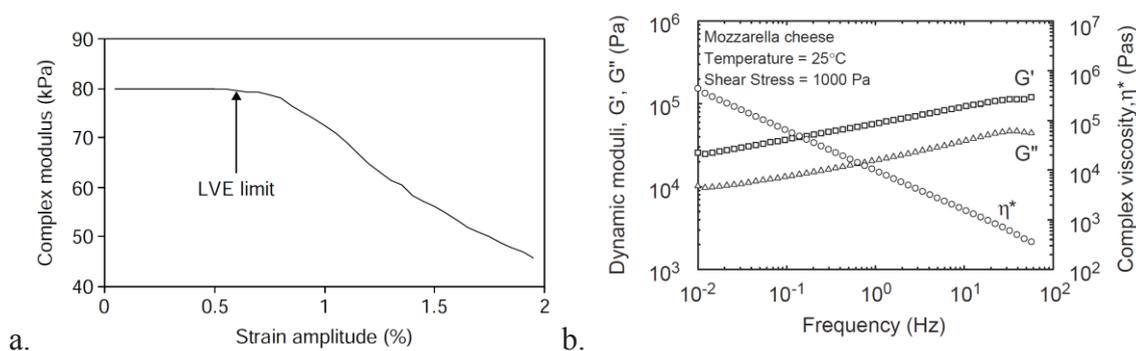


Figure 2.1 Linear viscoelastic measurements (G' , G'' , G^*) of Mozzarella cheese (a) Linear viscoelastic limit during strain sweep test at constant frequency (e.g., 1 Hz) (LVE stands for linear viscoelasticity.) (Ak and Gunasekaran, 1996.), (b) Frequency sweep (Muliawan and Hatzikiriakos, 2007).

SAOS measurements are performed in a number of ways e.g. strain (or stress) sweep, frequency sweep and temperature sweep. A **strain amplitude sweep** is used to determine the LVE limit and it involves measurement of dynamic moduli with change in amplitude keeping frequency constant. A **frequency sweep** continuously measures the dynamic moduli of the food material as a function of frequency at constant temperature and strain or stress level (Fig. 2.1b). This test is performed in the LVE range and is always considered a versatile test to characterize the viscoelastic behaviour and physical state of the food material. At low frequencies, e.g. 10^{-5} Hz, frequency sweeps take more than two days to complete one cycle. Time-temperature superposition (TTS) is an efficient way to shorten the test duration (Ak and Gunasekaran, 2003). At 25°C the elastic modulus in Mozzarella cheese was observed to be higher than the viscous modulus over the whole experimental frequency range which is considered as typical viscoelastic solid behaviour (Bahler, Back and Hinrichs, 2015; Muliawan and Hatzikiriakos, 2007; Subramanian and Gunasekaran, 1997a; Nolan et al. 1989; Diefes, Rizvi, and Bartsch, 1993). At higher temperatures during the frequency sweep, due to melting of fat and increased protein mobilization, cheeses start behaving like viscoelastic fluids, i.e. similar to the behaviour of a molten polymer (Dealy and Wissbrun, 1990). The frequency dependence of G' and G'' gives useful insights into material behaviour. It indicates the nature of interactions taking place in the viscoelastic network and helps in classification of weak or strong networks (Tunick, 2011).

A **temperature sweep** is conducted by measuring viscoelastic properties in the LVE range at varying temperatures while keeping strain or stress amplitude and frequency constant. The temperature sweep curves demonstrate the state of the material and its transition as a function of temperature. Mozzarella cheese at low temperature behaves like a solid viscoelastic material which converts into a viscoelastic fluid at a higher temperature. As a result of thermal softening the dynamic moduli (G' , G'' , G^*) decrease with increasing temperature (Joshi et al., 2004; Hsieh et al., 1993; Venugopal and Muthukumarappan, 2003; Guinee et al., 2002; Karoui et al., 2003). However, the rate of decrease of these moduli varies depending on the material characteristics e.g. composition, age, physicochemical properties etc. Because of melting of fat over a range of temperatures, e.g. 20 to 40°C, a steep/sharp decrease in dynamic moduli is observed (Guinee et al., 2002, Muliawan and Hatzikiriakos, 2007). However, at higher temperatures, e.g. range 50-80°C, a further decrease in elastic modulus is reported. The

probable reason for this is softening and mobilization of the protein matrix due to reduced protein-protein and protein-mineral interactions. As a result the viscous flow regime starts dominating.

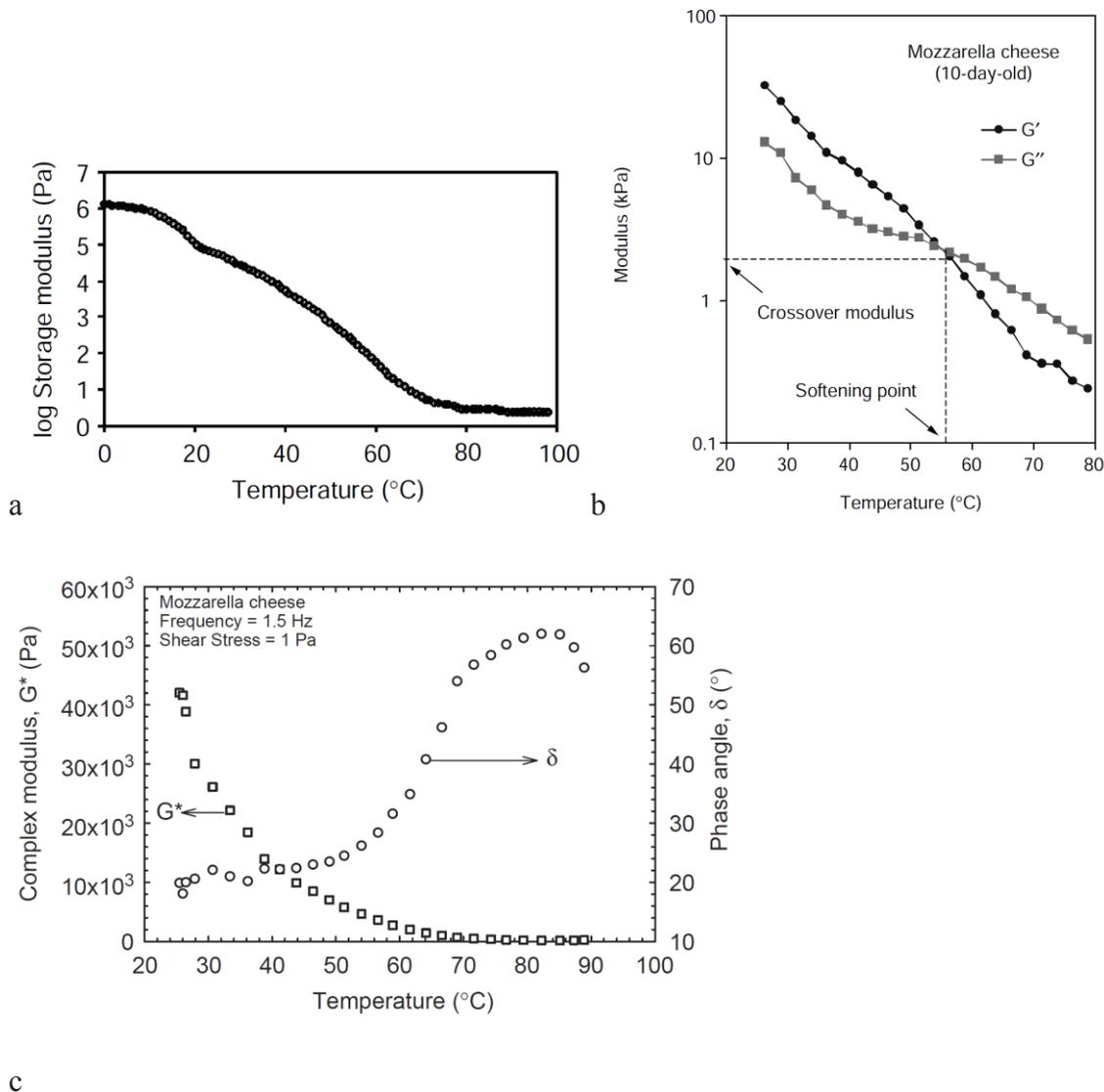


Figure 2.2 Temperature sweep tests on Mozzarella cheese showing (a) storage modulus (Wetton and Marsh, 1990.), (b) temperature at crossover modulus corresponding to cheese softening point (Gunasekaran et al., 2002), (c) effect of temperature on complex modulus and phase angle (Muliawan and Hatzikiriakos, 2007).

During a temperature sweep, the cross over point, i.e. the temperature at which viscous modulus equals elastic modulus ($G''=G'$, $\tan \delta=1$), is considered to signify the gel-sol transition (melting) point (Gunasekaran and Ak, 2000; Guggisberg, Butikofer, and Albrecht, 2007; Schenkel, Samudrala and Hinrichs, 2013; Bahler, Back and Hinrichs, 2015; Shima & Tanimoto, 2016), or transition from solid-like to fluid-like behaviour

(Muliawan and Hatzikiriakos, 2007), or an indication of the softening point of the cheese (Ma, Balaban, Zhang, Emanuelsson-Patterson & James, 2014) or an onset temperature of rapid melt and flow (Gunasekaran et al. 2002). Contradictory results were observed in two references from the same research group. Muliawan and Hatzikiriakos (2007) reported approximately 65°C (Fig. 2.2c) as the cross over temperature for Mozzarella cheese which was inconsistent with Muliawan (2008) reporting 55-56°C and with other research groups. Muliawan (2008) mentioned that at 60°C the Mozzarella cheese melts completely as seen on a temperature sweep curve (Fig. 2.2c).

Mounsey and O’Riordan (1999) assessed the usefulness of dynamic measurements as indicators of imitation cheese meltability and found a high correlation ($R^2 = + 0.96$) between maximum $\tan \delta$ values and meltability as assessed by an empirical method based on flow in a heated tube (Olson and Price, 1958). Ma et al. (2014) quantified pizza baking performance using image analysis and correlated this with cheese properties including rheology. Though melt and flow properties in Mozzarella-like cheese are closely related, the maximum loss tangent ($\tan \delta_{\max}$) on a temperature sweep curve can be regarded as an indicator of flowability (Guinee, Auty and Mullins, 1999; Schenkel, Samudrala and Hinrichs, 2013). Ma et al. (2013) determined flowability of Mozzarella cheese by determining the activation energy of flow from a temperature sweep using the Arrhenius equation for complex viscosity. There are number of methods available to test cheese metlatibility. These methods have been recently reviewed by Zhu (2013).

2.4. Time-Temperature Superposition (TTS)

Rheological properties of Mozzarella cheese are very dependent on temperature. Studying this temperature dependence is required to understand more fully the rheological behaviour of the cheese. For less complex materials (i.e. thermo-rheologically simple), linear viscoelastic properties measured at various temperatures can be shown on one plot known as a “Master Curve” by using the time-temperature superposition (TTS) procedure (Ferry, 1980; Dealy and Wissbrun, 1990; Honerkamp and Weese, 1993). The master curve can be used to derive viscoelastic properties at expanded timescales or frequencies that are otherwise not measurable experimentally. Cheese is not thermo-rheologically simple because of its multiple phases (fat, protein

and water) and phase changes with temperature. Nevertheless, some studies have been conducted on cheese for TTS (more precisely frequency-temperature superposition) (Taneya et al., 1979; Subramanian and Gunasekaran, 1997b; Muliawan and Hatzikiriakos, 2007). In a situation where the actual experiment could take a prolonged time or is not even possible because of sample stability, TTS can help by deriving data from experiments at shorter times and predicting the behaviour for longer times. This saves both time and resources. As per the TTS principle, viscoelastic properties (e.g. G' , G'' and $\tan \delta$) measured at various temperatures should have a constant ratio (known as shift factor, a_T) with the corresponding property measured at a reference temperature. According to Ferry (1980), three criteria should match to enable successful TTS application: a. The shapes of the adjacent curves should match, b. All viscoelastic functions should be able to superpose with the same value of empirical shift factors (a_T) c. Temperature dependence of a_T must have a consistent form (e.g. Arrhenius-type or William-Landel-Ferry (WLF) type relation). Subramanian and Gunasekaran (1997b) obtained master curves with 40°C as reference temperature for storage modulus using the temperature-frequency superposition principle. They successfully derived the variation of relaxation time and viscosity spectrum of cheese with age from the master curves using the generalised Maxwell model and nonlinear regression analysis. Muliawan and Hatzikiriakos (2007) obtained master curves for Mozzarella cheese with 60°C as the reference temperature from their experiments (Fig. 2.3). The horizontal shift factors, a_T were found to follow the Arrhenius equation. Due to the complex nature of Mozzarella cheese superposition below 40°C was not satisfactory. However, the results were good when fat was molten (>40°C) which also indicated that Mozzarella cheese can be considered as a thermo-rheologically simple material for $T > 40^\circ\text{C}$ (Muliawan and Hatzikiriakos, 2007).

Udayarajan et al (2007) studied the time-temperature superposition behaviour of part-skim Mozzarella cheese and of a fat free cheese during heating and cooling using Fourier transform mechanical spectroscopy. Master curves for G' , G'' and loss tangent (LT) were obtained using a reference temperature of 70°C. An Increase in temperature at which the maximum value of LT (LT_{\max}) occurred and decrease in absolute value of LT_{\max} was observed with an increase in frequency for both samples.

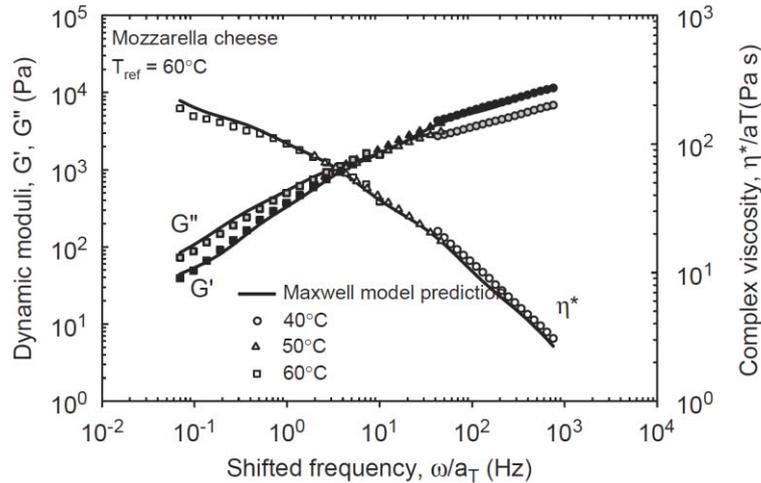


Figure 2.3 Time–temperature superposition of Mozzarella cheese linear viscoelastic data (G' , G'' and η^*) obtained at 40, 50, and 60°C at the reference temperature of 60°C. Solid lines represent the Maxwell model prediction of the data (Muliawan and Hatzikiriakos, 2007).

However, fat-free cheese exhibited higher values of LT_{\max} than with the part-skim Mozzarella cheese indicating a higher degree of bond mobility in the fat-free cheese. The authors hypothesized the cause of higher mobility as lower levels of total and insoluble Ca and higher moisture content in fat free cheese. Rheological data obtained in the low temperature region were found to obey TTS very well. Because of permanent structure formation between calcium and protein during heating, on subsequent cooling, the material exhibited a different rheological response.

2.5. Time-dependent behaviour

Because Mozzarella cheese is viscoelastic material, its structure and texture perception is a combined result of rheological and fracture properties. A viscoelastic material during deformation stores a part (elastic) of the total energy given and dissipates the rest (loss or viscous) in the form of heat or internal energy. The material response is time dependent because the ratio of dissipated to stored energy depends on the timescale of deformation. If the dissipated energy is above a certain value, it may leave some permanent deformation in the food material and also delay the food response to the external force applied (Steffe, 1996; Lucey et al. 2003). Thus, the duration for which a stress of certain magnitude and direction acts on the food material, may cause the response of the cheese to be more elastic or more viscous (van Vliet, 1999). This phenomenon can be significant during cooking and stretching of pasta-filata type

cheeses. Stretching at a faster rate for a short duration may result in earlier breakage of Mozzarella cheese fibres compared to a slower speed. At a shorter timescale, Camembert cheese behaves more elastically and is more amenable to cutting into pieces whereas over longer timescales it flows, even at refrigerated temperatures (van Vliet, 1999).

Time-dependency in food materials can arise from two effects: 1. changes in the structure of the material during the measurement, or 2. the viscoelastic effects discussed above. If the initial response of the material to the stress is instantaneous with viscosity then being time-dependent, it is due to the change in the structure of the material. This could either be strain thinning (thixotropic) or strain thickening (rheopectic). Time-dependency arising from viscoelastic effects results in a non-instantaneous (with lag time) response to the stress and should not be linked to structural change in the food material (Steffe, 1996). However, the time scale of thixotropic and visco-elastic effects may be quite different. Ideally, these two phenomena can be explored independently of the other but real food materials may exhibit both effects simultaneously which may make the food material characterisation even more difficult.

2.6. Cox-Merz rule

The Cox-Merz rule defines the empirical relationship between the shear rate dependence of the steady shear viscosity, $\eta(\dot{\gamma})$ and the frequency dependence of the complex viscosity, $\eta^*(\omega)$ (Cox and Merz, 1958).

$$\eta(\dot{\gamma}) = |\eta^*(\omega)| = \frac{G''}{\omega} \sqrt{1 + \left(\frac{G'}{G''}\right)^2} \Bigg|_{\omega=\dot{\gamma}} \quad (2.3)$$

The rule relates a linear viscoelastic property to a nonlinear property but has been found to be valid for a variety of polymer melts and solutions (Dealy and Wissbrun, 1990; Ferry, 1980). For a material having a yield stress, e.g. tomato paste, the rule is applicable using effective shear rates (Doraiswamy et al. 1991 and Rao and Cooley, 1992). The major utility of this rule is estimating steady shear viscosity particularly at high shear rates from oscillatory rheometric data (Gunasekaran and Ak, 2003).

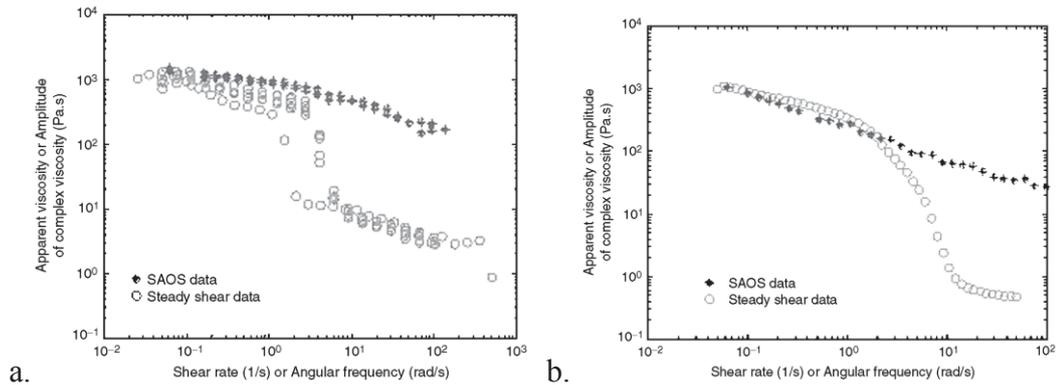


Figure 2.4 Steady shear apparent viscosity and complex viscosity data for (a) Mozzarella cheese and (b) processed cheese at 60°C. (Yu and Gunasekaran, 2001)

Experiments to examine the applicability of the Cox-Merz rule or its modified form to Mozzarella cheese and processed cheese revealed non-validity at 60°C (Fig. 2.4) (Yu and Gunasekaran 2001). The reasons for the failure of this rule on Mozzarella cheese and process cheese are not known. Sample slippage after melting of the cheese could be a possible reason for this failure.

2.7. Stress relaxation

A stress relaxation test is used to analyse viscoelastic behaviour by recording the decay of stress as a function of time at a fixed magnitude of strain or deformation applied as a step function (Fig. 2.5 a). In other words, the stress required to hold the deformation (instantly applied) constant is measured as a function of time. This experiment can be performed either in compression, tension, or rotational modes. The results obtained are expressed in terms of time dependent modulus $E(t)$ in tension or compression, $G(t)$ in shear, or $K(t)$ in bulk compression. A generalised Maxwell rheological model (with springs and dashpots) is used to interpret the stress relaxation behaviour.

The most important parameter obtained from this experiment is the stress relaxation time, the time at which the stress in a body resembling a simple Maxwell model (single exponential) decays to $1/e$ (0.37) times the initial stress (Fig. 2.5a). It is a measure of the rate at which a material dissipates stress after receiving a sudden strain. When a body is modelled by multiple Maxwell elements (invoking multiple time constants) connected in parallel, a spectrum of relaxation times exists from which one should select the mean relaxation time (Mohsenin 1996).

In compression mode the sample should not have friction with the upper plate. To prevent this, the specimen is either bonded to the plate or lubricated on the surface. Lubrication is usually more readily achieved. This test can be applied to polymers with chemically inert fillers or cross linked polymers, gels and dispersions containing showing a physico-chemical network (Mezger 2011).

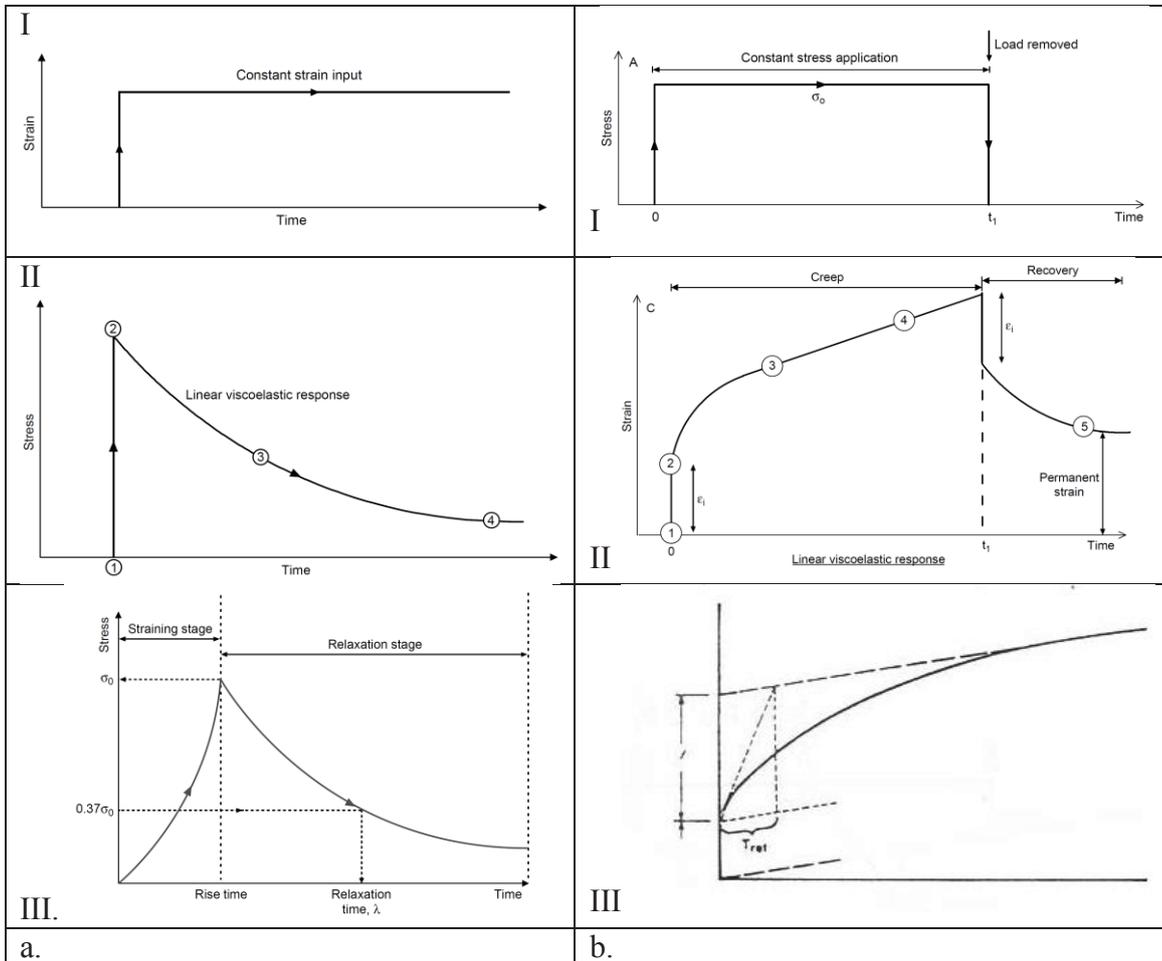


Figure 2.5 a. Step-strain input (I) and stress relaxation response (II) of a viscoelastic material. In an actual relaxation test, the strain is applied over a finite time (rise time) (III). The peak stress at the end of the straining stage is the initial stress (σ_0). The time taken for the stress to decay to $0.37\sigma_0$ during the relaxation stage is the relaxation time, λ . (Gunasekaran and Ak, 2003). b. Typical creep–recovery test. (I) application of instantaneous and constant stress (σ_0); (II) strain response of viscoelastic material. The time taken for the strain to build up to $0.63 \epsilon_0$ during the stress application stage is the retardation time, T_{ret} . (Mohsenin, 1996; Gunasekaran and Ak, 2003)

Bahler, Nagele, Weiss & Hinrichs (2015) studied force relaxation in compression mode at 60 °C using a custom built compression tool attached to an Instron texture analyzer. They used Deborah number as a criterion for structuring in the the pasta-filata type cheese curd. However, the results reported are not convincing as the estimation method

used to obtain relaxation time was empirical and the method was actually lubricated squeezing flow yet the results were discussed as relaxation in compression mode. The material should have crossed the LVE limit at the higher compression ratios used (16 fold) and so the Boltzmann superposition principle can not be applied. Relaxation models are therefore invalid for the data set. The sample was heated by immersing in a water bath; there would be a strong possibility of leaching water soluble components to the surrounding liquid if the sample was not hermetically sealed. The relaxation time reported was less than 2 s, yet authors recommended 300 s would be needed for compressing curd to obtain structural alignment. Experimental elongations were only 25% of theoretical values.

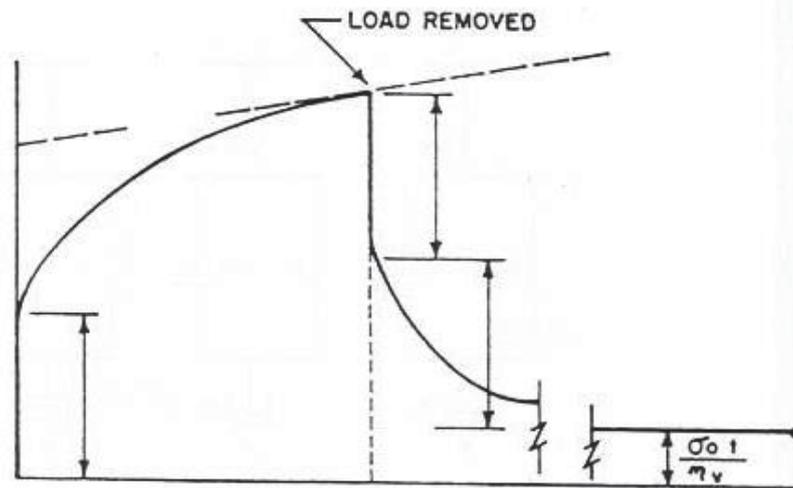


Figure 2.6 Typical Creep and recovery curve in a viscoelastic material exhibiting instantaneous elasticity, retarded elasticity and viscous flow (Mohsenin 1996).

2.8. Creep behaviour

A creep test involves sudden application of a constant load (stress) to the material. Deformation (strain) is then measured as a function of time at constant stress (Fig 2.5b; 2.6). The results are expressed in terms of time dependent constants such as $E(t)$ or its compliance $D(t)$ in tension or compression creep, $G(t)$ or $J(t)$ in shear creep, and $K(t)$ or $B(t)$ in bulk creep. The compliance is the reciprocal of modulus in each case. The creep behaviour in a viscoelastic material is often represented by a 4-element Burger model which is considered one of the best rheological models to predict the creep behaviour.

Typically the model comprises a spring and dashpot in series with another spring and dashpot in parallel. The model is also valid for a material exhibiting elasticity, retarded elasticity, and flow (Mohsenin 1996).

Several studies on Mozzarella type cheeses and similar casein-based materials have used creep-recovery methods (Subramanian, Muthukumarappan & Gunasekaran, 2003; Manski, van der Zalm, van der Goot & Boom, 2008; Olivares, Zorrilla & Rubiolo, 2009). Creep tests are more common for cheese rheology, as they are easier to perform, can describe material behavior more practically and are the best way of obtaining zero-shear viscosity. Olivares et al. (2009) conducted creep/recovery experiments on Mozzarella cheese to study the internal structure both for fresh and stored samples at various temperatures and also considered different sample orientations, i.e. parallel and perpendicular to protein fibre direction. They obtained viscous and elastic contributions from creep data using a 4-element Burger model. They reported that the viscoelasticity index (VI) derived by Kuo et al. (2000) (the instantaneous slope of the creep curve) was a good basis for objective evaluation of cheese meltability. Viscoelasticity index is discussed in more detail in section 2.9. Patel, Dumlu, Vermeir, Lewille, Lesaffer et al. (2015) studied transient viscoelastic properties of gel-in-oil-in gel type structured emulsions using a creep-recovery method and used maximum creep and relative recovery as structure related parameters. Melito, Daubert and Foegeding (2012) fitted a 4-element Burgers model to the creep data obtained for WPI/ κ -carrageenan gels with different structures and found correlation of the model parameters with large amplitude oscillatory shear (LAOS) creep behaviour.

2.9. Non-linear viscoelasticity

During small deformations a material is slightly disturbed from its equilibrium state - in response to this the material exhibits linear viscoelastic behaviour for which the rheological properties are independent of strain amplitude. However, if the deformation is large enough, causing (irreversible) structural changes in the material, we observe non-linear viscoelasticity, i.e. strain dependence. Material characterization in the linear regime is simple and predictable due to the validity of the Boltzmann superposition principle in the form of the generalised Maxwell model covering the experimental range. However, in actual processing conditions such as cooking, mixing, stretching, moulding and extrusion etc., materials like Mozzarella cheese experience large and/or

rapid deformations causing significant structural changes. This leads to complex non-linear viscoelastic behaviour. To fully characterize such complex materials, non-linear viscoelastic properties should also be measured. Most studies of non-linear phenomena measure the following properties: nonzero first normal stress difference (Weissenberg effect), shear rate dependence of steady state shear viscosity and dependence of the relaxation modulus on strain magnitude (Gunasekaran and Ak, 2003; Dealy and Wissbrun, 1990).

The major difference between linear and non-linear viscoelasticity is the rate and extent of deformation. Non-linear viscoelasticity as discussed above implies larger and faster deformations. The material response in the non-linear viscoelastic region depends on the size, rate and kinematics (time and pathway) of deformation. In order to repeat the same response these three parameters need to be matched. In a shorter range of deviations from linearity, the strain-amplitude relationship with complex modulus or complex viscosity is often studied (Ohta et al., 1987).

Relatively few studies have been conducted on non-linear viscoelastic behaviour of food materials using LAOS rheometry because instruments with sufficient precision are often not available and adequate theoretical background to describe and analyse the non-linear material response is often absent. LAOS rheological testing of food materials has been used mostly for assessment of fracture properties (Li et al., 1999; Truong and Daubert, 2000; Nishinari, 2004; Tabilo-Munizaga and Barbosa-Cánovas, 2005) and to estimate recoverable energy (van den Berg et al., 2008a, b; Sala et al., 2009a; 2009b; Cakir et al., 2011; de Jongh, 2011). However, the technique has been successfully used to study the viscoelastic properties of polymers and polymer melts beyond the LVE range, but prior to fracture. An instrument for LAOS testing must have the ability to generate large, uniform, transient deformations involving higher shear rates. Such an instrument would be suitable to study the time-dependent structure function relationship for complex materials e.g. Mozzarella cheese. The sliding plate rheometer (SPR) developed by Giacomini et al. (1989) generates large, uniform, transient deformations involving high shear rates with a homogenous flow field. This rheometer has been used by Tariq et al. (1998) for measuring non-linear viscoelasticity of cheese using LAOS. In a typical LAOS test on a reduced-fat Mozzarella cheese sample at 60°C, a sinusoidal strain of magnitude 6 was applied and the resultant stress was recorded with time. After 4 cycles, the stress amplitude became a standing wave (Tariq, 1998). Muliawan (2008)

studied non-linear viscoelastic behaviour of Mozzarella cheese at different temperatures (25, 40, 50, 60°C) using small and large step shear strain relaxation. He compared the non-linear relaxation process to linear relaxation and the damping function, a measure of the degree of non-linearity, was derived. The damping function could be described by a generalised Zapas model. Further studies confirmed that the linearity of the rheological behaviour of Mozzarella cheese increased with temperature because of the better flowability of the cheese at higher temperatures (Muliawan 2008). Melito, Daubert and Foegeding (2013a, b) studied whey protein/ κ -carageenan gels and commercial samples of Cheddar, Mozzarella, and American cheeses for their non-linear viscoelastic behaviour using LAOS and creep techniques and correlated them with sensory and oral processing characteristics. They concluded with the remark that non-linear viscoelastic properties of gels can be measured using both techniques. LAOS testing yields a more robust quantitative measure of the type and extent of nonlinear behaviour, while creep parameters indicate only the presence of nonlinear behaviour below LVE.

2.10. Extensional rheology

The coefficient of viscous traction, the term coined by Trouton in 1906, later became known as extensional viscosity (Petrie, 2006). Trouton obtained the relationship between (shear) viscosity, η , of an incompressible Newtonian fluid and the coefficient of viscous traction, η_T by resolving stress and shear rate tensors in appropriate directions, to reveal the following equation,

$$\eta_T = 3\eta \quad (2.4)$$

Extensional deformation in a material usually takes place under non-steady and non-uniform conditions which led researchers to make use of the terms transient extensional viscosity or biaxial growth coefficient. Like polymer processing, mozzarella cheese manufacture involves both shear and extensional deformation. Shear forces lead to breakdown of fat droplets while extensional forces stretch mainly the continuous protein phase causing it to orient in one direction. The structuring gives rise to unique melt and flow characteristics for Mozzarella cheese. However, relatively few studies using extensional rheometry have been conducted on cheese.

The relationship between transient elongational viscosity and shear viscosity is known as the Trouton ratio (T_R), η_T/η (Jones et al., 1987). The Trouton ratio for Newtonian fluids is 3, 4 and 6 under uniaxial, general (biaxial) and planar extensional deformation respectively (Jones et al, 1987; Barnes et al., 1989; Gunasekaran and Ak, 2003). According to Jones et al. (1987), any variation from the T_R values mentioned above for various deformations may be attributed to ambiguity arising from different definitions of strain rates and shear rates and also to viscoelastic effects. They proposed T_R relationships for non-Newtonian fluids under uniaxial and planar extensional flows with an unambiguous effect of viscoelasticity,

$$\text{Uniaxial flow} \quad T_R = \frac{\eta_E(\dot{\epsilon})}{\eta(\dot{\gamma} = \sqrt{3}\dot{\epsilon})} \quad (\text{Jones et al. ,1987}) \quad (2.5)$$

$$\text{Planar extension} \quad T_R = \frac{\eta_E(\dot{\epsilon})}{\eta(\dot{\gamma} = 2\dot{\epsilon})} \quad (\text{Jones et al. ,1987}) \quad (2.6)$$

Where η_E is the tensile or extensional viscosity, η is the shear viscosity, $\dot{\gamma}$ shear rate and $\dot{\epsilon}$ strain rate

For estimating T_R , shear viscosity should be measured at a shear rate equal to the numerical values $\sqrt{3}\dot{\epsilon}$ and $2\dot{\epsilon}$ for uniaxial and planar deformation respectively. For non-Newtonian and viscoelastic fluids the relationship converts into a limiting case, e.g. same shear/strain rate, at very small strain rates (Barnies et al., 1989; Dealy, 1995 and Petrie, 2006).

$$\eta_E(\dot{\epsilon})\Big|_{\dot{\epsilon} \rightarrow 0} = 3\eta(\dot{\gamma})\Big|_{\dot{\gamma} \rightarrow 0} \quad (2.7)$$

According to Munstedt et al. (1998) extensional flow offers great potential for polymer characterization as it is more sensitive to the molecular structure of the polymer material. Petrie (2006) highlighted the fact that extensional viscosity should be carefully defined and examined. Experiments should be designed based on the assumption that there may be non-steady and spatially non-uniform deformation taking place. This finally led to a term known as “transient extensional viscosity”. However, the use of this term is easy in terms of definition but during experiments it may create

confusion and inconsistent results. In his opinion, a better way to obtain consistent data is to use strain functions rather than strain rates. A strain function will also help in understanding changes in macromolecular structure in the sample. He still foresees a danger of overestimating the microstructural changes using strain measurements. Furthermore, for deriving material property functions the separation of elastic and viscous effects is required; this is a cumbersome exercise and prone to error in most extensional viscosity measurements.

Ak et al (1993) performed uniaxial horizontal extension tests on low-moisture part-skim Mozzarella cheese in a mineral oil bath to determine tensile properties as a function of storage time (7-28 days), temperature (10-40°C), and deformation rate (50-500 mm/min). They used a universal testing machine with modifications to translate the vertical cross head movement to the horizontal plane. With storage time, the tensile strength and deformability modulus decreased because of proteolysis of the protein matrix. However, a temperature rise increased the fracture strain many fold. Later Ak and Gunasekaran (1995) measured transient elongational viscosity of a Mozzarella cheese over a month interval of storage on a vertical extension apparatus by melting the sample in hot oil (60°C) and stretching in the same environment. They successfully achieved 400% extension. However, the temperature and strain rates increased during the measurement and this had a significant effect on measurements.

Muliawan (2008) measured uniaxial extensional properties of market Mozzarella cheese at various temperatures (25 to 60°C) using a Sentmanat Extensional Rheometer (SER) fitted to a convective oven in a strain controlled rheometer. The stress growth coefficients were plotted against time for various Hencky strains. Strain softening and ductile failure of specimens was reported at 60°C. However, the method followed in the study to obtain the data at 60°C is questionable, as it is difficult to perform tensile tests on a food material that is molten (Bourne, 1982; Luyten et al., 1992; Ak and Gunasekaran, 1995). For the comparison of extensional and shear measurements, dynamic moduli obtained in the LVE were used to predict of transient shear stress growth using a Maxwell model (Muliawan and Hatzkirakos, 2007). The model prediction did not agree with actual extensional measurements and discrepancy increased with increase in temperature. Loss of moisture at higher temperatures was suggested as the reason for poor fit.

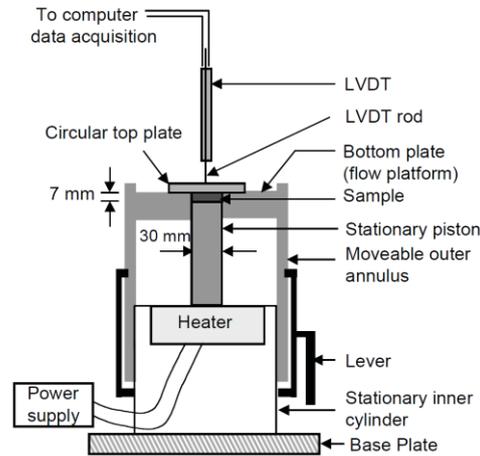
Lubricated squeezing flow (LSF) is considered as a potential technique for food characterization as it overcomes slip problems and also avoids any partial destruction of the microstructure during sample loading into coaxial rotational or capillary rheometers (Hoffner et al., 1998; Suwonsichon and Peleg, 1999). LSF has been used to characterize the melting behaviour of Mozzarella cheese (Casiraghi et al., 1985; Apostolopoulos, 1994; Ak and Gunasekaran, 1995). The test involves compressing the sample in one direction which leads to biaxial extension of the material in perpendicular directions. The ideal conditions include pure extensional deformation which could be achieved by lubricating the contact surfaces. The test can be performed under constant load, constant speed or constant stress conditions (Gunasekaran, & Ak. 2003). Constant speed tests have a drawback as strain rate changes during the test; it is difficult to remove the effect of time from the data. Huc, Mariette and Michon (2014) developed a constant strain rate LSF method on an MCR 301 rheometer (Anton Paar, Graz, Austria) by running the instrument in compression mode using parallel plates and allowing speed to decrease exponentially with time.

A modification of the LSF is the University of Wisconsin (UW) meltmeter which measures the cheese meltability by measuring the change in the height of a sample with time at a given temperature (Wang et al, 1998) (Fig. 2.7). The instrument can be run on its own in constant force mode or can be connected to a Universal testing machine to run in constant deformation rate mode. Under constant load, a sample is melted at the selected temperature and the decrease in height is converted to cross-sectional area of the specimen used for calculation of net stretching stress. Various velocity components, i.e. axial, radial and tangential, are used for deriving the biaxial strain rate using the method described by Dealy (1995) (Wang et al., 1998). Since steady conditions are not met in the UW meltmeter, the resistance of melted cheese to flow is represented as a stress growth coefficient or transient extensional viscosity.

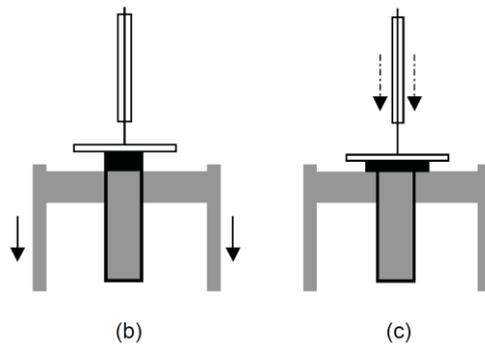
$$\text{Constant force mode} \quad \eta_B^* = \frac{2FH^2(t)}{H_o A_o \left(-\frac{dH(t)}{dt}\right)} \quad \begin{array}{l} \text{(Dealy} \\ \text{(2.8)} \end{array} \quad \begin{array}{l} \text{1995)} \\ \end{array}$$

$$\text{Constant deformation rate} \quad \eta_B^* = \frac{2F(t)(H_o - V_z t)^2}{H_o A_o V_z} \quad \begin{array}{l} \text{(Campanella et al., 1987)} \\ \text{(2.9)} \end{array}$$

Where H_0 height at time zero, A_0 initial cross sectional area of the specimen, F squeezing force, $H(t)$ height at time t , $F(t)$ squeezing force at time t , V_z constant deformation rate.



(a)



(b)

(c)

Figure 2.7 (a) Schematic diagram of UW meltmeter. Most components are made of aluminium. (b) At the start of a test the outer ring moves down (c) Sample position during a test; the circular plate squeezes the sample outwards (Wang et al, 1998 and Gunasekaran and Ak, 2003).

Zhu (2013) measured extensional viscosity of Mozzarella cheese using the UW melt meter at different temperatures. Kuo et al. (2000) conducted uniaxial creep tests on cheddar cheeses of different fat contents using a UW meltmeter with slight modifications. They used a 6-element Kelvin model to analyse creep data and compared it with meltability calculated from height v/s time UW data. The instantaneous slope of the creep curve, defined as the viscoelasticity index (VI), was proposed to predict cheese meltability as it distinguished Cheddar cheeses of various ages and fat contents

(Kuo et al., 2000). They obtained a reasonably strong correlation ($R^2=0.81$) between VI and the meltability (Fig. 2.8).

$$VI = \frac{dJ}{dt} \Big|_{t=0} = \frac{J_1}{\tau_1} + \frac{J_2}{\tau_2} + \frac{1}{\eta_v} \quad (2.10)$$

Where J_1 and J_2 ($1/kPa$), retarded compliances, τ_1 (s) and τ_2 (s), retardation times, η_v ($kPa \cdot s$), Newtonian viscosity

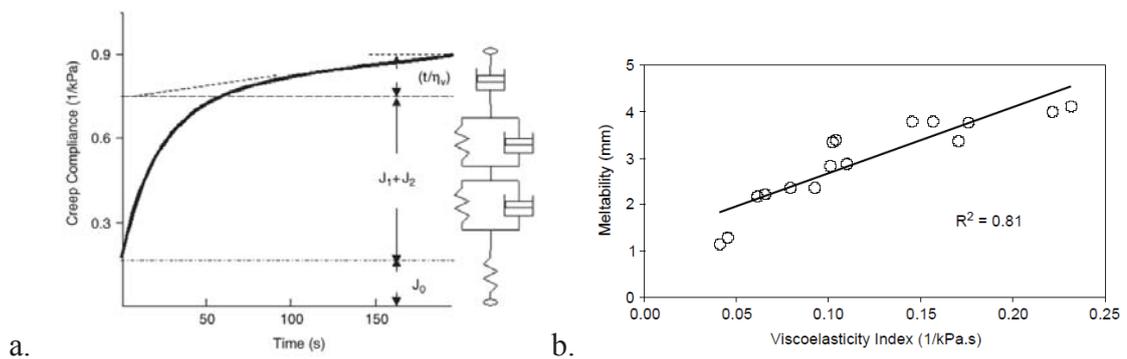


Figure 2.8 (a) Illustration of a typical creep curve showing correspondence to mechanical elements in the six-element Kelvin model (Kuo et al., 2000). (b). Linear relationship between the viscoelastic index and meltability determined by the UW Meltmeter test at 60°C (represented as change in sample height at 1 s after start of flow) for Cheddar cheese (Kuo et al., 2000).

2.11. Problems with measurement of Mozzarella cheese rheology

Because of its complexity, Mozzarella cheese poses significant challenges for rheological measurements. Cheese is very dynamic and undergoes various transitions during the early phases of its production and storage which result in substantial differences in functionality.

In all rheological investigations temperature control of the product is challenging. For example in a rotating parallel plate rheometer, problems arise because of heat loss through the upper metal plate to the environment. A combination of this heat loss and the heterogeneity and anisotropy of cheese structure makes it difficult to achieve steady flow conditions during rheological experiments. During temperature sweep experiments, fat melting and its movement governs the initial rheological behaviour of the material whereas softening and mobilisation of the protein network dominates at higher temperatures. At higher temperatures other structural changes such as increase in

free water content and variations in pore characteristics of the cheese matrix (Vogt, Smith, Seymour, Carr, Golding & Codd, 2015) will also affect rheological measurements. These temperature dependent rheological responses can create non-ideal conditions for material characterization especially during the manufacturing process. The unique process of creating pasta-filata structure by stretching, kneading and moulding in Mozzarella manufacture results in continuous changes in the rheological responses of the molten cheese.

Many of the problems encountered during rheometry arise because of product microstructure. For example, in concentrated pastes and polymer structures fracture inside the sheared sample may be observed and is generally attributed to localisation of shear known as shear banding (Fig 2.9a). Similarly, multiphase systems have a tendency to form a particle depleted low viscosity layer adjacent to rotating surfaces or walls under high shear conditions (Yoshimura et al, 1988; Barnes, 1995). The velocity gradient in the low viscosity zone is higher than in the remaining bulk phase which results in apparent slip. Under slip conditions, the apparent viscosity measured can be significantly lower than the actual viscosity (Yoshimura et al, 1988).

Shear induced structuring (SIS) is a relatively new area of research in polymer science. Molecular structures in the form of emulsions, gels, micelles and pleated sheets have tendencies to orient themselves to minimise free energy. These materials are complex and dynamic in nature especially during their processing and therefore are less predictable and more complex in rheological behaviour. SIS may cause shear thickening or work thickening (or thinning). SIS may also induce anisotropy (direction dependent properties) or may produce relatively stable or stronger intermediate or permanent structures leading to changes in rheological response. These shear generated structures may move at different velocities over each other showing different shear rates (Fig. 2.9a). This phenomenon is known as **shear-banding**. This generates a non-homogeneous flow regime in a conveying or mixing system. Sometime it is also linked to wall slip and apparent slip within the product and also to the edge effects in a parallel plate measuring system (Mezger, 2011). Wall slip is a phenomenon that refers to non-adherence or slip of the fluid at the wall thus violating the classical no-slip boundary condition (Muliawan and Hatzikiriakos, 2008). Patarin et al. (2014) studied the effect of interface adherence conditions on occurrence of wall slip in cheese samples using strain field visualization technique.

In order to minimize wall slip on the stationary wall of a rotating vane rheometer, use of either a slender gauze basket inside the outer cylinder (Barnes and Nguyen, 2001) or lining the inner wall with aluminium foil were found helpful (Yoshimura et al., 1987). Wall slip observed during yield stress measurements is reduced by the use of a vane geometry in rotational mode (Barnes and Nguyen, 2001; Patarin et al., 2014). Nolan et al. (1989) expressed serious concern about slip in dynamic rheological measurements of cheese because of lubrication at contact surfaces with fat melting at elevated temperatures. Nolan et al. (1989), Sutteerawattananonda & Bastian (1998) and Patarin et al. (2014) directly bonded cheese samples to the measurement geometry using cyanoacrylate ester adhesive. Rosenberg et al. (1995) used an upper rough surface to prevent slip in the LVE measurements of Cheddar cheese in oscillatory mode. Serrated plates for both contact surfaces were also used to avoid slip problems (Yun et al., 1994; Sutteerawattananonda and Bastian, 1998; Schenkel, Samudrala and Hinrichs, 2013; Ma et al., 2013). Gluing sand paper on parallel plate surfaces has also been tried successfully to solve the problem of slip (Subramanian and Gunasekaran, 1997a and 1997b; Muliawan, 2008; Patarin et al., 2014).

The use of normal (compression) forces before the rheological measurement is another approach to minimise slip, but, cheese samples should be allowed time for stress relaxation before starting the measurements (Nolan et al., 1989; Diefes et al., 1993; Ma et al., 1996; Mounsey and O’Riordan, 1999; Messens et al., 2000). Occurrence of slip is observed during non-linear viscoelastic measurements of melt polymers above a critical strain. It becomes very difficult in such situations to distinguish the effect of slip from viscoelastic response (Hatzikiriakos and Dealy, 1991). It is almost impossible to completely eliminate wall slip, but the above measures enable reliable rheological data to be obtained at higher shear rates (Ancey, 2005).

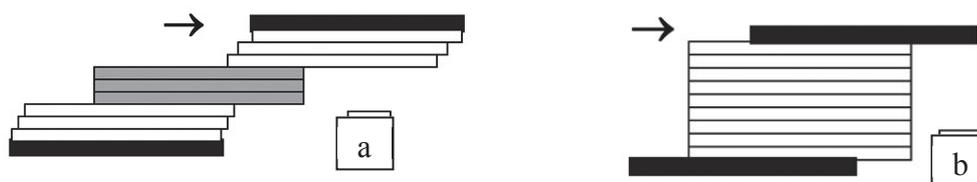


Figure 2.9 Schematic diagram of flow pattern in different flow conditions a. shear banding and b. wall slip (Mezger, 2011)

A schematic diagram of wall-slipped material is shown in fig. 2.9b, where the sample displays very pronounced cohesion while slipping along the walls without much adhesion. End effects also play a very important role during rheological measurements that require necessary correction factors while computing flow curves. End effects arise because of the viscoelastic nature of the material exhibiting compression or expansion of the flow stream near the entry and exit of a capillary. End effects in the length of a flow channel e.g. capillary, the bottom of a couette rheometer or the fluid surface at the peripheral free surface of a rotational parallel plate rheometer may induce significant variations in measured torque or pressure drop (Aucey, 2005).

In capillary rheometry, one of the important assumptions is the absence of wall slip through the die. However, products like cheese and polymers are known to show some slip above a critical shear stress value. Slip at a wall reduces shear rate at that wall. This may require applying appropriate wall slip correction factors such as Mooney's analysis and the Rabinowitsch correction for non-Newtonian fluids. Extrusion capillary rheometers offer an advantage over co-axial rotating rheometers because they can be operated at higher shear rates for highly viscous materials with less slip. However, at very high shear rates various rheological phenomena may be observed with complex materials like cheeses, e.g. flow instabilities, **spurt or stick-slip transitions** or melt fracture, gross extrudate distortion and sharkskin like surface roughness of the extruded mass (Wang and Drda, 1997; Koopmans et al., 2011). These phenomena are believed to arise for various reasons including molecular instability. The occurrence of wall slip during flow of polymer in a pipe may coincide with flow instabilities, extrudate distortion and stick-slip transitions.

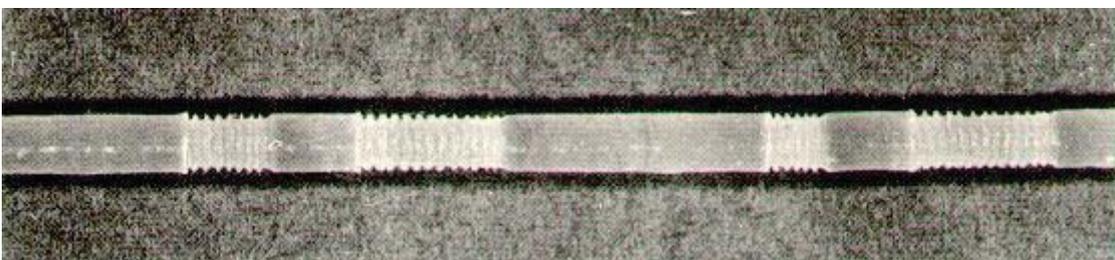


Figure 2.10 Bamboo looking extrudate (stick-slip type) distortion for linear polymer melt (Black, 2000).

Melt flow instability is described as pressure oscillations (sometimes called oscillatory flow) observed upon exit or entry of the polymer from an extrusion capillary above a

critical piston speed. This may result in alternating **bamboo-looking** (Fig. 2.10) smooth and rough surface distortions of the extrudates (Wang and Drda, 1997; Black, 2000). The frequency of pressure fluctuations sometimes may be linked to compression of the viscoelastic fluid under flow conditions. The precise causes and molecular mechanisms associated with distortion of extrudates are yet to be discovered. An attempt was made by Vinogradov and his group in Russia to correlate the periodicity of the stick-slip transition to the frequency of oscillatory measurement where the bulk transition from liquid like (G'') to rubber like (G') behaviour occurred (Vinogradov et al., 1972). Wang and Drda (1997) confirmed that the nature and origin of stick-slip transition was interfacial, rather than constitutive. They demonstrated that stick-slip transitions do not produce distorted extrudate or shear bands as is the case for constitutive instabilities. The starting time of the transition depends upon the critical stress and the temperature. At a molecular level this phenomenon may relate to a coil and stretch transition. Polymer chains close to the interface are adsorbed to the wall and some remaining chains become entangled to the adsorbed layer (Fig. 2.11a). The entangled state is called a **no-slip** hydrodynamic boundary condition (HBC). Above a critical stress, a **stick-slip** condition exists that involves stretching of adsorbed chains and disentanglement of non-adsorbed chains; their recoiling takes place and this creates an imaginary slip layer between the adsorbed and disentangled chains (Fig. 2.11b). The situation between state (a) and state (b) can also be associated with early flow inconsistencies or pressure fluctuations. Further, an increase of stress above a critical value will result in a **slip HBC** because of desorption by weakening adhesion between the wall and adsorbed molecular structures (Fig. 2.11c). The stick-slip transition and its magnitude should have molecular weight dependence. That means the larger chains should have a higher prevalence of stick-slip (Wang and Drda, 1997).

Extrudate distortion is often viewed as a manifestation of the **melt fracture** phenomenon. Melt fracture is considered to be a constitutive phenomenon that occurs because of breakdown of cohesive bonds within the test material. The actual phenomenon takes place inside the capillary but it is convenient to look at the extrudate to measure the effects. As a manifestation of this material failure, the extrudate's surface becomes rough and irregular. This situation may sometimes coincide with the occurrence of a stick-slip transition if the material is less cohesive in nature. Wang and

Drda (1997) tried to distinguish between the theories of melt fracture and extrudate distortion.

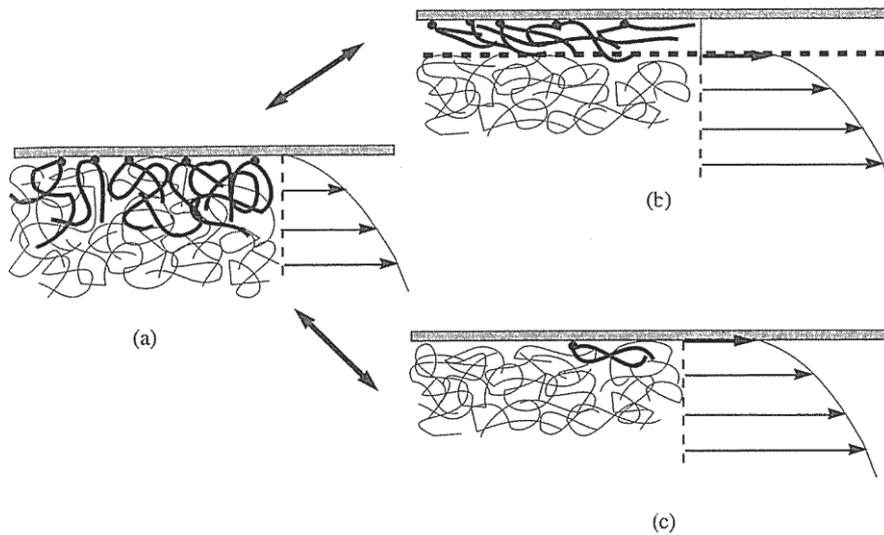


Figure 2.11 Various interfacial states before and after the stick-slip transition in capillary extrusion of polymer a. Entanglement; b. Stretch and disentanglement and; c. Desorption. (Wang and Drda, 1997)

Wang and Drda (1997) proposed that distortion in the extrudate may arise from entry effects to the die rather than from melt fracture. Possible causes of this phenomenon are compression and decompression cycles of polymer melt inside the barrel or an abrupt entry to the die leading to a convergent flow experiencing elongational forces that induce a coil and stretch phenomenon. The entry angle may influence the appearance of extrudate as well (Fig. 2.12). Entry at an angle may result in slower convergence thus allowing deformed coils but a stable flow state demonstrated by smooth extrudate appearance.

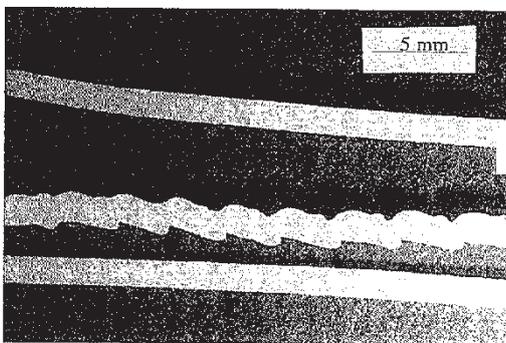


Figure 2.12 Extrudates of HDPE coming out of the same die a. top extrudate prior to stick-slip condition at entry angle 180°;

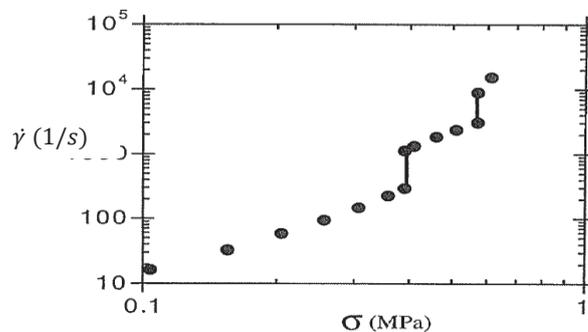


Figure 2.13 Two consecutive slip transitions in the same size of capillary with increase in the shear stress. First

b. middle extrudate above the stick-slip transition recorded as stick-slip type at condition at entry angle 180° ; c. bottom extrudate well above the critical stress in the same die but at 60° angle of entry (Wang and Drda, 1997).

According to Wang and Drda (1997), **melt fracture** is a cohesive failure of the material that is a “stress-induced disentanglement” of polymer bulk chains. Melt fracture can be mapped in rheological terms as a (slip-slip) transitional behaviour and can be regarded as a material characteristic. During post stick-slip transitions, at higher stresses, some material may exhibit another slip, the extent of which is independent of die diameter and results in cohesive gross breakdown of the material (Fig 2.13, 2.14.). Such a condition can be defined as “melt fracture” (Wang and Drda, 1997).

Formation of sharkskin like structures on the surface of extrudates is another type of flow instability that precedes the stick-slip transition and is also known as surface melt fracture. It shows very small amplitude distortions (less than 10% of thickness of the sample) on the surface at relatively lower flow rates or stresses with high molecular weight linear polymers e.g. HDPE (Fig. 2.15a) and LLDPE (Fig. 2.15b) (Wang and Drda, 1997; Black, 2000).



Figure 2.14 Melt fracture or gross distortion of extrudate passing through a die well above its critical stress (Black, 2000).

Wang and Drda (1997) envisaged this as a **local phenomenon** involving both wall slip near the exit of the die and cohesive failure by disentanglement of bulk polymer chains from adsorbed chains at the interfacial wall of the die near the exit (Fig. 2.15c).

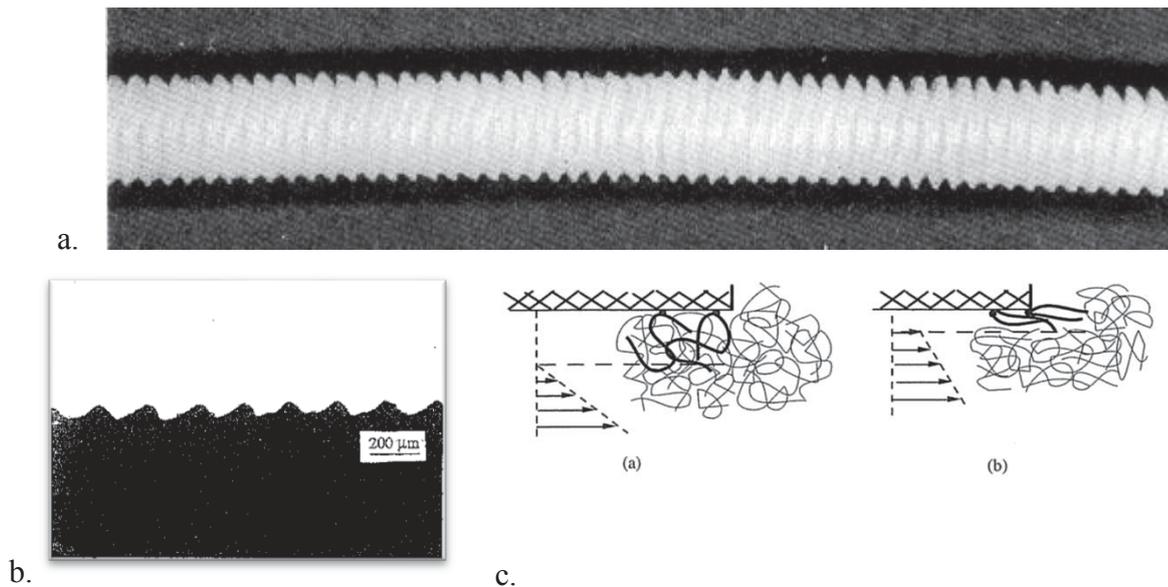


Figure 2.15 Sharkskin (rough surface) formation a. sharkskin extrudate of HDPE (Black, 2000) b. Micrograph of sharkskin extrudate of LLDPE; c. Localised molecular instabilities in sharkskin formation (a) relaxed state of adsorbed chains (b) stretched state of adsorbed chains with slight loss of molecular entanglement with departing chains producing local interfacial slip (Wang and Drda, 1997).

Muliawan and Hatzikiriakos (2008) reported distorted extrudates from capillary extrusion of mozzarella cheese at low temperatures ($<50^{\circ}\text{C}$) even at low shear rates through capillaries of various L/D ratios (15, 30, 40) attached to the bottom of a 9.525 mm wide by 305 mm long barrel (Fig. 2.16). These distortions in Mozzarella cheese extrudates resulted in pressure fluctuations or transients (Bahler and Hinrichs, 2013). At low temperature, Mozzarella cheese tends to behave like a solid material demonstrating fracture during the extrusion process. At higher shear rates and higher temperatures, a probable cause for these pressure fluctuations is protein-fat phase separation (Bahler and Hinrichs, 2013). However, at still higher temperatures (60°C), the extrudates were smooth. Both research groups suggest that the starting point of pressure oscillations is temperature dependent and the extent of the oscillations becomes less if the temperature of the material is maintained above the gel-sol transition point. Muliawan and Hatzikiriakos (2008) further reported that variance of steady state pressure values resulted from heterogeneity of the samples and the nature of the extrusion process. At lower and higher shear rates, Mozzarella cheese samples didn't extrude smoothly but exhibited a stick-slip type of extrusion even at low temperatures. Non-smooth extrudate and pressure fluctuations in capillary flow can be attributed to spurt or stick-slip type melt-fracture (Bahler and Hinrichs, 2013; Muliawan and Hatzikiriakos, 2008)

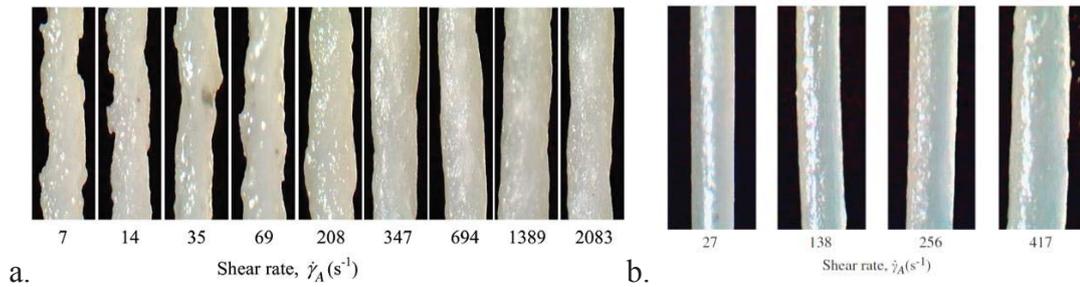


Figure 2.16 Typical extrudate appearance obtained from capillary die extrusion of mozzarella cheese at various apparent shear rates, $\dot{\gamma}_A$, at a. 25°C using capillary die with a diameter of 0.813 mm, L/D of 15, and an entrance angle of 180° b. 60 °C using capillary die with a diameter of 0.711 mm, L/D of 82 and an entrance angle of 180° (Muliawan and Hatzikiriakos, 2008).

2.12. Process-structure-property relationship

Rheology and texture is one external manifestation of the microstructure of a food material. The associations at a molecular level give rise to various types of food structure e.g. suspensions, gels, emulsions and solutions. The molecular structure of ingredients used to formulate the food material play an important role. The microstructural arrangement of food constituents e.g. fat, protein and water leads to rheology and sensory texture. Rheology of food materials depends on the type of molecular structure of ingredients used and the process of manufacture. Timescales and length scales of both factors, i.e. molecular interactions and processing, are different. Food molecules may utilize sub-micron length and sub-millisecond time scales for their associations and arrangements e.g. food proteins. Most food manufacturing processes operate at higher time and length scales and involve hydrodynamic effects e.g. dispersion, mixing. The influence of processing conditions on food microstructure and properties can be measured in terms of rheological response; however, retrieving molecular level information may be a more difficult task. Rheological characterization of food materials is different from polymer rheology because of the complex nature of the food constituents. Length and time scales are dependent on the application, e.g. Mozzarella for block formation or for pizza making. Therefore, the rheology of complex food materials should be assessed considering the individual roles of the food constituents as well as their interactions with the food matrix (Fischer et al., 2009). Based on various structural arrangements of the food constituents, there are three length scales where food rheology can be considered, i.e. phenomenological, flow behaviour and colloidal (Fig 2.17)

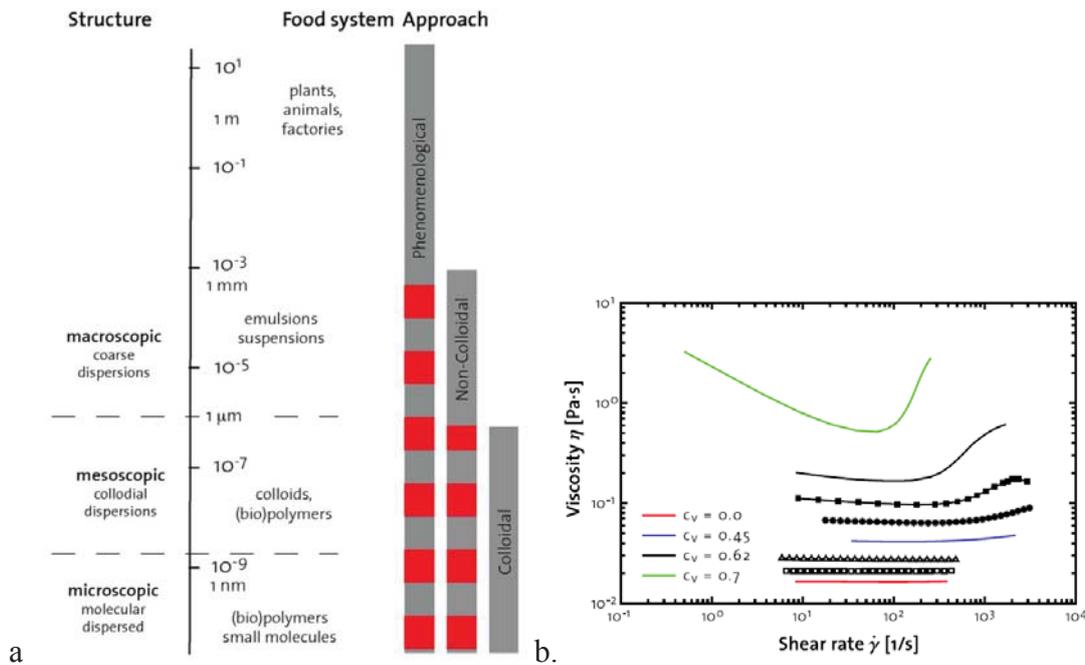


Figure 2.17 a. Length scales relevant for food structures and associated levels of abstraction in food rheology. The shaded areas in the ‘phenomenological’ and ‘non-colloidal’ models are generally not considered in these approaches even though transport processes occurring on those length scales may influence the measured rheological properties. b. Viscosity as a function of shear rate for different total volume concentrations c_v of TiO_2 aggregates in polyethylene glycol. Depending on the aggregate concentration Newtonian, shear thinning and shear thickening can be observed (Fischer et al., 2009)

Phenomenological studies are based on rheological flow models e.g. Cross, power law, Bingham, Herschel Bulkley etc. Macroscopic–level interaction between constituents is expected but the models use basic theories of particle interactions. Concentrated, dispersed food systems are more difficult to characterize as they can exhibit various non-Newtonian phenomena with increase in volume fraction at different shear rates, e.g. shear thinning; shear thickening and sometimes transient responses (Fig 2.17b). More complex and concentrated systems like Mozzarella cheese may demonstrate yield stress, wall slip and shear-induced phase transitions. Such systems cannot be described by phenomenological models over a wide range of shear rates because such models cannot map the structural changes taking place at a molecular and colloidal level during shear. To characterize such complex food systems an empirical approach should be adopted where the applicability of these types of models should be assessed for a given shear application and product composition (Fischer et al., 2009).

Windhab (2009) envisaged rheology as a (length-scale related) representation of dynamic structural (micro to macro level) properties which can be generated, transformed or modified by processing. He proposed a triangular relationship (S-PRO²) between structure-process-properties (Fig. 2.18.). Structuring (S) can take place during processing (PRO) or during product property (PRO) evaluation e.g. in a rheometer or product utilization, (PRO) e. g pizza baking. Altering the process may create a desired structure or rheological response to the finished product to deliver the desired functional property.

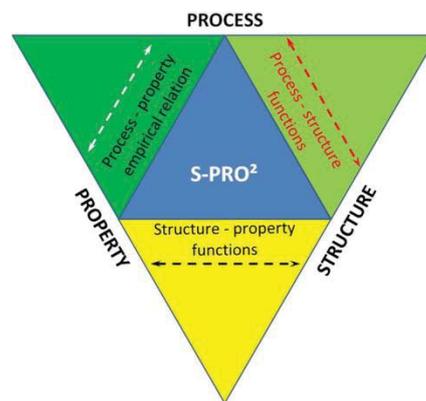


Figure 2.18 Structure-Process-Property relationship proposed by Windhab (2009)

During Mozzarella manufacture, heating and stretching plays an important role in fibrous structure formation (McMahon, Fife, & Oberg, 1999). At early stages, the curd is melted to attain a plastic and workable consistency. This happens above the gel-sol transition temperature of the protein phase. Subsequently, the molten curd is worked, either, in a single or twin screw mixer/worker or in a swept surface mixer known as a Rotatherm (Kindstedt et al., 2010; Bahler, Ruf, Samudrala, Schenkel & Hinrichs, 2015). Stretching of mozzarella curd converts an amorphous 3-D protein network to aligned protein fibres as observed with microscopic techniques (Fig. 2.19) (Taneya, Izutsu, Kimura & Shioya, 1992; Oberg et al., 1993; McMahon et al., 1999; Auty et al., 2001; Lucey, Johnson & Horne, 2003; Yang, 2013). Formation of macroscopic protein fibres is attributed to the nature of fat as it physically hinders fusion of protein strands (McMahon, Paulson and Oberg, 2005). The presence of fat also affects the amount of free water in the cheese matrix (McMahon, Fife, & Oberg, 1999). Mozzarella structure consists of bundles of protein fibres having open channels in between with fat and serum aligned in the direction of the protein fibres. The presence of the fat-serum channels between protein fibers helps to deliver the desired melt functionality for pizza

applications (Rudan & Barbano, 1998) as it helps in moisture and fat migration to the surfaces during pizza baking thus preventing surface drying. These types of serum pockets are also reported in nonfat Mozzarella cheese (Paulson, McMahon and Oberg, 1998; McMahon et al., 1999; Pastarino, Dave, Oberg and McMahon, 2002; McMahon et al. 2005;). Because of this structural alignment of protein fibres as well as fat and serum, Mozzarella cheese is expected to be anisotropic in rheological and mechanical response (Ak and Gunasekaran, 1997; Yang, 2013). However, there is conflicting information available in the literature on anisotropic behaviour of Mozzarella type cheeses (Muliawan, 2008; Olivares et al., 2009). Stretching in the process yields an elastic and springy mass that melts during pizza baking and gives rise to the desirable fibrous, chewy and stringy texture.

Heat induced changes in the structure of pasta filata type cheese has been extensively studied. Pastarino et al. (2002) determined the effect of temperature on structure and opacity of nonfat Mozzarella cheese and linked opacity to the re-association of β -casein and calcium upon heating. Increased size of protein aggregates was suggested to cause more reflectance of light and thus opacity.

Flow structuring has been used in the past to create similar structures to Mozzarella cheese from protein dispersions. Fat filled protein anisotropic structures were created by shearing calcium caseinate-fat dispersions in a shear cell in the presence of the cross linking enzyme transglutaminase (Manski, van der Zalm, van der Goot & Boom, 2008). Manski et al. (2008) showed that shearing led to structural orientation initially and then with a further increase in shear rate resulted in failure of the material. El-Bakry, Duggan, O'Riordan & Sullivan (2011) also reported disappearance of the fibrous character of imitation cheese with prolonged processing time during shearing in a twin screw cooker. Manski, van der Goot and Boom (2007) observed anisotropy at micro and macro scales in sheared and cross linked Ca-caseinate dispersions as determined by SEM and tensile testing. Structural alignment of nonfat cheese was observed by Mizuno and Lucey (2005).

The amount of energy given to the cheese curd mass during stretching can be treated as a critical control variable in attaining the desired functionality (Mulvaney et al., 1997). Energy can be delivered by using various time-temperature-shear combinations. Control of residence time in the cooker/stretcher and the temperature of curd during and at the

end of stretching are also important factors (Kindstedt et al., 2010). Modification of chemical composition is another approach to deliver similar structure and functionality characteristics (Lawrence et al., 1987). The rate and temperature of cooling determines the residual rennet and starter activity, and thus functionality change during storage. As described above, the quantification of structural changes taking place during stretching or working of Mozzarella curd can be done by observing the rheological response of the material throughout the processing. The optimal process time to deliver the right amount of energy to the given mass can be suggested by indicating optimal structuring in terms of rheological signatures.

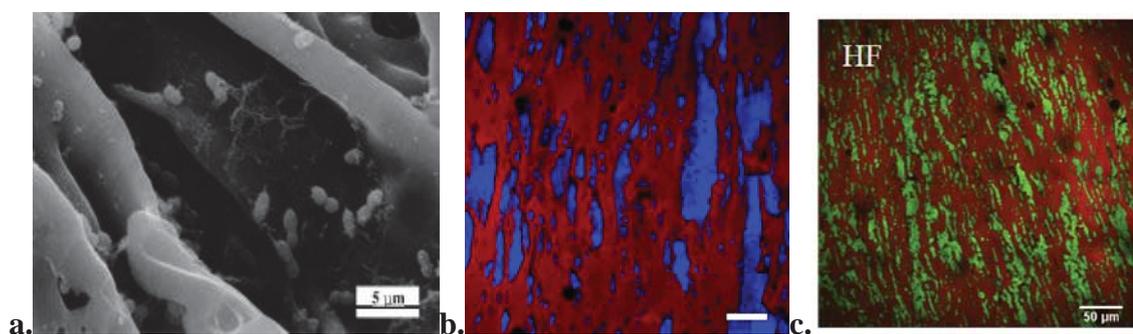


Figure 2.19 a. Scanning electron micrograph of commercial part-skim Mozzarella cheese taken immediately after hot-water stretching. Cheese matrix proteins appear as smooth, elongated fibres separated by serum channels, which contain fat, water, and bacteria. Bacterial cells and residual fat globule membrane material adhere to the fat-serum channel walls (McMahon et al., 1999); b. Confocal micrograph of Mozzarella cheese showing the protein phase as red, the fat phase as blue, and the serum phase as black; scale bar 25 µm (Rowney, et al., 2004) c. confocal image of high fat mozzarella cheese (fat in green, protein in red) (Ma et al., 2013).

The thermo-mechanical treatment received by Mozzarella cheese in a cooker/stretcher is a critical step in delivering the desired functionality to the cheese for pizza application. Mulvaney et al. (1997) made an attempt to control the rheological and functional properties of low moisture, part-skim Mozzarella cheese by manipulating process variables, e.g. mixer screw speed and barrel temperature. Specific mechanical energy input was determined for each screw speed at constant temperature. Initial modulus in stress relaxation experiments was taken as the rheological response variable indicating the elastic properties of cheese (Fig. 2.20a). They suggested including closed loop control systems for specific mechanical energy and melting temperature. The study concluded that it was possible to control rheological and functional properties of Mozzarella cheese by manipulating process variables. In a similar study on low

moisture, part-skim Mozzarella, Yu and Gunasekaran (2005) concluded that mechanical and thermal treatments had a significant effect on the composition and functional properties of cheese (Fig. 2.20b). Optimization of cheese yield was possible and the formation of the characteristic fibrous microstructure was strongly dependent on specific mechanical energy input and temperature of exit of the stretched curd. However, neither study, discussed about the individual effects of barrel temperature and specific mechanical energy input.

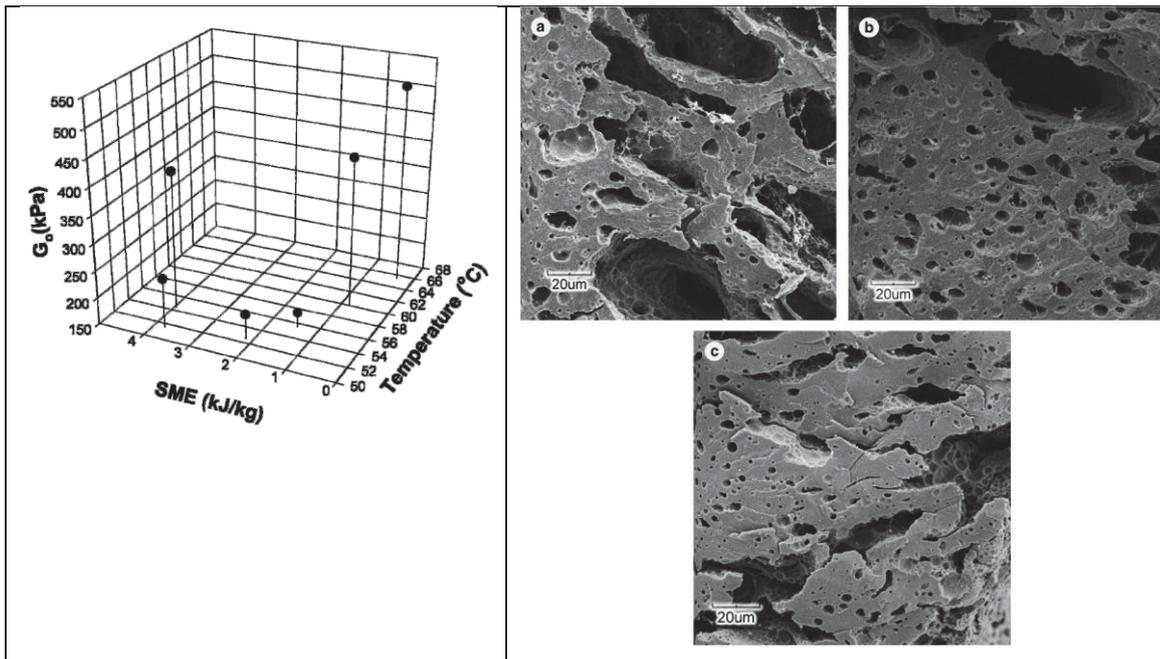


Figure 2.20 a. Initial modulus in stress relaxation (G_0) as a function of specific mechanical energy (SME) and cheese exit temperature (Mulvaney et al., 1997); B. Microstructure of Mozzarella cheese samples obtained at a barrel temperature of 72°C and different screw speeds of (a) 15rpm; (b) 25rpm; and (c) 40rpm (Yu and Gunasekaran, 2005).

2.12.1. Strain hardening and work thickening

Work thickening is defined as increase in mechanical strength of the material upon working. Strain hardening is a phenomenon in which stress required to deform a material increases more than proportional to the strain (at constant strain rate and increasing strain) (Kokelaar, van Vliet & Prins, 1996; Van Vliet, 2008, van Vliet, 2014). Mozzarella cheese is expected to work thicken and may also strain harden. However, to the best of our knowledge neither phenomenon has been reported for Mozzarella cheese or for any other cheese. Strain hardening has been reported for some other dairy protein systems such as fine stranded whey protein isolate gels (Lowe, Foegeding & Daubert,

2003), weak β -lactoglobulin gels (Pouzot, Nicolai, Benyahia & Durand, 2006) and gels formed by acidifying transglutaminase cross-linked casein (Rohm, Ullrich, Schmidt, Lobner & Jaros, 2014).

Like Mozzarella cheese, bread is also a structured food system that consists of air cells dispersed into the gluten network. The analogy between these two products can assist rheological characterization and quality control of both products. The extensional rheology of dough is important in air incorporation and in stabilization of bubbles. Strain hardening produces a rapid increase in the viscosity with increased extensional deformation. This is generally linked to extensional rheology. Formulation and processing conditions are determining factors for structural development. Gluten polymer structure and molecular weight determine the extent of development of extensional strain hardening that stabilizes the foams inside the gluten matrix and is also responsible for dough expansion. Processing conditions, e.g. mixing and kneading of dough, introduce air into the gluten matrix and thus affect the rheology of the dough. Design and operation of the mixer can create different texture and structure in the finished product. The nature of mixing action can also influence the viscoelastic properties of gluten and thus the rheology of bread dough. The rheology in turn affects the time and energy input required for achieving optimal structure development. Thus, modification of mixing conditions can be considered as a critical control variable for optimising the functional properties of bread dough (Dobraszczyk, 2008). A similar approach can be adopted for Mozzarella cheese as it is a dispersion of fat and moisture into a structured 3-D protein network. Stabilization of the product can be achieved by giving the right energy and time inputs during mixing and stretching of the cheese curd. This will yield optimal quality product in terms of the functional attributes e.g. melt, flow and stretch.

In bread dough making providing the optimal work input (mixing speed and energy) to the dough will not only incorporate the right amount of air but also develop stable structure, functionality and baking performance in the bread because of strain hardening (van Vliet et al. 1992; Kokelaar et al. 1996; Dobraszczyk and Morgenstern 2003). However, overworking may lead to breakdown of the structure of the gluten network which in turn may result in functional failure of the material. It would be interesting to investigate such phenomena in Mozzarella cheese because of the different nature of the proteins and their networks. Mixing in food systems can be achieved by two means,

shear flow and elongational flow. Elongational flow mixers are considered more energy efficient because of the fact that extensional flow results in enhanced strain hardening thus more structural development at lower power inputs (Dobraszczyk, 2008). According to polymer theory, molecular size, structure and molecular weight distribution of polymers affects rheology and end use performance greatly (McLeish and Larson, 1998). Above a critical molecular weight, polymer chains start entangling with each other and providing physical constraints. This may result in rapid increases in viscosity, relaxation time and strain hardening with further increases in molecular weight. These effects become more pronounced with branched polymer chains (Dobraszczyk, 2008) (Fig. 2.21).

In comparison with dynamic shear properties, extensional rheological properties are more sensitive to molecular weights, polymer structures and polymer orientations (Mundstedt et al., 1998, Dobraszczyk, 2008). For example, longer and more highly branched polymer chains tend to entangle more, and thus significantly increase the extensional viscosity. On the other hand, branched polymer chains do not increase the shear viscosity of the polymer melt to a great extent (Fig. 2.21).

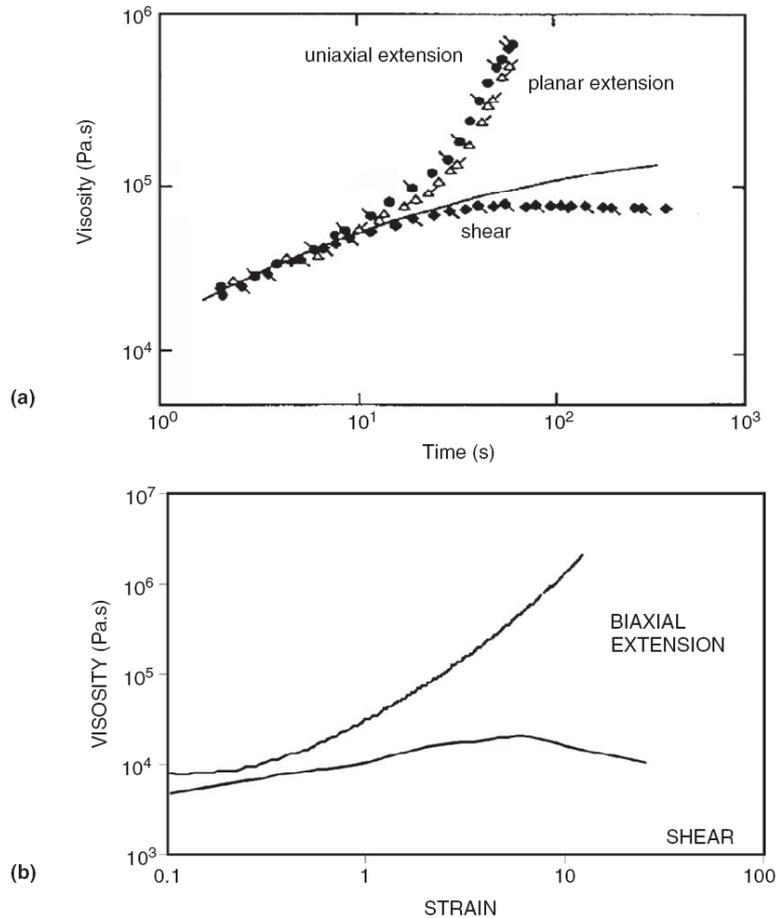


Figure 2.21 Effect of polymer chain branching on shear and extensional viscosities of (a) branched LDPE (low-density polyethylene) melts, and (b) dough. (Dobraszczyk, 2008)

In dough development, the deformation is biaxial, large strain and at low strain rate; by contrast Mozzarella cheese manufacture involves uni or biaxial, large strain at large strain rates during cooking, mixing and stretching (Dobraszczyk and Morgenstern, 2003). For dough rheology appropriate tests are bubble inflation or lubricated squeezing flows. However, for Mozzarella appropriate methods need to be devised which mimic the actual processing conditions. Rheological tests to assess the quality of Mozzarella cheese should be performed at similar conditions to those of processing or application.

2.13. Conclusions

Despite the difficulty in measuring Mozzarella cheese rheology at because of wall slip, structural changes and time dependency during measurements, available literature in polymer rheology indicates that these problems can be overcome if surface modification is done and time dependency is addressed. There are large number of techniques

available in the literature which can be employed on Mozzarella type cheese for small strain and large strain rheology.

The working of cheese influences quality characteristic of finished product. Specific energy input or shear work can be used as a processing variable to control texture and melt quality of Mozzarella type cheese. Structural changes taking place during working of Mozzarella cheese as explained in literature can be correlated well with rheology and functionality in the similar lines to the dough rheology.

3.0 Measurement techniques for steady shear viscosity of Mozzarella-type cheeses at high shear rates and high temperature

Chapter Summary

While measuring steady shear viscosity of Mozzarella-type cheeses in a rotational rheometer at 70°C, three main difficulties were encountered; wall slip, structural failure during measurement and viscoelastic time dependent effects. Serrated plates were the most successful surface modification at eliminating wall slip. However, even with serrated plates shear banding occurred at higher shear rates. Because of the viscoelastic nature of the cheeses, a time dependent viscous response occurred at shear rates $<1 \text{ s}^{-1}$, requiring longer times to attain steady shear conditions. Prolonged continuous shearing altered the structure of the molten cheeses. The effects of structural change were greatly reduced by minimising the total accumulated strain exerted on the sample during flow curve determination. These techniques enabled successful measurement of steady shear viscosity of molten Mozzarella-type cheeses at 70°C at shear rates up to 250 s^{-1} .

3.1. Introduction

Accurate measurement of the rheological properties of food materials is important for equipment design, product development, quality control and process modelling. Mozzarella cheese is rheologically complex over its processing and consumption conditions because it is viscoelastic, exhibiting varying amounts of solid- and liquid-like character depending upon temperature, rate of deformation and extent of working during manufacture. Many studies have been reported on small angle oscillatory shear measurements on Mozzarella-like cheeses (Tunick et al., 1993; Hsieh, Yun, & Rao, 1993; Ak & Gunasekaran, 1996; Subramanian & Gunasekaran 1997a; Guinee, Feeney, Auty & Fox 2002; Venugopal & Muthukumarappan, 2003; Karoui, Laguet & Dufour, 2003; Joshi, Muthukumarappan & Dave, 2004; Rock et al., 2005; Udayarajan, Horne & Lucey, 2007; Hussain et al., 2012; Ma, Balaban, Zhang, Emanuelsson-Patterson & James, 2014) as it is relatively easy to perform such experiments on rotational rheometers. However, there are fewer reports on steady shear viscosity measurements on such cheeses with rotational rheometers because of the difficulty in performing steady shear experiments (Lee, Imoto & Rha, 1978; Ruegg, Eberhard, Popplewell & Peleg, 1991; Guinee & O' Callaghan 1997; Yu & Gunasekaran, 2001). Most of the

steady shear reports were conducted using empirical methods or devices, and did not produce data at shear rates $>10 \text{ s}^{-1}$. Capillary rheometers have been used successfully to achieve higher shear rates but various flow instabilities were noted during their use (Smith, Rosenau & Peleg, 1980; Cavella, Chemin, & Masi, 1992; Taneya, Izutsu, Kimura & Shioya, 1992; Muliawan & Hatzikiriakos, 2008; Bahler & Hinrichs, 2013).

A study on the cooking/stretching stage in Mozzarella manufacture was also commenced as it is poorly understood. The deformation regime in rotational rheometers is closer to the processing conditions in this process stage than that in capillary rheometers. Reported average shear rates during cooking/stretching vary from $\sim 40 \text{ s}^{-1}$ for a batch pilot-scale twin screw cooker (Glenn & Daubert, 2003; Glenn, Daubert, Farkas & Stefanski, 2003) to $70\text{-}150 \text{ s}^{-1}$ for a laboratory scale, single impeller mixing device (Lai, Steffe & Ng, 2000; Kapoor, Lehtola & Metzger, 2004). We estimate maximum shear rates between the screw tip and the wall in a batch pilot-scale Blentech cooker to be about 200 s^{-1} . It is therefore useful to determine shear viscosity of molten cheese at higher shear rates.

The presence of a no-slip condition at the wall is an important pre-requisite in accurately measuring steady shear viscosity. In the case of Mozzarella-like cheeses in the molten state, liquid fat at the cheese surface starts lubricating the wall (Ruegg et al., 1991; Muliawan & Hatzikiriakos, 2008). This lubrication violates the classical no-slip boundary condition, leading to erroneous viscosity data (Yoshimura & Prud'homme, 1988) particularly at shear rates $> 10 \text{ s}^{-1}$.

A rheologically complex material such as Mozzarella cheese exhibits time dependency arising from two separate phenomena: 1. The viscoelastic nature of the material which is important at low shear rates ($<1 \text{ s}^{-1}$) and 2. Structural change as a result of shearing which is important after prolonged shearing at higher shear rates (Steffe, 1996; van Vliet, 2014). We use the term viscoelastic time dependency to refer to the first and structural change to refer to the second. Because of these difficulties of wall slip and time dependency, a limited amount of work has been conducted on steady shear rheology of Mozzarella cheese.

It is desirable to have a method that takes account of the viscoelastic time dependency and measures the viscosity before significant structural change has occurred. The main aim of this study was to develop such a method that is suitable at higher shear rates and

higher temperatures. A secondary aim was to understand the physical phenomena that occur during shear viscosity measurement as these same phenomena will also occur in processing equipment that imparts shear.

3.2. Materials and Methods

3.2.1. Materials

Samples of commercial Mozzarella cheese, a model Mozzarella cheese and renneted casein gel were obtained as frozen blocks from Fonterra Co-operative Group Limited, Palmerston North, New Zealand. Model Mozzarella cheese was prepared by mixing and working renneted casein gel, cream, water and salt at 70 °C in a twin screw batch cooker (Blentech, model CC-0045, Blentech Corporation, Rohnert Park, CA, USA). Renneted casein gel was a dewatered, renneted and acidified curd made from skim milk. The compositions of the cheeses were determined by the Analytical Services Group of Fonterra Research and Development Centre (Table 3.1). Each cheese block was thawed at 4 °C for at least 24 h before use in experiments. Cheese cylinders of 20 mm diameter were drawn from a cheese block using a cork borer. Discs 2-3 mm thick were cut from the cheese cylinder using a wire cutter. Cheese discs were wrapped in food wrap to prevent moisture loss and stored at 4 °C.

Table 3.1 Composition of Experimental Cheeses

	Model Mozzarella	Commercial Mozzarella	Renneted Casein Gel
Moisture (g 100 g ⁻¹)	53.9	48.7	56.1
Fat (g 100 g ⁻¹)	22.2	22.4	0.2
Protein (g 100 g ⁻¹)	22.3	24.7	41.6
Salt (NaCl) (g 100 g ⁻¹)	1.13	1.25	-
pH	5.5	5.4	5.6

3.2.2. Rheological properties

Initial experiments (as noted in figure legends) were conducted on a stress controlled AR-G2 rheometer (TA Instruments, New Castle, DE, USA) using parallel plate geometry (diameter 20 mm). Unless otherwise noted in figure legends, all other

rheological measurements were conducted on a MCR 301 rheometer (Anton Paar, Graz, Austria) using a Peltier temperature hood (H-PTD 200), a 20 mm serrated parallel plate geometry and using the following conditions.

Cheese discs were equilibrated to room temperature (21 °C) for at least 30 min and then placed between the parallel plates of the rheometer. To ensure good rheometer/sample contact the measurement gap was set by closing the gap at room temperature until the normal force was 5 N. While closing the gap, the velocity of the rheometer moving head was 50 $\mu\text{m/s}$. The sample was then heated to 70 °C using the in-built Peltier heating system for both the bottom plate and the upper temperature hood. The sample was then held at 70 °C for 2 min to ensure isothermal conditions and to allow some stress relaxation. To avoid drying soybean oil was applied around the edges of the cheese disc. All rheological measurements were performed at 70 °C. Oscillatory rheological measurements on renneted casein gel were conducted in the linear viscoelastic range using 1 Hz frequency and 0.5% amplitude at 70 °C.

Temperature gradients across the samples were explored using a temperature probe (Q1437 digital thermometer, Dick Smith Electronics, Auckland, New Zealand) and a high viscosity standard oil (Viscosity reference standard N4000, Cannon Instrument Company, State College, PA, USA).

3.2.3. Image acquisition to illustrate wall slip

To visualize wall slip a digital camera, Canon EOS 650D (Canon, Tokyo, Japan), was used in video mode. The camera was operated remotely by computer using software EOS digital version 25.2 (Canon, Tokyo, Japan). The camera was fixed at the same height as the rotating plate and sample and was able to capture the wall slip event. A reference mark was drawn vertically on the sample and upper plate. The sample was heated to 55 °C using the in-built Peltier heating system. The rheometer was run at a shear rate of 0.04 s^{-1} . A video-clip was captured at 50 frames s^{-1} and 1280 x 720 pixels resolution for 69 s. Still images were extracted from the video using the software Windows Live™ Movie Maker (Microsoft Corporation, Redmond, WA, USA).

3.2.4. Environmental scanning electron microscopy

To explore the effect of shearing on microstructure, environmental scanning electron microscopy (ESEM) was conducted on cheese samples obtained before and after

shearing in the rheometer. For the unsheared sample, a specimen was cut assuming random protein fibre orientation. However, for the sheared fibrous-looking samples, protein fibre orientation was assumed to be along the length of the sample and the specimen was cut in the longitudinal direction. Specimens were cut with dimensions 4x4x1 mm. ESEM was conducted in a variable pressure FEI Quanta 200F scanning electron microscope (FEI, Hillsboro, OR, USA) equipped with a Schottky field emission gun and a Peltier cooling stage in environmental mode. Water vapour (imaging gas) was used as a gas medium for secondary electron signal amplification. The chamber was pumped for four to five cycles with minimum pressure 3.2 Torr and maximum pressure 7 Torr to stabilise the water vapour pressure. A spot size of 3 mm, accelerating voltage of 10 kV and working distance of approximately 5-6 mm were used. In order to ensure wetness of the sample, the relative humidity of the chamber was maintained at 60% by controlling pressure at 3.2 Torr and temperature at 2.0 °C.

3.3. Results and Discussion

3.3.1. Wall slip and shear banding

In an initial attempt to determine a flow curve of model Mozzarella cheese at 70 °C using smooth plate geometry, a flow discontinuity was observed, evidenced by a sudden drop in apparent viscosity and shear stress at shear rates $\sim 5 \text{ s}^{-1}$ (Fig. 3.1). Yu and Gunasekaran (2001) reported a similar drop in viscosity in the shear rate range 2-5 s^{-1} while measuring steady shear viscosity of Mozzarella cheese at 60 °C. For Mozzarella-like cheeses at temperatures above 30 °C, the fat will be molten and so the cheese surface will tend to be slippery (Ruegg et al., 1991; Muliawan & Hatzikiriakos, 2008). The molten fat and slippery surfaces are likely to cause loss of grip resulting in early wall slip.

To confirm the hypothesis that molten fat was causing wall slip, a movie was filmed using a high resolution camera (Fig. 3.2). Even at the very low shear rate of 0.04 s^{-1} , the images show that the mark at the top of the cheese became displaced from that on the top plate by 35 s and was progressively displaced further after 45 s and 55 s. This displacement was a clear visual indication of wall slip.

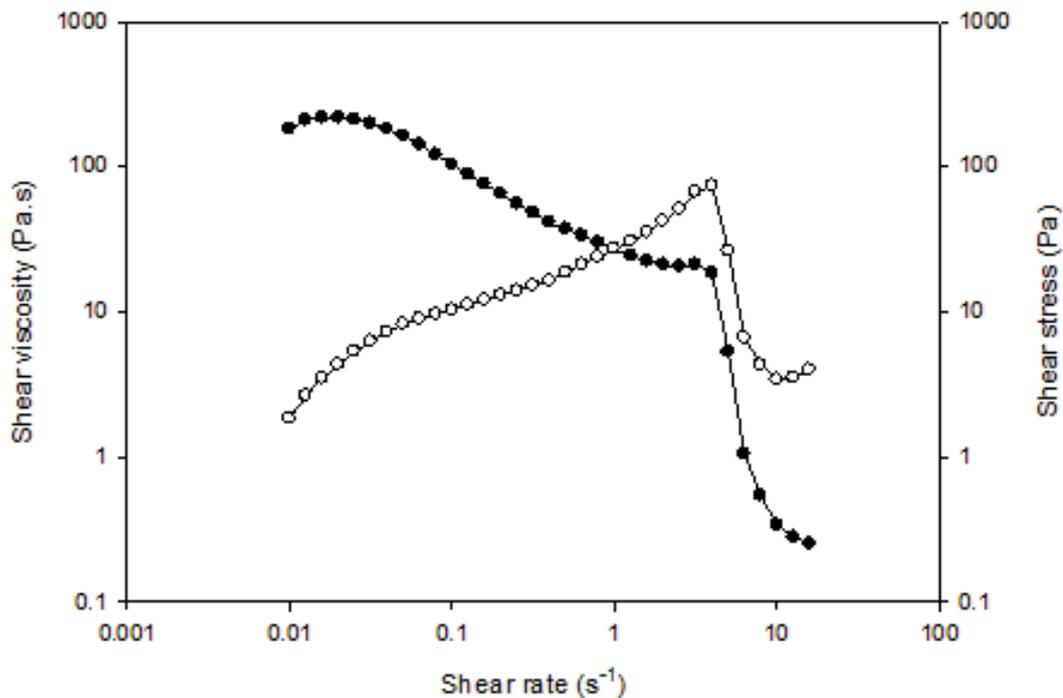


Figure 3.1 Flow curves of model Mozzarella cheese showing wall slip obtained in the AR-G2 (TA Instruments) rheometer using smooth plates. Shear viscosity (●), Shear stress (○).

From the polymer literature, the picture of wall slip is that of accumulated strain/stress building up in polymeric chains, reaching a critical value and eventually leading to permanent detachment from the interface. The molten fat in Mozzarella cheese may have worsened this situation. For an isothermal sample of a Newtonian liquid or Hookean solid the marker line would be expected to be linear. We suggest the curve formed with little distortion near the upper plate (Fig. 3.2) is caused by a temperature gradient in the sample from 55 °C on the lower Peltier plate to a lower temperature at the upper plate.

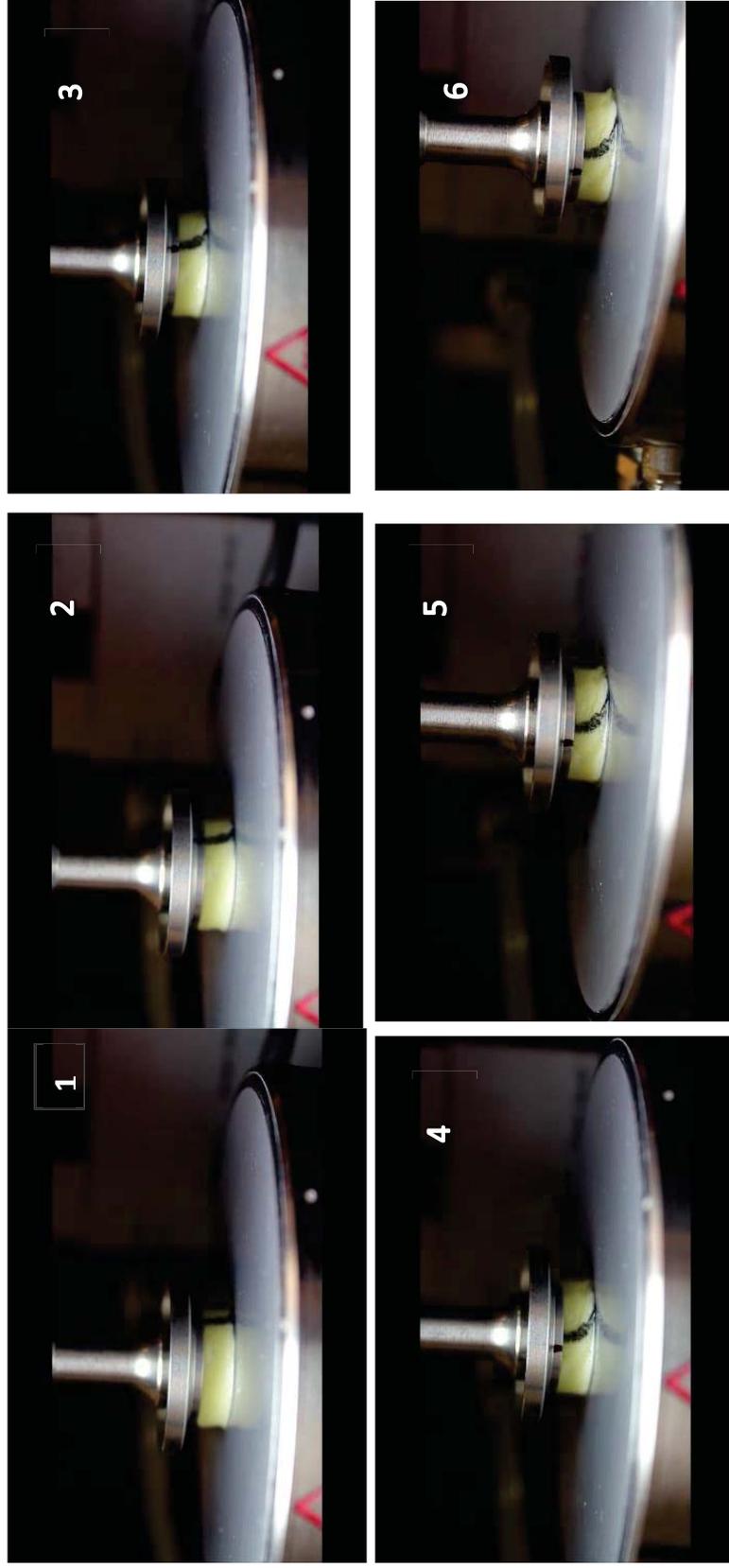


Figure 3.2 Visualization of wall slip of model Mozzarella cheese at 55 °C using smooth parallel plates on the AR-G2 (TA Instruments) rheometer at 0.04 s⁻¹ shear rate. The black marker line on the cheese becomes displaced from that on the upper plate suggesting wall slip. The images were taken at 0, 10, 25, 35, 45 and 55 s from the start of shearing.

We modified the cheese contact surfaces in an attempt to eliminate or minimize the wall slip effect. The best results were obtained with serrated plates, followed by sandpaper and then sandblasted plates (Fig. 3.3). The viscosity values with the serrated plates (Fig. 3.3) are about 5 times those with smooth plates (Fig. 3.1) even at low shear rates indicating the large effect of wall slip on the results. Flow discontinuities were still observed at shear rates $> 100 \text{ s}^{-1}$, so surface modification has just changed the location of the apparent slip or sample fracture from the walls to within the material. Patarin, Galliard, Magnin and Goldschmidt (2014) described similar behaviour as macroscopic failure or fracture when a cheese sample having good contact with upper rotating and bottom stationary plates was sheared in a rheometer. This apparent slip or fracture within the sample is referred to as shear banding, shear localisation or melt fracture in the polymer literature (Ancy, 2005).

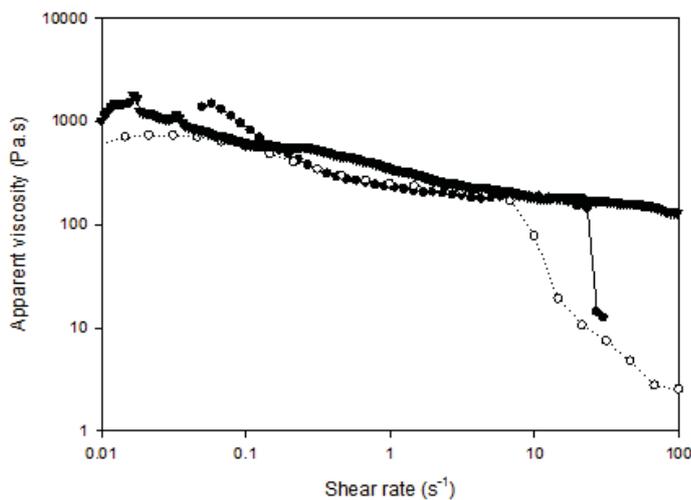


Figure 3.3 Effect of surface modification of the rotating parallel plates on the flow curves of model Mozzarella cheese at $70 \text{ }^{\circ}\text{C}$. Experiments used 40 grit sand paper (\bullet) on the AR-G2 (TA Instruments) rheometer and sandblasted plates (\circ) or serrated plates (∇) on the MCR301 (Anton Paar) rheometer.

A temperature gradient across the samples was observed while conducting tests on the AR-G2 rheometer. When the temperature of the Peltier bottom plate was $70 \text{ }^{\circ}\text{C}$ a temperature gradient of $\sim 5 \text{ }^{\circ}\text{C}$ was recorded across the sample with the temperature probe. The high viscosity standard oil showed that the measured viscosity was accurate

at 20 °C but high at 70 °C indicating that the average sample temperature was lower than the 70 °C set temperature.

3.3.2. *Transient viscoelastic effects and measurement duration*

At low shear rates ($<0.1 \text{ s}^{-1}$) and 70 °C, model Mozzarella cheese exhibited a transient viscosity peak, a localized maximum of viscosity on the flow curve (Fig. 3.3). Viscoelastic materials exhibit non-steady state flow conditions at low shear rates ($<1 \text{ s}^{-1}$) if the timescale of deformation is too small. This effect is related to the slow rate of stress dissipation within the material resulting in slow development of steady flow conditions. In the literature, these effects are termed start-up effects or time-dependent transition effects (Mezger, 2011; van Vliet, 2014).

The apparent viscosity of Mozzarella cheese at 0.01 s^{-1} and 70 °C was found to be time dependent (Fig. 3.4). Viscosity increased with measurement time and eventually reached a relatively constant steady state value at around 100 s. At a higher shear rate (10 s^{-1}) apparent viscosity attained a constant value in less than 2 s.

Mezger (2011) proposed a rule of thumb that measurement duration at each point should be at least as long as the reciprocal of shear rate, i.e. $t > 1/\dot{\gamma}$. Fig. 3.4 agrees with this rule of thumb. Attaining steady shear conditions at each shear rate step is important for obtaining an accurate flow curve for viscoelastic materials such as cheese. Van Vliet (2014) provides an excellent description of the role of time scale in food rheology including cheese examples. The duration of shear rate application plays a vital role in the stress response of the material. Reaction to applied stress is nearly instantaneous for a rigid elastic material but is time-dependent for soft solids (van Vliet, 2014; Malkin, 2013). For viscoelastic materials time dependency is related to the disruption and reformation of molecular interactions and to the spectrum of relaxation times of these processes (van Vliet, 2014).

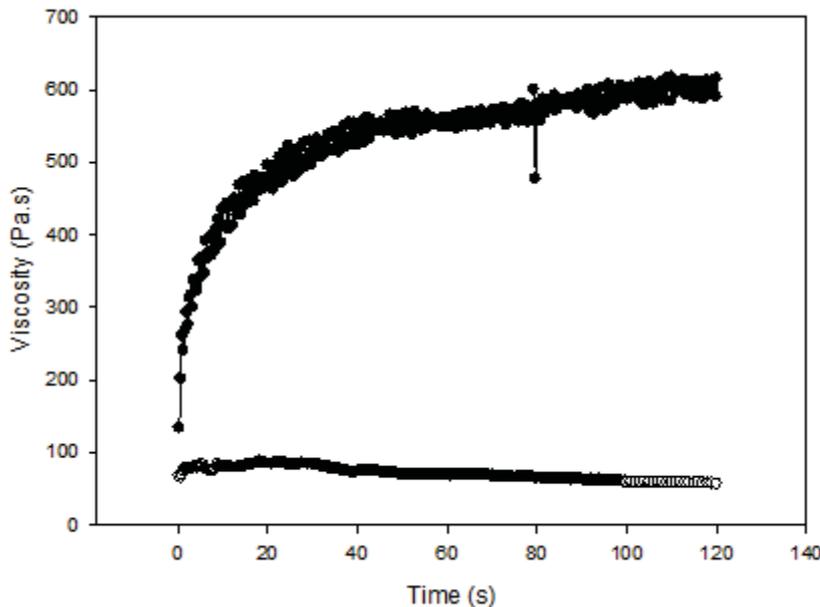


Figure 3.4 Transient start-up effects on shear viscosity of commercial Mozzarella cheese at constant shear rates 0.01 s^{-1} (\bullet) and 10 s^{-1} (\circ) and at $70 \text{ }^\circ\text{C}$.

3.3.3. Structural changes/failure during shearing

Continuous shearing of Mozzarella cheese at higher shear rates eventually resulted in structural failure and expulsion of some of the sample from the rheometer measurement gap in the form of a thick strand (diameter about the same as the measurement gap) with aligned protein fibres. The unsheared sample had a random ESEM structure (Fig. 3.5). On the other hand, the sheared sample exhibited alignment of the protein and fat structure, presumably in the direction of shearing. These observations plus observations on model Mozzarella cheese manufactured in pilot-scale equipment led to the conclusion that shearing of Mozzarella-type cheese led to changes in the structure of the material. Similar observations were reported by Manski (2007), who created fat filled protein structures by shearing calcium caseinate-fat dispersions and showed that shearing led to structural orientation initially and then with a further increase in shear rate also resulted in failure of the material.

Steady-state viscosity measurement for Mozzarella cheeses therefore changes the structure of the cheese thus changing the viscosity that we are trying to measure. Although viscosity measurements can be used as probing tools for changes in structure we wish to measure steady shear viscosity before any significant structural change has occurred.

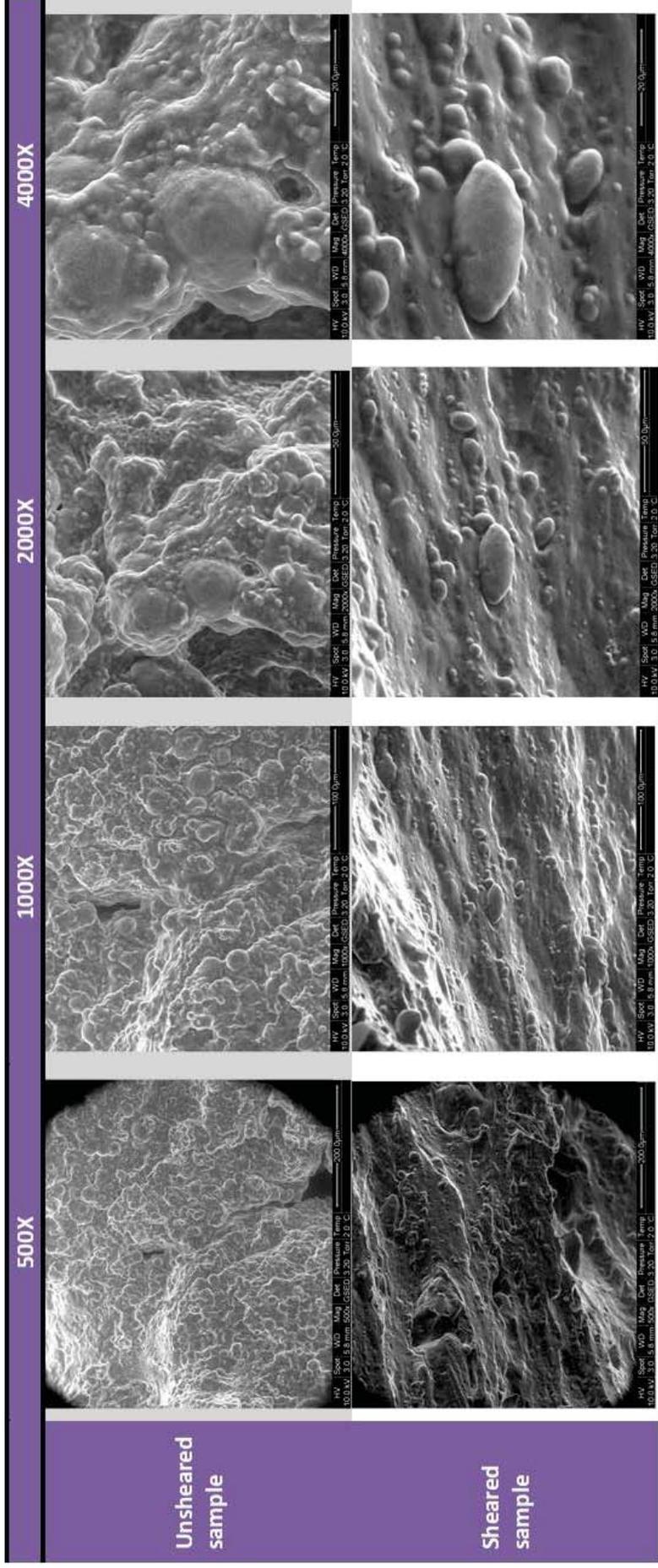


Figure 3.5 ESEM images of unsheared model Mozzarella cheese (upper row) and of a thick strand of sheared cheese that had come out from the measurement gap of the rheometer after shearing for 3529 strain units (bottom row). Globular structures represent fat globules.

3.3.4. Optimum flow curve

One way to limit structural changes during rheological measurement is to minimize shearing of the sample during the measurement. Fig. 3.6 indicates that this strategy was certainly an improvement. The default shear rate settings for flow curve determination resulted in an early breakdown of the flow curve at a shear rate near 10 s^{-1} . The flow curve with only 5 shear rate steps with shorter measurement durations resulted in successful measurement of shear viscosity up to a shear rate of 150 s^{-1} . The power law model fitted the data well ($R^2=0.998$). The practical limit of the method appeared to be about 150 s^{-1} with the chosen steps as only one reliable data point was obtained at 150 s^{-1} from the triplicate runs.

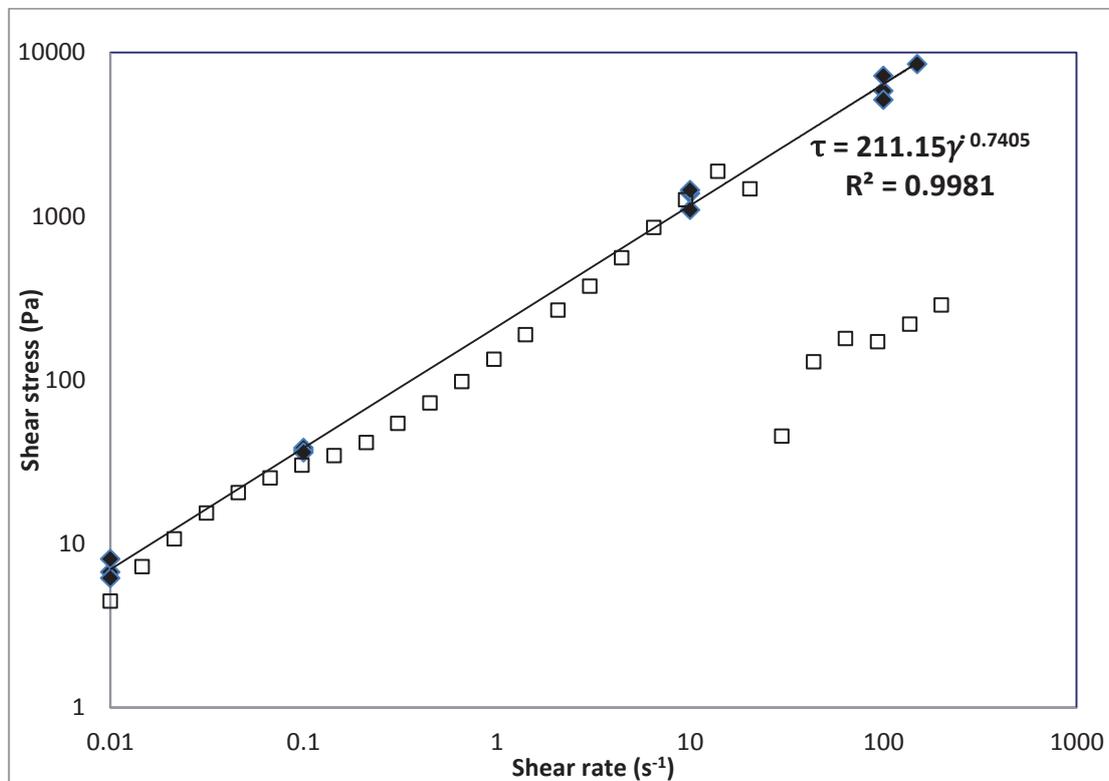


Figure 3.6 Two flow curves for commercial Mozzarella cheese using different shear rate sequences: continuous flow curve using default settings of the Anton Paar MCR301 to give uniform spacings on the log shear rate axis (\square) and flow curve using only five selected shear rates (\blacklozenge). For the default settings measurement duration decreased at a logarithmic rate from 25 s to 2 s as the shear rate increased from 0.01 to 200 s^{-1} accumulating 1572 total strain units. The selected shear rates were 0.01 , 0.1 , 10 , 100 and 150 s^{-1} for measurement durations of 100, 25, 5, 0.1 and 0.05 s respectively accumulating only 71 total strain units. For clarity only one flow curve is shown using the default settings. The flow curve with selected shear rates was performed in triplicate.

To further check the robustness of the method two different shear step series with lower accumulated strain units were attempted on commercial Mozzarella cheese. The data obtained from both series also fitted very well to the power law model ($R^2=0.998$) and the flow curves for the two series were virtually identical (Fig. 3.7). The maximum shear rate achieved was 250 s^{-1} . Thus, a smooth flow curve up to 250 s^{-1} was obtained for Mozzarella cheese by allowing longer measurement durations at low shear rates to avoid transient viscoelastic effects and selecting only a few shear rate steps in order to limit total accumulated shear strain (<50 strain units).

Values of the flow behaviour index for commercial Mozzarella cheese were similar (~ 0.74) in figures 3.6 and 3.7 indicating similar moderate shear thinning behaviour. However, the consistency coefficient shown in Fig. 3.7 is lower ($\sim 122 \text{ Pa}\cdot\text{s}^n$) than that in figure 6 ($\sim 211 \text{ Pa}\cdot\text{s}^n$). Fig. 3.7 was performed on the same material as Fig. 3.6 but after storage at 4°C for two weeks. The lower consistency coefficient in Fig. 3.7 was possibly attributed to softening caused by proteolysis during storage of the cheese at 4°C . The effect of proteolysis on softening of Mozzarella cheese is well reported (Metzger, Barbano & Kindstedt, 2001; Guinee *et al.*, 2002; Kindstedt, Hillier & Mayes, 2010).

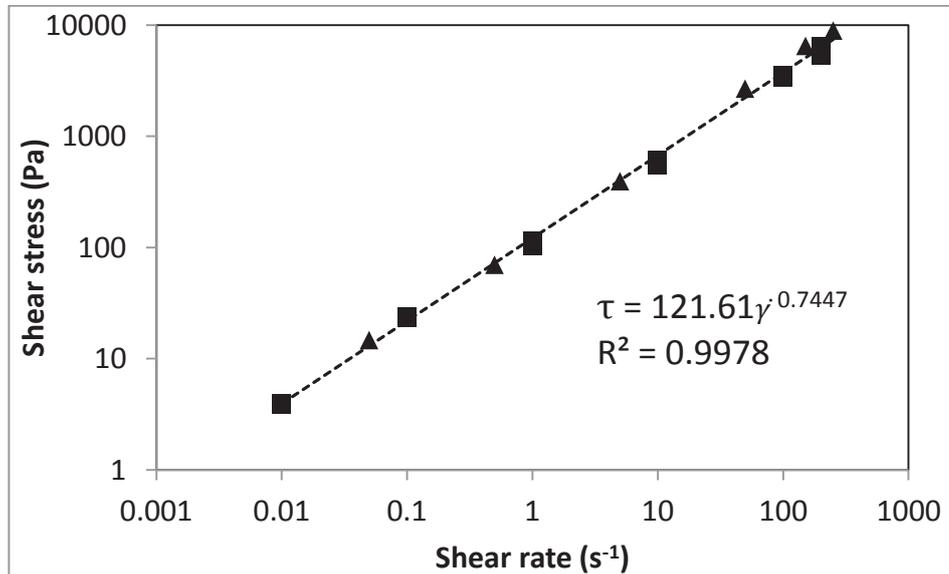


Figure 3.7 Two flow curves of commercial Mozzarella cheese using different series of shear rate steps; Series 1: 0.01-0.1-1-10-100-200 s^{-1} (■) with measurement durations of 100, 12.5, 5, 0.05, 0.05, 0.05 s respectively, performed in duplicate; Series 2: 0.05-0.5-5-50-150-250 s^{-1} (▲) with measurement durations of 50, 6.25, 2.5, 0.05, 0.05, 0.05 s respectively. The dotted line represents the power law regression model fitted on the pooled series. Total accumulated strain was 23 units for series 1 and 41 units for series 2.

Rheological data obtained by Muliawan & Hatzikiriakos (2007) for Mozzarella cheese at 25 °C using both capillary and sliding plate rheometers were described by the Herschel-Bulkley model (i.e. Power law with yield stress) with a higher consistency coefficient ($K=3.34 \text{ kPa}\cdot\text{s}^n$), a lower flow behaviour index ($n=0.25$) and a yield stress (1.93 kPa). Muliawan & Hatzikiriakos (2007) reported the absence of a yield stress above 60 °C and suggested this was because of complete melting of the protein structure and hence easier initial flow of the cheese. The fit of data to the power law model therefore agrees with Muliawan & Hatzikiriakos (2007) but absolute values of the model parameters were different because of our higher test temperature.

3.3.5. Flow properties of molten renneted casein gel

Some experiments were conducted with molten renneted casein gel, effectively a fat-free, low salt Mozzarella cheese, to explore the role of the protein phase in the flow instabilities such as wall slip or structural failure. Fig. 3.8 indicates a discontinuity occurred in the flow curve for molten renneted casein gel at around 25 s^{-1} with a sudden decrease in viscosity. Fat is absent here so the occurrence of flow instability suggests breakdown of protein structures upon shearing rather than wall slip.

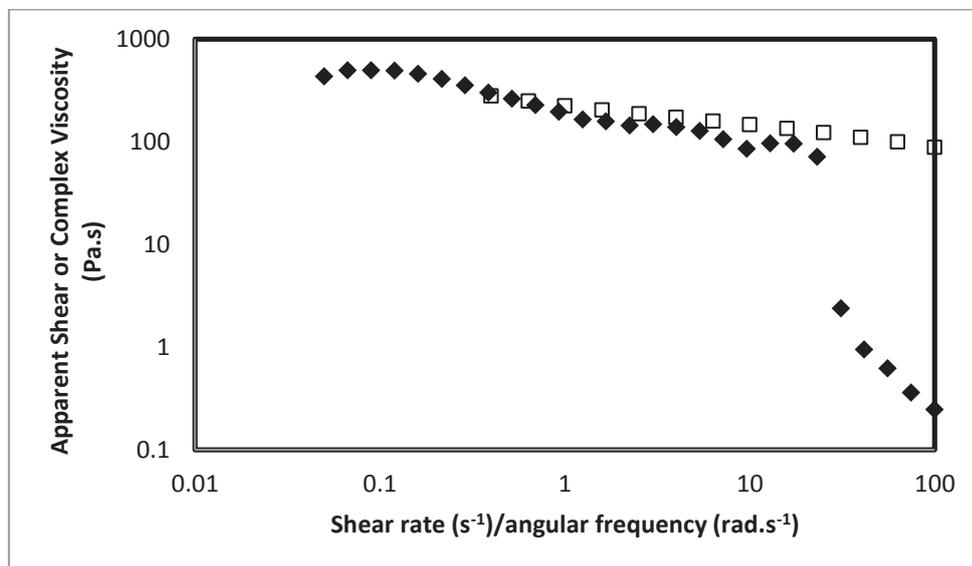


Figure 3.8 Shear viscosity, η (◆) and complex viscosity, $|\eta^*|$ (□) as a function of shear rate and angular frequency to explore the applicability of the Cox-Merz rule to molten renneted casein gel. For oscillatory measurements the strain amplitude was 0.5%.

3.3.6. Applicability of the Cox-Merz rule

The Cox-Merz rule is an empirical rule that seeks to relate oscillatory rheological data to steady shear data. The Cox-Merz rule is represented by following equation:

$$\eta(\dot{\gamma}) = |\eta^*(\omega)| = \frac{G''}{\omega} \sqrt{1 + \left(\frac{G'}{G''}\right)^2} \Bigg|_{\omega = \dot{\gamma}} \quad (3.1)$$

Where, $\eta(\dot{\gamma})$ is shear viscosity in Pa.s, $\eta^*(\omega)$ complex viscosity in Pa.s, ω rotational speed in rad.s^{-1} , G' , storage modulus and G'' loss modulus in Pa.

Reasonably good agreement was observed between complex viscosity and shear viscosity with our data almost superimposing over the shear rate range $0.01\text{-}25 \text{ s}^{-1}$ (Fig. 3.8). This agreement suggested that the Cox-Merz rule was applicable to renneted casein gel and also suggested the possible use of oscillatory data to estimate shear viscosity at higher shear rates beyond which wall slip or structural breakdown would have occurred in rotational steady shear mode. Muliawan & Hatziriakos (2007) compared complex and shear viscosities of Mozzarella cheese at various temperatures from $25 \text{ }^\circ\text{C}$ to $60 \text{ }^\circ\text{C}$ and reported poor agreement at temperatures up to $50 \text{ }^\circ\text{C}$ suggesting non-compliance to the Cox-Merz rule. They suggested this lack of agreement was caused by the solid-like structure and by the presence of a yield stress at temperatures of $50 \text{ }^\circ\text{C}$ and below. However, at $60 \text{ }^\circ\text{C}$ or above where the cheese is more molten Muliawan & Hatziriakos (2007) reported agreement between oscillatory and steady shear data in agreement with Fig. 3.8.

3.3.7. Structural origins of rheological behaviour

Flow instabilities have been widely reported and discussed in the polymer melt rheology literature. Two of the most common terms used for structural failure of the material during rheological measurement are shear banding and melt fracture. Entangled polymeric chains or aggregated gel networks both show shear banding in simple shear (Boukany & Wang, 2010). Polymer chain entanglement and disentanglement (also known as the coil and stretch phenomena) in concentrated polymer dispersions are the usual phenomena that have consequences for rheological measurements (Ferry, 1980; Graessley, 1974; Boukany & Wang, 2010). Entanglement of polymer chains may lead to an initial elastic deformation before the molten material actually starts flowing. If the rate of external deformation is higher than the chain relaxation rate, the chain or gel

network may collapse to facilitate flow. This collapse may be a localized event giving rise to a shear banding type of flow discontinuity. This is a complex type of time dependency in that it is shear rate dependent and also results in structural change with time. Shear banding could also arise from breakage of polymeric interactions above a critical shear stress or shear rate (Callaghan & Gill, 2000). Melt fracture is a stress induced structural failure of material perhaps arising from stress-induced disentanglement among bulk polymer chains (Wang & Drda, 1997; Koopmans, den Doelder & Molenaar, 2011).

Casein structures in Mozzarella-like cheeses can be viewed as entangled polymers, as while stretching at higher temperature, they form macroscopic fibers because of calcium mediated casein-casein interactions (Lucey, Johnson & Horne, 2003). These polymeric chains may also have cross links to further strengthen the protein network. Self-association of α and β -caseins may form worm-like polymeric chains and hedgehog-like micelles, respectively (Horne, 1998). Casein gels have also been considered as a heterogeneous network structure consisting of strands of aggregated casein particles (van Vliet, Roeffs, Zoon & Walstra, 1989). In relatively concentrated and close packed conditions such as cheese these casein aggregates may interact with neighboring casein aggregates through entanglement (Horne, 1998).

The fact that casein structures in Mozzarella-like cheeses can be considered as either entangled polymers or aggregated gel networks suggests that insights from the polymer literature are relevant. High shear rates applied to molten Mozzarella cheese in a capillary rheometer result in melt fracture, which can be caused either by fat-protein separation or by a stick-slip type of behaviour (Muliawan & Hatzikiriakos, 2008; Bahler & Hinrichs, 2013). However, a critical shear stress or shear rate was necessary to cause melt fracture. Yu & Gunasekaran (2001) reported a sharp drop in shear viscosity at $2\text{-}5\text{ s}^{-1}$ for molten Mozzarella cheese and suggested that the cheese undergoes structural breakdown above some critical shear rate.

3.4. Conclusions

Steady shear viscosity measurements are possible on molten Mozzarella-like cheeses at higher shear rates. The best methods to obtain reliable and consistent data up to 250 s^{-1} on steady shear viscosity of Mozzarella cheese were: 1. Use of 20 mm serrated plates with a Peltier temperature hood; 2. Using longer measurement duration for low shear

rates and; 3. Using fewer shear rate steps in the flow curve to limit the total accumulated shear strain. The flow curves obtained for Mozzarella-type cheeses at 70 °C were found to follow the power law model. At higher shear rates flow inconsistencies may arise from the combined effect of wall slip and structural failure of the material. The Cox-Merz rule was found to be applicable for renneted casein gel at 70 °C and is recommended as a possible tool to predict steady shear viscosity from oscillatory rheological data.

4.0 Effect of shear work input on steady shear rheology and melt functionality of model Mozzarella cheeses

Chapter Summary

Model Mozzarella cheeses with varied amounts of shear work input were prepared by working molten cheese mass at 70 °C in a twin screw cooker. Rheology and melt functionality were found to be strongly dependent on total shear work input. A non-linear increase in consistency coefficient (K from power law model) and apparent viscosity and decrease in flow behaviour index (n from power law model) were observed with increasing amounts of accumulated shear work, indicating work thickening behaviour. An exponential work thickening equation is proposed to describe this behaviour. Excessively worked cheese samples exhibited liquid exudation, poor melting and poor stretch. Nonfat cheese exhibited similar but smaller changes after excessive shear work input. It was concluded that the dominant contributor to the changes in properties with increased shear work was shear induced structural changes to the protein matrix. A good correlation was found between the steady shear rheological properties and the melting properties of the cheeses.

4.1. Introduction

The Mozzarella cheese process includes a cooking and stretching step that gives a plastic appearance to the curd and promotes formation of a fibrous protein network (Lucey, Johnson & Horne, 2003). The stretching step not only brings about desirable textural changes but also helps in redistributing the fat-serum channels within the cheese matrix and this is important for attaining optimum melt characteristics during pizza baking (Rudan & Barbano, 1998). Traditionally Mozzarella cooking was accomplished by melting the curd in hot water (60-85°C) and then working the molten curd by stretching and kneading manually until the desired texture was achieved. Modern processes use mechanical workers/mixers that perform stretching and kneading action by rotation of single or twin screws in the presence of hot water (Noronha, O’Riordan & O’Sullivan, 2008). The mechanical process rapidly transforms curd particles into a heterogeneous but continuously flowable mass (Mulvaney, Rong, Barbano & Yun, 1997). However, the process may cause heterogeneous distribution of moisture at a microstructural level in fresh Mozzarella cheese (Kuo, Gunasekaran,

Johnson & Chen, 2001) making it a non-equilibrium system as the cheese may subsequently undergo further changes during processing and storage such as salt and moisture migration (McMahon, Fife & Oberg, 1999). Blentech, Stephan and Brabender Farinograph are common small scale cheese cookers that have recently been used to study preparation of imitation cheeses (Noronha et al., 2008; El-Bakry, Duggan, O'Riordan & O'Sullivan, 2010a, b) and process cheese (Glenn, Daubert, Farkas & Stefanski, 2003; Kapoor, Lehtola & Metzger, 2004). These recent studies mainly focused on the effect of processing variables on melt functionality and firmness of cheese. The Brabender Farinograph has been used for preparing imitation cheeses with or without chelating salts and the torque responses and functional characteristics of the cheeses have been reported (El-Bakry et al., 2010a, b; El-Bakry, Duggan, O'Riordan & O' Sullivan, 2011a, b, c).

The rheology, stretch and melt functionality of cheeses at higher temperatures is determined by the strength and number of casein-casein interactions (Park, Rosenau & Peleg, 1984) mainly hydrophobic, calcium cross-linking, electrostatic interactions and hydrogen bonds. The overall effect of increasing temperature on these interactions is weakening of the cheese matrix. Lucey et al. (2003) reviewed the chemistry and physics of cheese stretching which is relevant both to the stretching stage during manufacture and to stretch functionality during application on a pizza. There is a critical level of energy storage and dissipation that enables melt and stretch of a casein network. This energy level is related to the relaxation times of casein-casein interactions. If the interactions are too strong then internal stresses generated in the cheese during stretching are not easily released and the fibres are more likely to break rather than stretch or flow. If the interactions are too weak then the cheese will not stretch but will act as a viscous liquid. For cheese to stretch well the casein molecules must interact closely but the bonds must relax and reform very quickly.

Two studies were found considering the effect of working on rheology and functionality of Mozzarella cheese (Mulvaney et al., 1997; Yu & Gunasekaran, 2005). Both studies investigated the effect of thermomechanical treatments on rheological and functional properties and used temperature of working and specific mechanical energy as their system variables. Both studies concluded that it was possible to create the desired functionality by manipulating these two system variables. However, the very small range of shear work applied (2-6.5 kJ/kg) led to a narrow range of melt.

Mozzarella cheese has recently been shown to strain harden during tensile testing and to work thicken during a manual rolling process (Bast, Sharma, Easton, Dessev, Lad & Munro, 2015). Work thickening was defined as an increase in mechanical strength when a material is worked. The structure of three Mozzarella-type cheeses has also been shown to change during rheological testing in a rotational rheometer (in Chapter 3 of this thesis). It was therefore interesting to explore whether work thickening occurred in a Mozzarella cheese mechanical cooker. A novel technique (Chapter 3) was developed to successfully measure steady shear viscosity of Mozzarella cheese at relatively high shear rates ($\sim 200 \text{ s}^{-1}$) at $70 \text{ }^\circ\text{C}$. Our objectives were to study the effect of shear work on steady shear rheology and melt functionality of model Mozzarella cheeses. Shear work inputs were extended well beyond normal working times to exaggerate any work thickening effects. Nonfat cheese was included in the study to observe rheological and structural changes occurring in the protein matrix in the absence of fat.

4.2. Materials and Methods

4.2.1. Materials

Renneted and acidified protein gel manufactured from skim milk was obtained at $-20 \text{ }^\circ\text{C}$ 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 $11 \text{ }^\circ\text{C}$ 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.

4.2.2. 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 specified working volume of this cooker was 29.45 L and the batch size in this study was 25 kg cheese, based on previous experience at FRDC. Three types of model Mozzarella cheese were made - full fat, nonfat and full fat with 0.5 % TSC as a chelating agent. Using TSC is atypical for Mozzarella cheese but its addition created another model system that gave useful

insights. 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. Nonfat cheese used the same protein/moisture and protein/salt ratios as full fat cheese. 31 batches of model Mozzarella cheese were prepared using the conditions outlined in Table 4.1.

Previous experience at FRDC with the cooker plus preliminary experiments had shown that a preworking treatment was useful in order to create macroscopically homogenous and workable molten cheese with all the cream and water incorporated. The preworking involved mixing of curd, cream, salt and water in the cooker with direct steam injection till the temperature reached 70 °C, typically after 320 - 350 s. The expected mass of condensate added by steam injection was included in the target composition calculations.

Preworking was followed by working the molten cheese mass using different screw speeds for various times (Table 4.1). Temperature was maintained at 70 °C by indirectly injecting steam intermittently into the Blentech jacket by manually opening a needle valve. A load cell was fixed to the Blentech frame 0.2 m from the axis of one of the mixer shafts and set up to enable torque on the shaft to be continuously logged. Cheese samples from the experiments were placed in plastic containers or plastic bags and cooled rapidly in salt-ice-water slurry. Samples were then either frozen and stored at -20 °C or were tested after storage at 4 °C for up to 7 d.

4.2.3. *Shear work calculation*

Force measured by the load cell was converted to torque, M , using

$$M(t) = [F(t) - F_0] \times L \quad (4.1)$$

where $F(t)$ is the force at time t , F_0 is the baseline force value and L is the distance of the force sensor from the shaft axis, 0.2 m. F_0 was measured at 70 °C and at each screw speed used in the trials both with water and with air in the Blentech to measure frictional force created by the bearings and mechanical seals when wet. F_0 values in air were used in the calculations. The load cell calibration was checked periodically using standard weights.

Table 4.1 Blentech cooker run conditions and product compositions.

Run no.	Screw speed rpm	Date	Sampling time ^e s	Shear work kJ/kg	Moisture g/100g	Fat g/100g	Protein g/100g	Salt g/100g	Ash g/100g
1	50	17-10-2013	520	2.80	54.3	20.6	20.61	1.4	-
2	50	17-10-2013	650	3.87	53.6	22.6	20.93	1.4	-
3	50	17-10-2013	780	4.98	53.4	22.9	21.31	1.41	-
4	50	17-10-2013	1680	6.92	52.6	24.0	20.16	1.39	-
5	50	17-10-2013	3180	13.78	53.1	23.1	21.12	1.41	-
6	50	17-10-2013	4185	14.49	52.9	23.4	20.80	1.42	-
7	50	2-7-2014	315	1.32	54.0	22.4	20.67	1.4	2.22
8	50	2-7-2014	430	2.99	53.9	22.6	20.80	1.4	2.18
9	50	2-7-2014	640	2.85	54.1	22.5	20.86	1.39	2.2
10	50	2-7-2014	1590	5.91	53.5	23.1	20.92	1.42	2.24
11	50	2-7-2014	2735	8.17	53.6	23.1	21.05	1.42	2.21
12	50	2-7-2014	3980	12.03	53.4	23.1	20.86	1.41	2.22
13	150	23-1-2014	375	3.30	53.7	22.7	20.8	1.40	2.13
14	150	23-1-2014	395	4.29	52.9	23.2	20.9	1.42	2.18
15	150	23-1-2014	635	8.80	52.8	23.2	20.7	1.43	2.21
16	150	23-1-2014	1515	26.26	52.5	23.5	20.9	1.42	2.19
17	150	23-1-2014	3035	58.23	53.1	23.3	20.7	1.41	2.20
18	150	23-1-2014	3950	73.71	52.5	23.7	20.6	1.43	2.21
19	150	29-5-2014	370	2.61	53.7	23.0	20.93	1.39	2.20
20	150	29-5-2014	425	2.20	53.9	22.9	20.74	1.41	2.21
21	150	29-5-2014	635	6.62	53.8	23.1	20.74	1.40	2.21
22	150	29-5-2014	1525	21.46	53.7	23.3	20.67	1.41	2.21
23	150	29-5-2014	2745	45.30	53.6	23.4	20.48	1.43	2.17
24 ^a	150	29-5-2014	3955	68.44	-	-	-	-	-
	150	29-5-2014	4850	81.32	54.0	23.0	20.48	1.38	2.19
25 ^a	Liquid ^d	29-5-2014	4850		93.6	1.27	0.76	2.69	3.02
	250	31-7-2014	380	3.49					
	250	31-7-2014	1020	25.95					

Run no.	Screw speed rpm	Date	Sampling time ^e s	Shear work kJ/kg	Moisture g/100g	Fat g/100g	Protein g/100g	Salt g/100g	Ash g/100g
	250	31-7-2014	1645	53.87	-	-	-	-	-
	250	31-7-2014	3685	166.05	50.6	23.2	23.54	1.47	2.46
26	250	3-9-2014	335	4.89	53.6	22.7	21.37	1.41	2.31
27 ^a	250	3-9-2014	1520	62.08	-	-	-	-	-
	250	3-9-2014	3080	159.21	-	-	-	-	-
	250	3-9-2014	3690	185.09	53.3	23.0	21.56	1.42	2.30
	Liquid ^d	3-9-2014	3690		95.4	0.22	0.49	2.75	2.92
28 ^{a,b}	150	31-7-2014	470	6.75	-	-	-	-	-
	150	31-7-2014	3025	60.49	-	-	-	-	-
	150	31-7-2014	4400	98.88	-	-	-	-	-
	150	31-7-2014	5285	128.10	63.8	0.06	32.86	1.79	2.56
	Liquid ^d	31-7-2014	5285		95.8	-	0.54	2.82	3.05
29 ^{a,b}	150	3-9-2014	330	4.85	-	-	-	-	-
	150	3-9-2014	1480	27.01	-	-	-	-	-
	150	3-9-2014	2680	50.59	-	-	-	-	-
	150	3-9-2014	3920	83.68	66.2	0.28	30.81	1.85	3.08
	Liquid ^d	3-9-2014	3920		95.7	0.19	0.52	2.84	3.06
30 ^{a,c}	150	31-7-2014	430	4.37	-	-	-	-	-
	150	31-7-2014	1570	24.52	-	-	-	-	-
	150	31-7-2014	2775	50.69	-	-	-	-	-
	150	31-7-2014	3970	80.90	52.6	22.7	21.95	1.35	3.08
31 ^{a,c}	150	3-9-2014	1570	21.40	-	-	-	-	-
	150	3-9-2014	3965	70.76	53.7	22.9	20.99	1.41	2.56

^aSamples taken for rheological and functionality measurement during the run. Shear work calculation was corrected for the reduced mass later in the run.

^bThese runs were for nonfat cheese.

^cTrisodium citrate was added to these runs.

^dExuded liquid sample at end of run.

^eCheese samples unless noted as liquid.

Shear work input (Steffe & Daubert, 2006) to the molten cheese mass was obtained by calculating area under the torque-time curve and applying the following equation.

$$\text{Shear work, } W_s \text{ (J/kg)} = \frac{\omega}{m} \int_0^t M(t) dt \quad (4.2)$$

where ω ($\text{rad}\cdot\text{s}^{-1}$) is the screw speed, m (kg) is the mass of cheese in the Blentech, M (Nm) is the torque calculated at time t (s) using Eq.4.1. Numerical integration of the torque-time curve with respect to time was conducted using the trapezoidal rule in Microsoft Excel. The shear work calculation included the shear work input during the preworking period. As it was a twin screw mixer with independent motor drives for each shaft and a load cell was fitted on only one shaft, shear work was multiplied by 2.

4.2.4. Meltability of Mozzarella cheese

The modified Schreiber test (Muthukumarappan, Wang & Gunasekaran, 1999) was used with some variations. Shreds of approximate size 15x5x5 mm were prepared from a block of cheese using wire and a roller cutter. For each test, 5 g shredded cheese was formed into a ~40 mm diameter disc by placing shredded cheese into a hollow plastic cylinder aligned vertically in a petri dish and compressing using a piston with 5 kg mass on it for 15 s. The petri dish was covered, placed at 4 °C for 10 min for temperature equilibration, then placed for 5 min in a forced-air convection oven at 232 °C. After removing from the oven, molten cheese samples were cooled on a flat surface for ~30 min. The extent of spread, expressed as melt score, was measured using the method suggested by Park et al. (1984). Cheese spread on the petri dish was read on a target-type concentric-circle graph with melt score starting as 0 at 40 mm diameter and increasing by 1 melt score unit for each radial distance increase of 2.54 mm. To correct for non-uniformity of melt spread, i.e. non-circular shape, six readings were obtained from six equally spaced radial vectors and averaged. For each cheese sample, the meltability test was conducted in quadruplicate.

4.2.5. Steady shear rheology

Steady shear rheological measurements on the cheese samples were conducted 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). A cheese cylinder was drawn from a block of cheese stored at 4 °C using a cork borer.

Cheese discs 20 mm diameter and ~2 mm thick were cut from this cylinder using a wire cutter. Cheese discs were wrapped in plastic and stored at 4 °C to prevent moisture loss. Discs were equilibrated to 21 °C, placed in the rheometer gap and the gap closed till normal force reached 5 N. The sample was heated to 70 °C and equilibrated for 120 s before rheological measurement started. A ring of soybean oil was put at the outer periphery of the sample to prevent moisture loss. The flow curve method described in Chapter 3 was used for measuring steady shear rheology at 70 °C. Shear rates from 0.01 to 200 s⁻¹ were applied with measurement times as follows: 60 s at 0.01 s⁻¹, 6.25 s at 0.1 s⁻¹, 0.5 s at 1 s⁻¹, 0.05 s at 10 s⁻¹, 0.05 s at 100 s⁻¹, 0.05 s at 200 s⁻¹. A power law model was used to fit the flow curve and calculate the consistency coefficient, K, and flow behaviour index, n (Chapter 3). Steady shear rheological measurements on each sample were conducted in triplicate.

4.2.6. Statistical analysis

Descriptive statistics, non-linear regression and correlation analysis were conducted on the data using SPSS software (version 13). Test of significance for correlation coefficients was conducted using a two-tailed t- test at P<0.01.

4.3. Results

4.3.1. Processing characteristics and physical properties of model Mozzarella cheese

Table 4.1 shows the total mixing times and compositions of all samples and the corresponding values of shear work. Torque-time curves during mixing at three screw speeds are presented in Fig. 4.1. The end of the preworking period when the temperature reached 70 °C is indicated by a vertical straight line. Accumulated shear work during the preworking period varied between runs but was always in the range 1.2-2.0 kJ/kg.

Post-preworking, the torque at 50 rpm remained relatively constant for the whole working time, accumulating shear work in the range of 12 kJ/kg (run 12) to 14.5 kJ/kg (run 6). In contrast at 150 and 250 rpm the torque progressively increased with time to a maximum, then decreased steeply and finally increased a second time after prolonged working. The rate of increase of torque with time was higher at 250 rpm. The increase of torque with time indicates work thickening of the cheese mass while the steep decline coincided with rapid changes to the macroscopic cheese structure. The maximum values

of torque for 150 and 250 rpm occurred at 3820 s and 2685 s (Fig. 4.1) corresponding to shear work inputs of 66 and 129 kJ/kg respectively. An earlier start of torque decline at 250 rpm indicates earlier onset of macroscopic structural breakdown. Throughout the working time, torque values were highest for 250 rpm followed by 150 rpm and then 50 rpm. At short times this may be attributed to higher inertial forces at higher speeds, but at longer times the higher work thickening at higher speeds also makes a contribution. Manski, van der Zalm, van der Goot and Boom (2008) reported similar changes in torque with time as a result of structure formation and breakdown during prolonged shearing of fat-containing Ca-caseinate dispersions.

Periodically the appearance and characteristic elongation of the cheese was used as an assessment of the pasta filata quality (Figs. 4.2 & 4.3). Insufficient shear work input results in a runny cheese often with the presence of buttery liquid indicating that the cream has not yet been well incorporated into the protein matrix (Fig. 4.2a). Retention of fat and moisture in the finished product is an important consideration in achieving desired functionality (Rizvi, Shukla & Srikiatden, 1999). The cheese had developed a typical pasta filata structure (Figs. 4.2b & 4.3a) after 635 s at 150 rpm corresponding to shear work inputs of 6.6-8.8 kJ/kg (runs 15 & 21). Prolonged shearing led to a damaged and short texture with an oatmeal like appearance and very limited stretch (Figs. 4.2d & 4.3b). The long fibrous strands typical of Mozzarella-type cheeses were absent. Emergence of this grossly overworked structure coincided with expulsion of small amounts of a watery fluid (whey like in appearance) from the cheese (Fig. 4.2d).

Nonfat cheese prepared by working at 150 rpm showed similar behaviour to full fat cheese with torque increasing then declining with time and with expulsion of watery fluid after overworking. Expelled serum after overworking for both full fat and nonfat cheese had a low total solids content of 4.5 - 6.0 % with salt as the main solid component and little fat (Table 4.1).

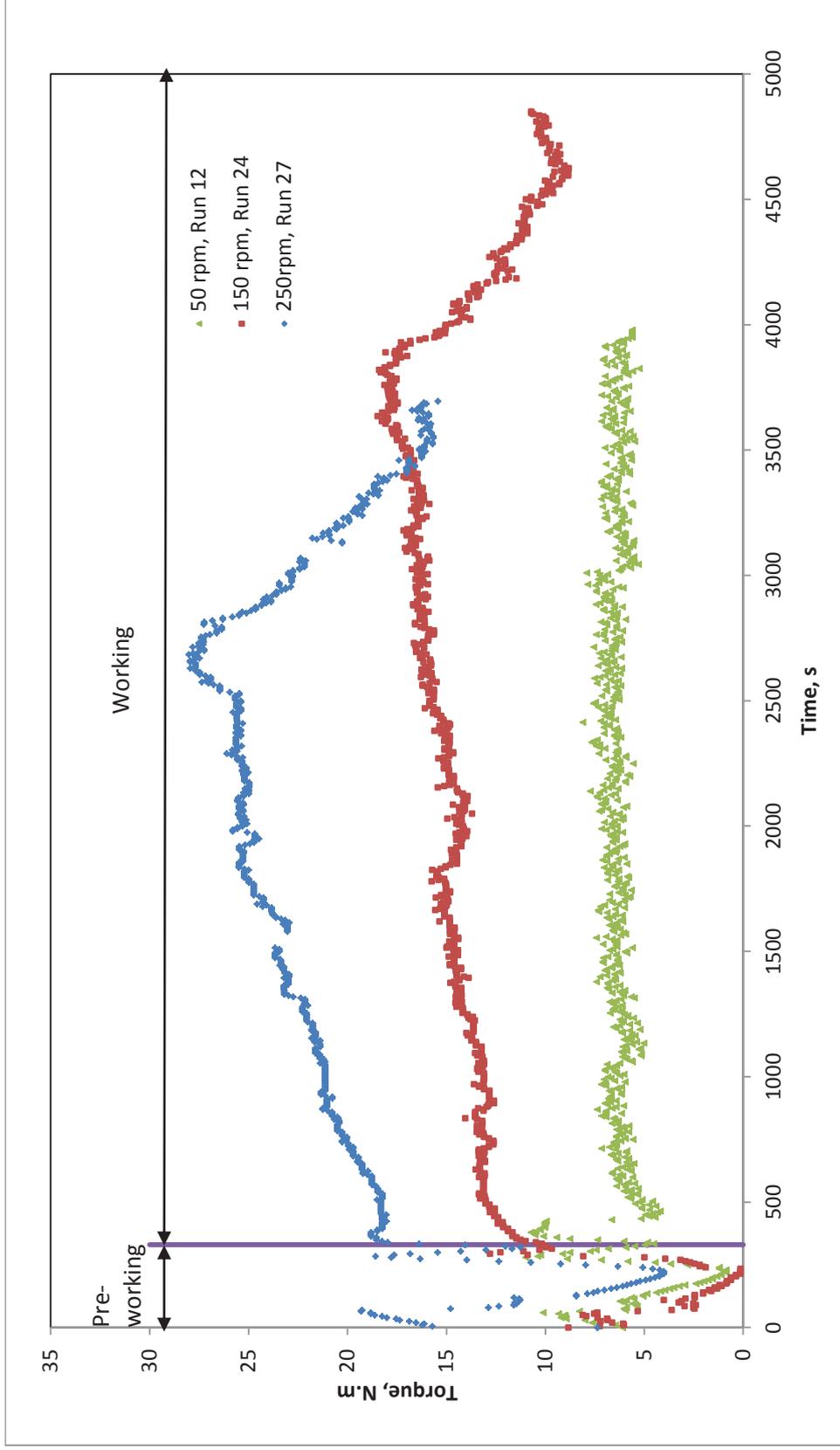


Figure 4.1 Evolution of torque with time during mixing of model Mozzarella cheese in the Blentech cooker at 70 °C and three screw speed.

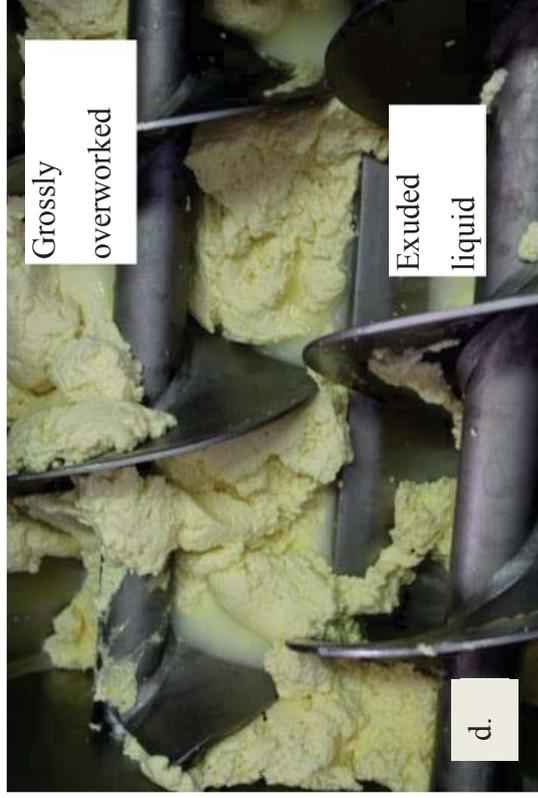


Figure 4.2 Visual appearance of model Mozzarella cheese in the Blentech cooker after various levels of shear work input at 70 °C and 150 rpm; a. 3.3, b. 6.6, c. 58.2 d. 73.7 kJ/kg



Figure 4.3 Stretch appearance of model Mozzarella cheese after working in the Blentech cooker at 70 °C and 150 rpm. a. Optimum melt functionality after shear work input of 6.6 kJ/kg; b. Damaged texture after excessive shear work input of 73.7 kJ/kg

Cheese prepared at 150 rpm with the addition of TSC showed uniform torque versus time with less fluctuation than the curves in Fig. 4.1. El-Bakry et al. (2010 a, b; 2011a, b, c) also reported relatively stable torque readings after attaining a homogenous mass during mixing at constant temperature for cheeses with added chelating salts. The typical fibrous structure of Mozzarella cheese was absent and the cheese showed a grainy visual appearance instead. TSC would be expected to chelate some calcium resulting in reduced casein-casein interactions (Mizuno & Lucey, 2005). This may result in dispersion of caseins (Brickley, Govindaswamy-Lucey, Jaeggi, Johnson, McSweeney & Lucey, 2008). Cheese with TSC added was very sticky. It stuck to plastic bags, rubber gloves and stainless steel. This could be attributed to the tendency of para-casein to stick to hydrophobic materials such as nylon gloves (Paulson, McMahon & Oberg, 1998). Brickley et al. (2008) attributed this stickiness to a decrease of hardness upon addition of TSC as a result of weakening of the casein matrix because of sequestering of calcium and increase of pH resulting in increased electrostatic repulsion.

4.3.2. Effect of accumulated shear work on steady shear rheology of model Mozzarella cheese

Preliminary experiments included comparison of rheological parameters for fresh samples direct from the Blentech, samples stored at 4 °C for 3-7 d and samples frozen then thawed. No significant difference was observed between the sample treatments for either K or n. Samples stored at 4 °C for 3-7 d were therefore used for most runs though for runs 25, 28, 30 and 31 frozen and thawed samples were used. Cervantes, Lund and Olson (1983) also reported no significant effect of a freeze-thaw cycle and frozen storage on textural and sensory properties of Mozzarella cheese. However, Dahlstrom (1978) reported changes in textural attributes of Mozzarella cheese immediately after thawing, but the cheese regained its original properties some weeks after thawing. We thawed frozen samples by storing at 4 °C for 3-7 days. This holding period may allow the structure to approach equilibrium with uniform distribution of moisture, avoiding any possible effects from localised moisture pockets.

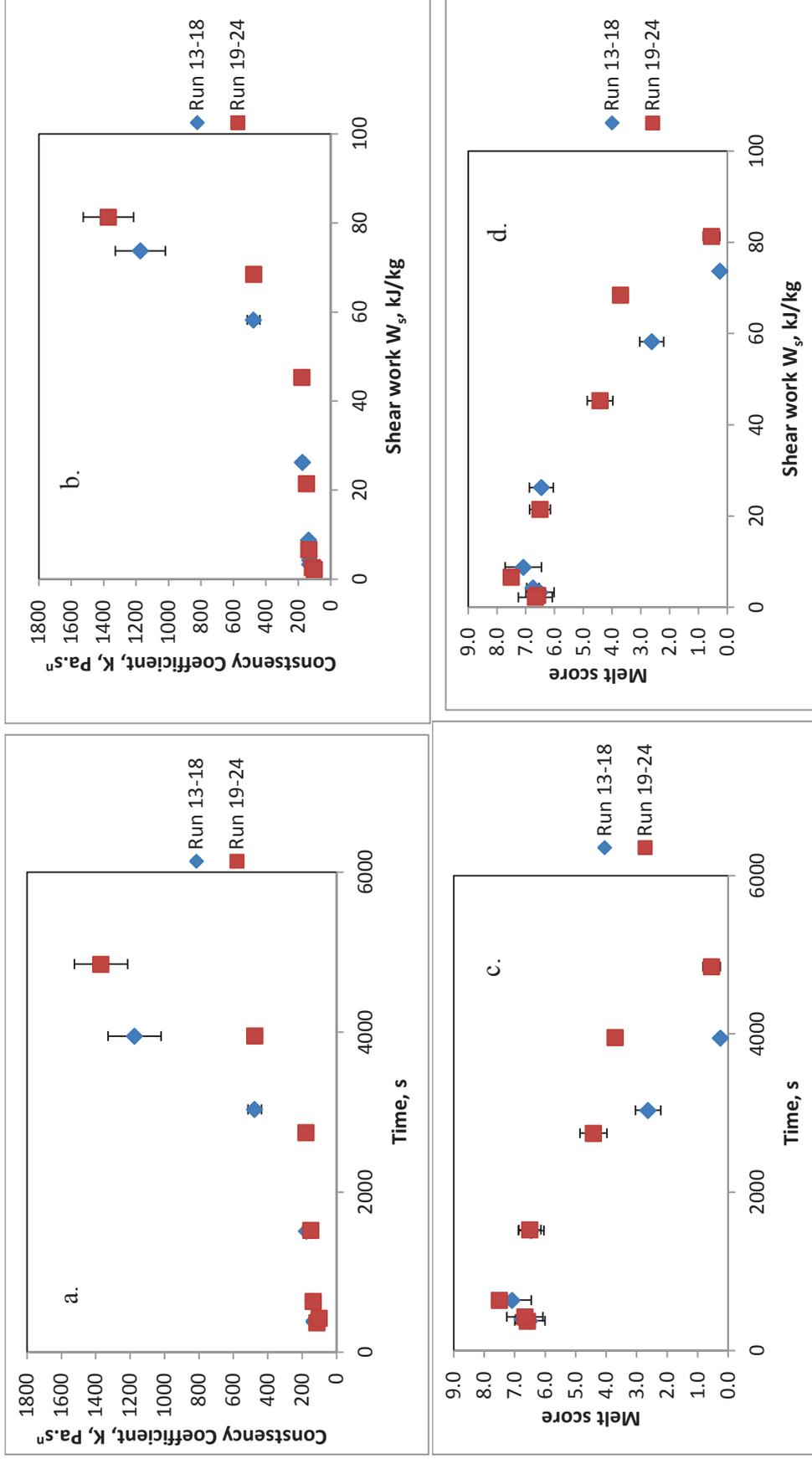


Figure 4.4 Changes in Consistency coefficient (K from power law model) and meltability of model Mozzarella cheese versus time (a,c) and shear work (b,d). Model Mozzarella cheese was manufactured in the Blentech cooker at 150 rpm screw speed and 70 °C. Error bars represent one standard deviation.

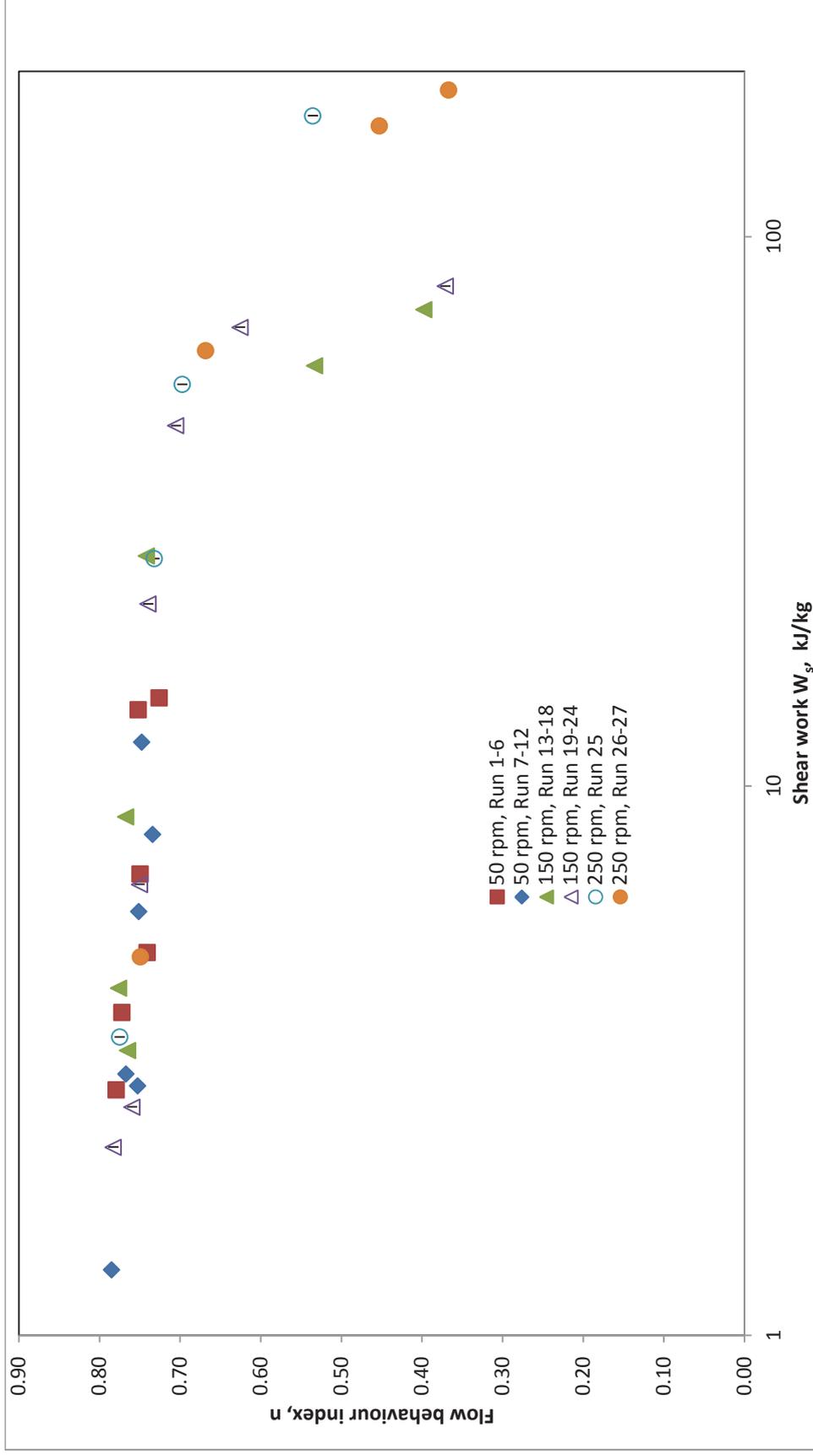


Figure 4.5 Effect of shear work on flow behaviour index (n from power law model) of model Mozzarella cheese at 70 °C. Error bars represent one standard deviation.

K and melt score showed better reproducibility between the two sets of runs at 150 rpm when plotted as a function of shear work input than when plotted as a function of shearing time (Fig. 4.4). Further plots were therefore done as a function of shear work. A further advantage of plotting versus shear work is that results at different screw speeds can be plotted together. Runs 19-24 took a longer time and higher shear work level to produce a given change in either consistency coefficient or melt score than runs 13-18. One reason for this difference was that the mean moisture content of runs 19-24 was higher (53.8%) than runs 13-18 (52.9%). This higher moisture content is probably because the ambient temperature was much colder in May than in January and the steam used for direct injection was therefore probably wetter. However, the protein gel and cream are both natural raw materials so some daily and seasonal variation is also expected.

Figs. 4.5 and 4.6 show the flow behaviour index and apparent viscosity obtained for all the full fat cheeses without TSC plotted together as a function of shear work. For determination of apparent viscosity (Fig. 4.6), a shear rate of 0.01s^{-1} was chosen as low strain rates produced on the cheese by gravity are relevant during the modified Schreiber melt test and also during baking on a pizza (Zhu, Brown, Gillies, Watkinson & Bronlund, 2015). Data from all 33 samples show definite trends with shear work and also fitted reasonably well on one correlation line. K ($K = 137.82e^{0.02W_s}$ with $R^2=0.85$) and apparent viscosity at 0.01 s^{-1} (Fig. 4.6) both increased exponentially with shear work input whereas n (Fig. 4.5) declined with shear work input. All three variables changed relatively slowly up to a shear work input of about 45 kJ/kg, but much more rapidly above that. The rapid decline in n at high shear work input (60-68 kJ/kg) corresponded well with the stage when peak torque was attained (66 kJ/kg, Fig.4.1) and when the macroscopic structural breakdown occurred (Fig. 4.2).

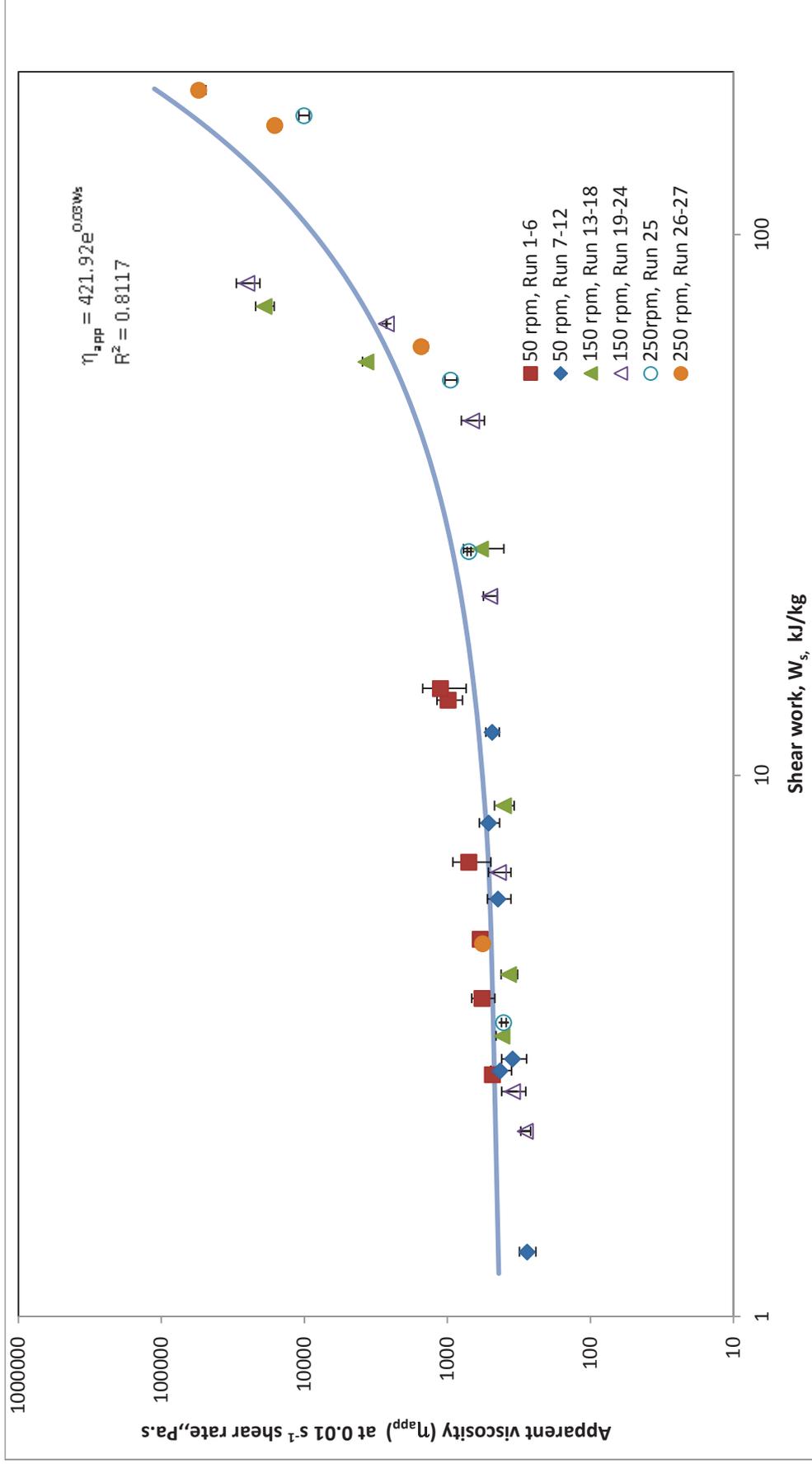


Figure 4.6 Effect of shear work on apparent viscosity of model Mozzarella cheese at 0.01 s⁻¹ shear rate and 70 °C. Error bars represent one standard deviation.

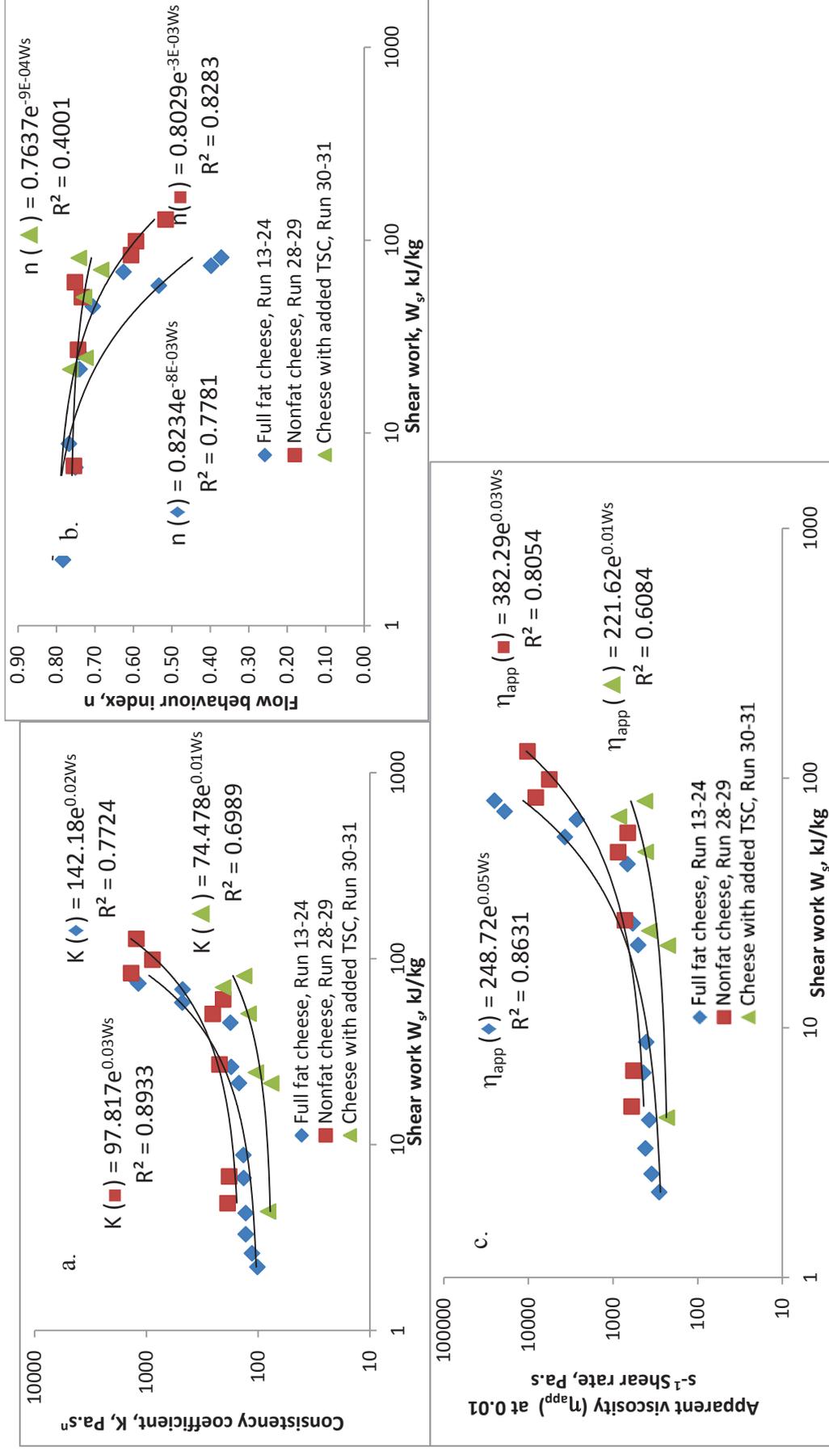


Figure 4.7 Effect of shear work on (a) consistency coefficient (K from power law model); (b) flow behaviour index (n from power law model) and (c) apparent viscosity at 0.01 s^{-1} , all at $70 \text{ }^\circ\text{C}$, for three model Mozzarella cheeses prepared in the Blentech cooker using 150 rpm speed.

Over the shear work range considered K increased from 97 to 2,928 Pa.sⁿ, n decreased from 0.78 to 0.37 and apparent viscosity at 0.01 s⁻¹ increased from 277 to 54,700 Pa.s. The 30-fold increase in K and 198-fold increase in apparent viscosity at 0.01 s⁻¹ indicate very significant work thickening over the shearing period. The decrease in n indicates that on working the cheese, was progressively becoming more shear thinning.

Rheological parameters for nonfat cheese and cheese with added TSC are compared with those of full fat cheese in Fig. 4.7. Shear work input had a very small effect on the rheological parameters of cheese with added TSC, i.e. no significant work thickening. Nonfat cheese exhibited significant work thickening with a big increase in both K and apparent viscosity at 0.01 s⁻¹ as shear work increased. Shear work input had the biggest effect for full fat cheese.

4.3.3. Effect of accumulated shear work on melt functionality of model Mozzarella cheese

Melt scores for all full fat samples without TSC demonstrated reasonable alignment plotted versus shear work (Fig. 4.8), suggesting the validity of shear work as a process control variable to achieve the desired product functionality. Melt scores did not change significantly with shear work below 10 kJ/kg, but decreased with shear work above 10 kJ/kg reaching very low values in the range 0.2-0.5 at shear work inputs >70 kJ/kg.

Melt score versus shear work curves for nonfat cheese and cheese with added TSC are compared with that for full fat cheese in Fig. 4.9. Melt score changed very little with shear work for cheese with added TSC. Nonfat cheese was much less shear work sensitive than full fat cheese reaching a lowest melt score of 3.7 after prolonged working compared to 0.2 for full fat cheese.

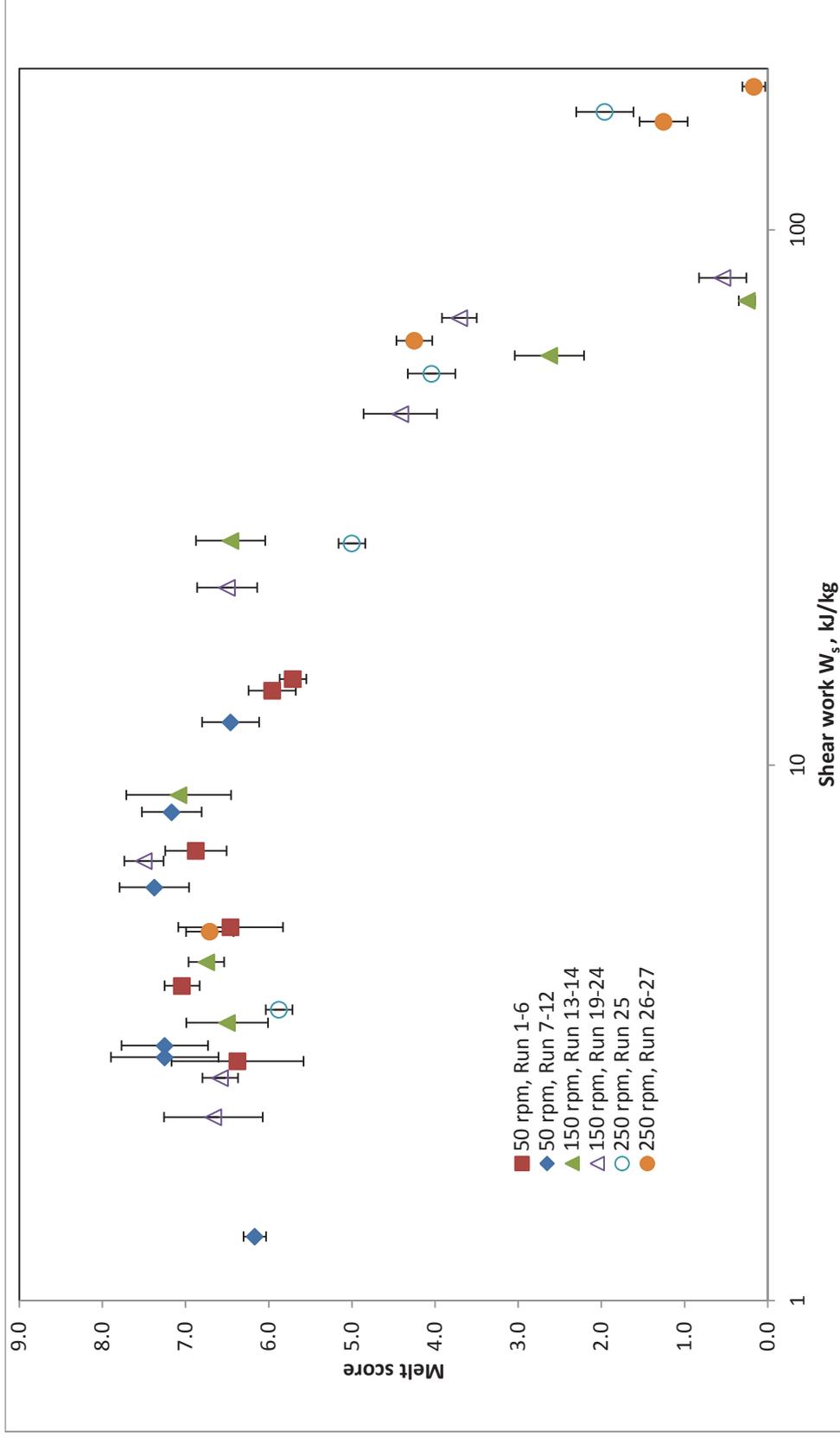


Figure 4.8 Effect of shear work on meltability of model Mozzarella cheese prepared at 70 °C in the Blentech cooker. Error bars represent one standard deviation.

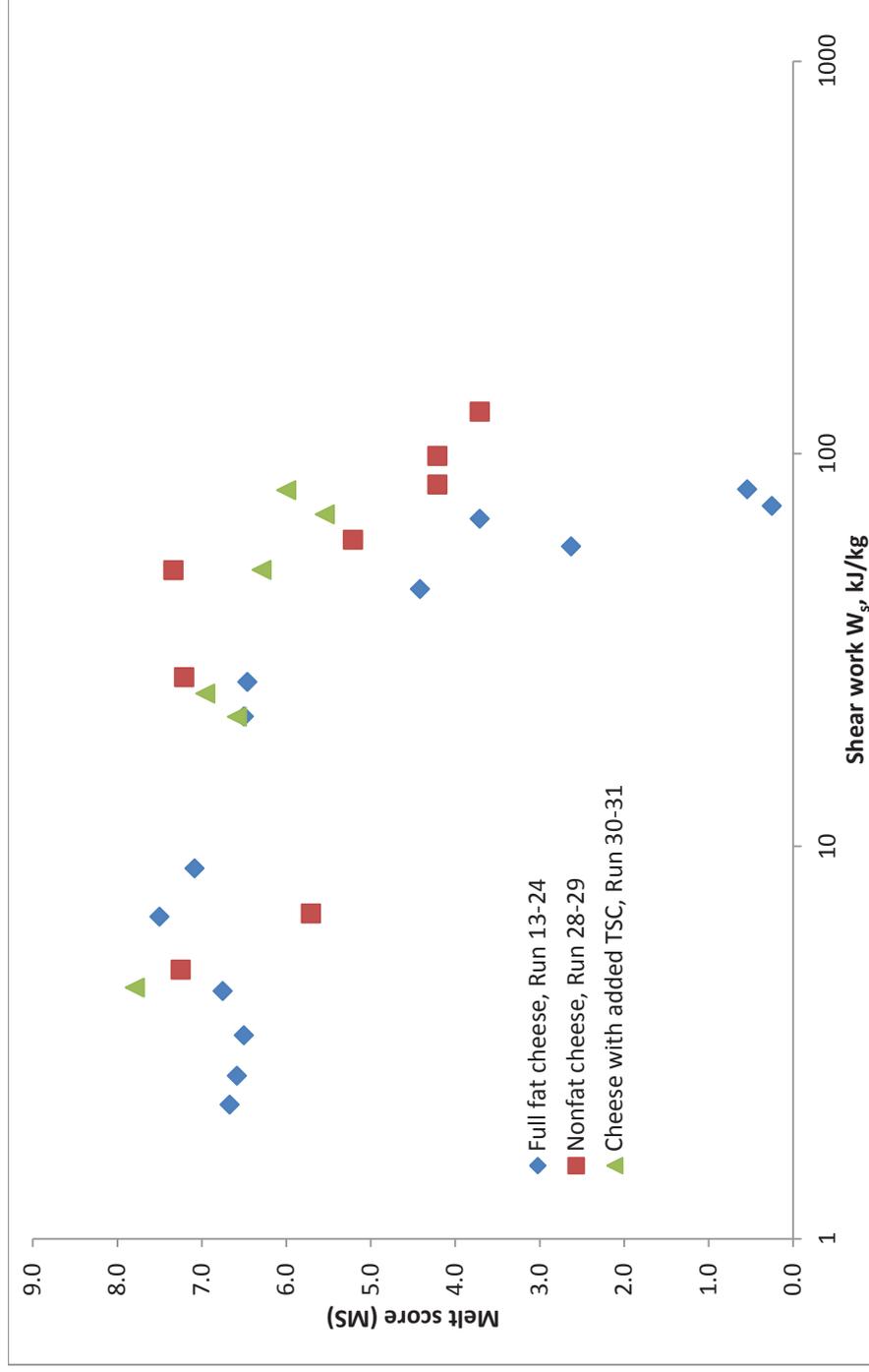


Figure 4.9 Melt score versus shear work input for model Mozzarella cheeses prepared in the Blentech cooker using 150 rpm screw speed.

Fig. 4.10 shows the appearance of several cheese samples after melting and then cooling in the modified Schreiber melt test. Optimal working (Fig. 4.10a) resulted in good melting, a reasonable spread, browning around the periphery and the desired free oil release. The series of events during the melt test for cheese with optimal functionality would be expected to be similar to those described by Rudan and Barbano (1998) during pizza baking. First fat started melting within the shreds and then travelled to the heat exposed surfaces. The rate of fat migration to the surface is very important because fat prevents surface drying and skin formation (Rudan & Barbano, 1998). This enables shreds to melt and then fuse together to make one molten mass followed by spreading or flow of the molten cheese by biaxial expansion/stretching under gravity.

However, excessively worked ($>70\text{kJ/kg}$) samples did not exhibit this behaviour. Some of the shreds retained their individual identity throughout the heating regime, fusion was limited and flow across the petri dish was very small (Fig. 4.10b). One possible reason for this behaviour could be that toughening of the protein matrix by excessive working is limiting fat migration through the protein matrix to the shred surface resulting in surface drying and skin formation thus inhibiting shred fusion and cheese flow. To check this possibility a sample of excessively worked cheese was coated with a vegetable oil spray before placing in the oven. This increased the melt score slightly from 0.2 to 0.5 (Fig. 4.10c) but did not give flow anywhere near that of the samples with low shear work input. This suggested that changes to the protein matrix itself were having a major role in limiting the melting and flow of the excessively worked samples.

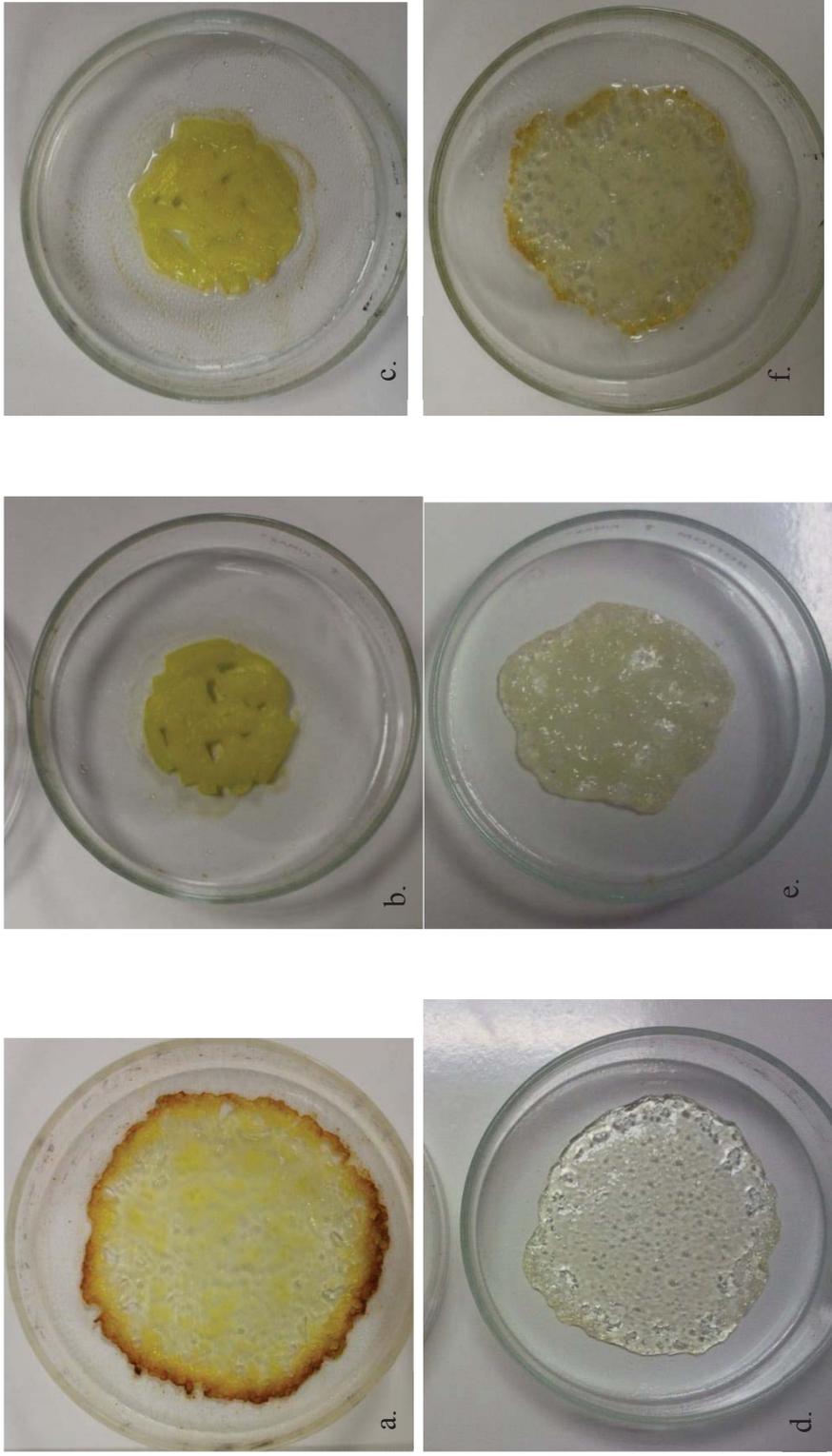


Figure 4.10 Photographs of cooled model Mozzarella cheese after the modified Schreiber melt test. a. Optimum functionality full fat cheese after 6.6 kJ/kg shear work input, melt score 7.5; b. Full fat cheese after excessive shear work input 166 kJ/kg, melt score 0.2; c. Full fat cheese after excessive shear work input 166 kJ/kg and with vegetable oil sprayed on the surface before melting, melt score 0.5; d. Nonfat cheese after 6.75 kJ/kg shear work, melt score 5.7; e. Nonfat cheese after 128 kJ/kg shear work, melt score 3.7; f. Nonfat cheese after 128 kJ/kg shear work and with vegetable oil sprayed on the surface before melting, melt score 4. Melt score given is the mean of the quadruple tests for each batch.

Nonfat cheese with low shear work input melted and flowed well (Fig. 4.10d). These melting results for nonfat cheese were surprising given the explanation above of the role of fat in preventing surface drying and skin formation and contrast with the pizza baking results of Rudan & Barbano (1998), who reported that low fat Mozzarella cheeses had limited melting. The melt of nonfat cheese was less impacted by excessive shear work (Fig. 4.10e) than that of full fat cheese. Again coating with vegetable oil spray had only a small positive effect on melt score (Fig. 4.10f). This suggests that there is a significant contribution of the fat phase to the poor melting properties of excessively worked full fat cheese. Changes to the protein matrix alone cannot explain the results. Molten nonfat cheese was white in appearance while still hot but transformed into a transparent plastic like sheet upon cooling to room temperature (Fig. 4.10d). Pastorino, Dave, Oberg and McMahon (2002) reported increased opacity of nonfat Mozzarella cheese upon heating from 10 °C to 50 °C and attributed this to heat induced changes in protein interactions as manifested by structural changes in the cheese. Heating would favour hydrophobic interactions and would possibly allow re-association of β -casein and calcium within the protein matrix, causing enhanced protein-protein interactions (Pastorino et al., 2002). Translucency of nonfat Mozzarella cheese in the presence of salt has also been attributed to limited light scattering from pockets of free serum distributed in the protein matrix (Paulson et al., 1998). Translucency of cheese has been attributed to the formation of a fine stranded protein network that allows light to pass through (Brickley et al., 2008).

4.3.4. Relationships between melt functionality and steady shear rheology

Apparent viscosity at 0.01 s^{-1} (Fig. 4.6) was able to distinctly differentiate the effect of shear work input for all the full fat cheese samples without added TSC. Therefore, an attempt was made to establish a relationship between melt functionality and apparent viscosity at 0.01 s^{-1} . A significant ($P < 0.01$) negative correlation fitted by an exponential relationship with good fit ($R^2 = 0.91$) was found for apparent viscosity at 0.01 s^{-1} as a function of melt score (Fig. 4.11). Brickley et al. (2008) also reported an inverse relationship between meltability and hardness of cheese.

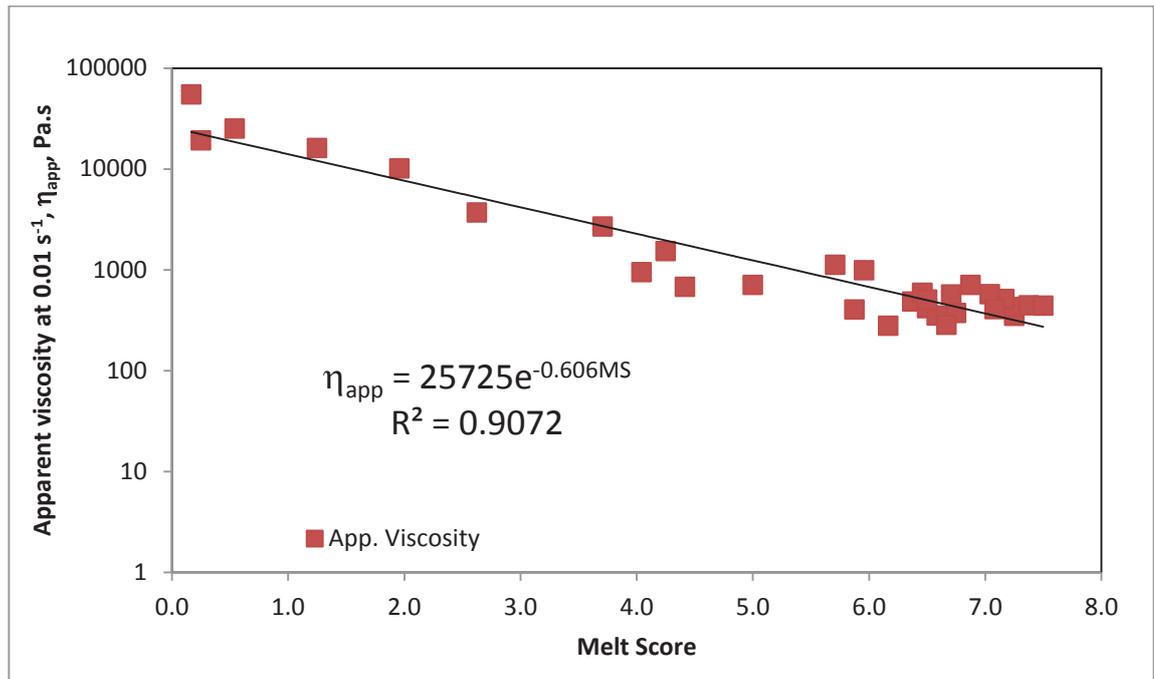


Figure 4.11 Correlation of apparent viscosity at 0.01 s^{-1} with melt score for the model Mozzarella cheese at $70 \text{ }^\circ\text{C}$.

4.4. Discussion

The torque profiles (Fig. 4.1) were different to those observed for imitation cheese by El-Bakry, Beninati, Duggan, O’Riordan & O’Sullivan (2011). They typically observed a peak-trough-peak profile and linked the first peak to hydration of dry rennet casein and the second peak to emulsification. However, the protein gel in this study had never been dried and so was already hydrated. Also emulsification was not expected to be a major event in our mixing as we used dairy cream instead of vegetable fat. Different torque profiles might therefore be expected.

The results give two main sets of evidence for very significant work thickening during working of the model Mozzarella cheeses: increase of torque with time at the 150 rpm and 250 rpm screw speeds (Fig. 4.1), and 198 fold increase in apparent viscosity at 0.01s^{-1} (Fig. 4.6) with increasing shear work input from 6 kJ/kg to $>70 \text{ kJ/kg}$. The detailed chemical basis for the work thickening is not clear. Cheese rheological properties are largely governed by the strength of casein-casein interactions (Lucey et al., 2003). Work thickening is a result of the strengthening of these interactions upon shearing at $70 \text{ }^\circ\text{C}$. The presence of shear is a critical element - merely holding at $70 \text{ }^\circ\text{C}$ would cause little change as even at 50 rpm shearing for 4,000 s (shear work input of 14 kJ/kg) caused little change in rheology. The presence of calcium is also critical as the

model cheese with added TSC to chelate calcium showed almost no work thickening even with shear work input of 81 kJ/kg (Fig. 4.7). The importance of calcium cross-linking to the casein-casein interactions in cheese and the changes in cheese properties on adding chelating salts have both been well covered in many publications (Brickley et al., 2008; Lucey & Fox, 1993; Lucey et al., 2003; Mizuno & Lucey, 2005; El-Bakry et al., 2010 a, b; 2011a, b, c). Some of the factors suggested by Bast et al. (2015) as important for strain hardening of Mozzarella cheese are also likely to be important for work thickening – movement of the casein fibres past one another and the “stickiness” of the adjacent casein chains both increasing casein-casein interactions.

What type of reaction or mechanism results in an exponential increase in apparent viscosity (Figs. 4.6, 4.7) with shear work? Particle growth by coalescence is one of the few process models that exhibits this functional form and is based on the distortion and kneading of the fluid eddies that bring the particles together (Levenspiel, 1996). There are parallels between the Blentech mixing-shearing action and interaction of fluid eddies. Let us define the rate of work thickening as $d\eta/dt$. Work thickening in the molten cheese system is caused by increases in the number or strength of effective molecular interactions, specifically protein-protein interactions. Fundamentally, viscosity is a measure of the strength of intermolecular interactions providing a resistance to flow. It is therefore reasonable to propose that

$$d\eta/dt \propto \eta \quad (4.3)$$

Rearranging and integrating between limits

$$\eta = \eta_0 e^{at} \quad (4.4)$$

As it has been found that shear work input (W_s) as an independent variable is a stronger predictor than time of the outcome with respect to rheology and melt functionality (Fig. 4.4), and also enables the results of different shear rates to be correlated on a single curve (Figs. 4.5-4.6), we can integrate with respect to W_s rather than time.

$$\eta = \eta_0 e^{bW_s} \quad (4.5)$$

where η_0 is viscosity before significant work thickening, η is viscosity at time t or shear work W_s during work thickening and a , b are work thickening constants.

Equation 4.5 is in exactly the form of the equations on Figs. 4.6 and 4.7c for apparent viscosity versus shear work. The results give b values at 150 rpm of 0.05 kg/kJ for full fat cheese, 0.03 kg/kJ for nonfat cheese and 0.01 kg/kJ for full fat cheese with added TSC (Fig. 4.7c).

An alternative approach to explain the exponential nature of work thickening with time or shear work arises from the nature of the laminar mixing process occurring in the Blentech with the extremely viscous cheese (Szalai, Alvarez & Muzzio, 2004). In the chaotic flow occurring in laminar mixing the stretching of a fluid element, λ , from length l_0 to length l_n after time t is given by

$$\lambda = l_n / l_0 = e^{\Lambda t} \quad (4.6)$$

where Λ , the Lyapunov exponent of the flow, represents the average rate of stretching after a given time. The stretching and folding action in laminar mixing not only gives an alternative theoretical basis for the exponential nature of work thickening, but also forms the structural basis for the creation of striated cheese microstructures such as those observed in Mozzarella cheese.

Torque versus time curves similar to those in Fig. 4.1 occur during mechanical development of dough which is a structurally similar food system where work thickening behaviour has been well explored (Zheng, Morgenstern, Campanella & Larsen, 2000; Peressini, Peighambardoust, Hamer, Sensidoni & van der Goot, 2008; Peighambardoust, van der Goot, van Vliet, Hamer & Boom, 2006). During mechanical development of dough, a fibrous gluten network is formed during the mixing process and moisture is also distributed uniformly within the developed protein network (Bloksma & Bushuk, 1988; Zheng et al., 2000). During dough development torque reaches a peak value indicating maximum resistance to deformation and subsequently declines, possibly indicating depolymerisation of the gluten network or some solubilisation of the gluten proteins (Hoseney, 1998; Bekes, Gras, Gupta, Hickman & Tatham, 1994). The chemistry of dough development is very different to that of cheese with S-H to S-S interchange reactions forming new covalent bonds between two adjacent gluten chains but there are many parallels in physical behaviour. Supramolecular polymer networks also display work thickening behaviour above a critical shear rate with the critical shear rate being experimentally correlated with the lifetime of crosslinking bonds (Xu, Hawk, Loveless, Jeon & Craig, 2010).

With large shear work inputs (>70 kJ/kg) work thickening became more extreme and along with the changes in rheological properties other major changes in cheese properties occurred including loss of stretch (Fig. 4.3), syneresis leading to liquid exudation (Fig. 4.2) and loss of melt (Fig. 4.8, 4.10b). All three of these changes are caused by very strong casein-casein interactions meaning that attractive forces have become dominant over repulsive forces. Stretch can be regarded as the ability of the casein network to maintain its integrity, i.e. not break, upon continuous application of tensile stress to molten cheese. It requires flexibility of interactions between casein molecules enabling them to relax applied stresses within the time scale of deformation. Loss of stretch is an indication that casein-casein interactions have become too strong (Lucey et al., 2003).

Liquid exudation upon prolonged working is likely to be caused by a change in the structure of the cheese. Various micro-structural models proposed the presence of fat-serum channels within the fibrous casein network of Mozzarella cheese (McMahon et al., 1999). Working of Mozzarella cheese allows coalescence of proteins to form larger strands (fibres) that are oriented in the direction of deformation. The rearrangement of casein fibres results in redistribution of water and fat during working with larger protein strands being separated by channels containing water, water soluble substance, bacteria and fat globules. These channels not only improve the water holding capacity of cheese matrix but also contribute to the melting behaviour of cheese (McMahon et al., 1999). It is apparent that prolonged working increases casein-casein interactions leading to a more compact structure with diminished fat-serum channels so leading to exudation of liquid and poor melting. After prolonged working the fat globule size is small (results not shown – fat size distribution and microstructure will be covered in Chapter 6) so the fat is well locked into the protein structure and the fat content of the exuded liquid is very small (Table 4.1).

The modified Schreiber melt test assesses the ability of cheese to lose individual shred identity and then flow and spread. For optimum melting a balance of attractive and repulsive casein-casein interactions is required (Lucey et al., 2003). The decreasing tendency to melt after prolonged working is another strong indication of excessive associative protein-protein interactions either from increased hydrophobic interactions or from absence of repulsive (electrostatic) interactions or both (Lucey et al., 2003).

Overall the results suggest that the protein phase is dominant in the work thickening process and in the resulting changes in rheological and melt behaviour, though the fat phase also makes a contribution. Nonfat cheese showed similar though smaller changes in rheological behaviour (Fig. 4.7) and melt score (Fig. 4.9) to full fat cheese, and in the nonfat cheese it is clearly the protein phase that is changing. Comparing melting behaviour in Fig. 4.10b (excessively worked cheese) with Fig. 4.10c (excessively worked cheese with vegetable oil on the surface) indicated that excessive shear work had changed the protein phase and it was not just poor fat migration to the surface that was preventing melting.

Some aspects of the results are not yet well understood. First, torque versus time curves at 150 and 250 rpm (Fig. 4.1) show a maximum torque implying a maximum in cheese viscosity whereas the rheological measurements (Figs. 4.5-4.6) show no maximum. We suggest this is caused by wall slip in the Blentech as liquid exudation begins – the rheological methods used in the rheometer were chosen to minimise or eliminate wall slip (Chapter 3). Second, although rheological measurements (Figs. 4.5 and 4.6) and melt score (Fig. 4.8) versus shear work fitted reasonably well on one line the points for 250 rpm are further right than those at 150 rpm suggesting that shear work at 250 rpm is less damaging, i.e. more shear work is needed at 250 rpm to cause a given change in rheology or melt score. Similarly the maximum torque in Fig. 4.1 occurs at 66 kJ/kg for 150 rpm and at 129 kJ/kg for 250 rpm. It is suggested that this is related to the viscoelasticity of the material and the time scale of the deformation. Shear rates are higher at 250 rpm so the material behaves more elastically and less energy goes into viscous flow which is probably more important for changing the structure. Pulling Mozzarella cheese slowly causes stretching and effective interactions between the fibres whereas pulling it fast tends to break the cheese instead (Lucey et al., 2003). An alternative explanation is that the flow pattern in the Blentech is somewhat different at 250 rpm with build up of cheese against the wall at the discharge end of each screw so that some cheese stays out of the shearing zone for a time. Third, it is puzzling that the melt behaviour of nonfat cheese was less impacted by excessive shear work than that of full fat cheese (Figs. 4.9 & 4.10) and that nonfat cheese work thickened less than full fat cheese (Fig. 4.7). A possible explanation is that maybe a filled gel such as full fat cheese work thickens more than a single phase gel such as nonfat cheese. Fourth, the manual rolling process of Bast et al. (2015) caused huge work thickening in just 18 s of

rolling, i.e. an increase in tensile fracture stress of 5.7 times parallel to the fibres and 2.1 times perpendicular to the fibres. However, shearing in the Blentech at 50 rpm for 4000 s caused only small changes in steady shear viscosity. It is suggested that the one dimensional elongational flow caused by rolling is far more effective than shear flow at causing work thickening. Another possible explanation is that the shearing process in the Blentech causes repeated rupture of any new casein-casein interactions that are formed whereas the rolling operation is a slow and gentle process where the new structure formed remains intact. The four features of the results described in this paragraph warrant further study.

4.5. Conclusions

Shear work input significantly increased steady shear viscosity and decreased melt score of model Mozzarella cheese. The rheological changes indicate very significant work thickening. The work thickening process showed unusual isothermal kinetics in that the viscosity increased exponentially with either time or shear work input. At shear work inputs > 70 kJ/kg expulsion of serum liquid and loss of cheese stretch were also observed. All these changes are caused by increasingly strong, calcium-mediated, attractive casein-casein interactions after prolonged working. An inverse relationship was found between melt score and apparent viscosity. Nonfat cheese also work thickened suggesting that shear induced changes in the protein phase of the cheese are a major contributor to the effects.

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

Chapter summary

The effect of shear work was investigated 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 °C in a twin screw Blentech cooker. G' at 70 °C increased with shear work input suggesting work thickening. At lower shear work inputs (<30 kJ/kg), 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 at 150 rpm). Excessive shear work levels (>70 kJ/kg) 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.

5.1. 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, 1997a; 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 flowability (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 this 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) and studied the combined effect of thermal and mechanical energy. Chapter 4 reported changes in steady shear rheology during the mechanical working of cheese in a Blentech twin-screw cooker. 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, indicating strong work thickening behaviour. Good negative correlation ($R^2=0.90$) was found between apparent viscosity and melt score. The objective in this study is to explore the effect of shear work input on the oscillatory properties of three model Mozzarella cheeses. The 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) was used to exaggerate any work thickening effects and changes in structure.

5.2. Materials and Methods

5.2.1. Materials

The materials used are outlined in section 4.2.1.

5.2.2. Manufacture of model Mozzarella cheeses

The manufacture of the model Mozzarella cheese is outlined in section 4.2.2. Shear work input was calculated as in section 4.2.3. Melt functionality was tested as in section 4.2.4.

5.2.3. 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) (Chapters 3, 4). 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 °C (Chapter 3). 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 °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°C to 90°C. To ensure nearly isothermal conditions during temperature sweeps, the rate of temperature rise of the Peltier heating system was maintained at 1.8°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°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).

$$G' = k_{\text{elastic}}\omega^n \quad (5.1)$$

$$G'' = k_{\text{viscous}} \omega^n \quad (5.2)$$

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.

5.2.4. 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 tested for significance using 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.

5.3. Results

5.3.1. Linear viscoelastic limit

Strain amplitude sweeps for cheese samples having shear work in the range 4.9-185 kJ/kg indicated that the limit of the LVE range was about 10 % strain for all samples (Fig. 5.1). G' at low strains increased from ~194 Pa at shear work 4.9 kJ/kg to 3890 Pa at shear work 185 kJ/kg, 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.

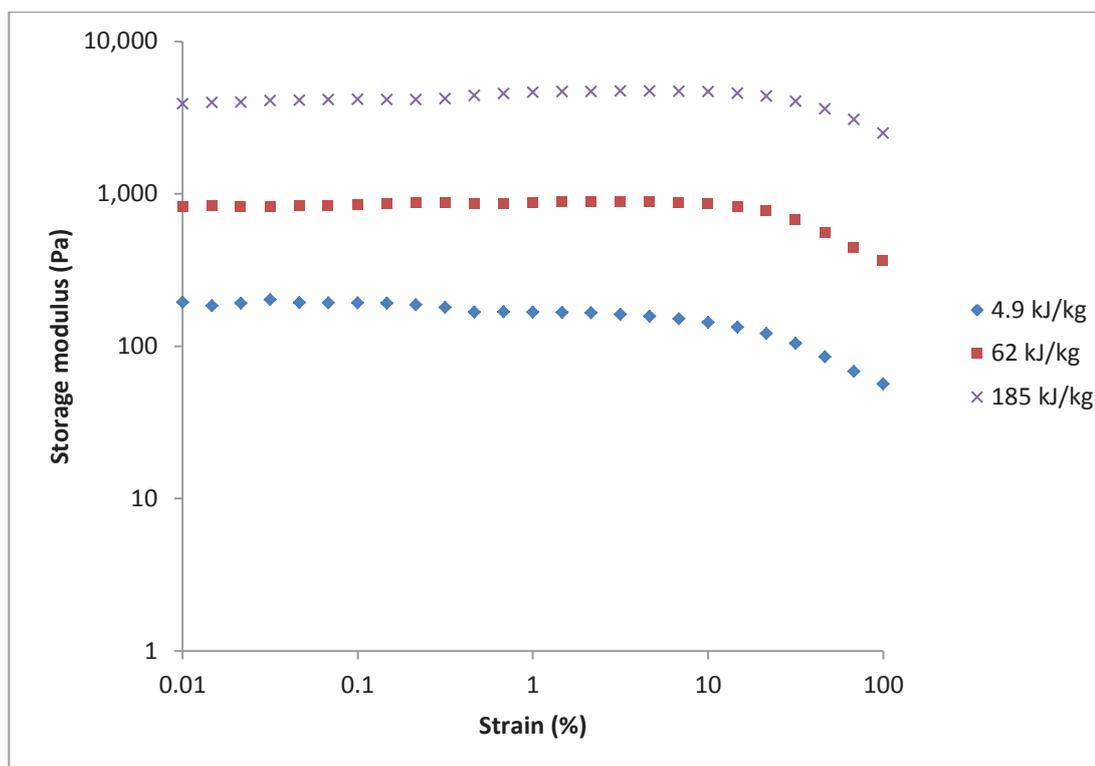


Figure 5.1 Strain sweeps of model Mozzarella cheeses subjected to different amounts of shear work. Experiments were conducted at a frequency of 1 Hz and 70 °C. Model Mozzarella cheeses were manufactured in the Blentech cooker at 250 rpm screw speed (Day 2, Table 4.1)

5.3.2. Frequency dependence of viscoelastic properties

Frequency sweeps on model Mozzarella cheeses (Fig. 5.2) 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), 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, Fig. 5.2b). 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, the frequency dependence of G' was much lower ($n=0.54$). Coincidentally, G' and G'' followed almost the same path with respect to frequency dependence for this cheese (Fig. 5.2c). 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 criterion 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°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, Fig. 5.2d) 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'' throughout 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.

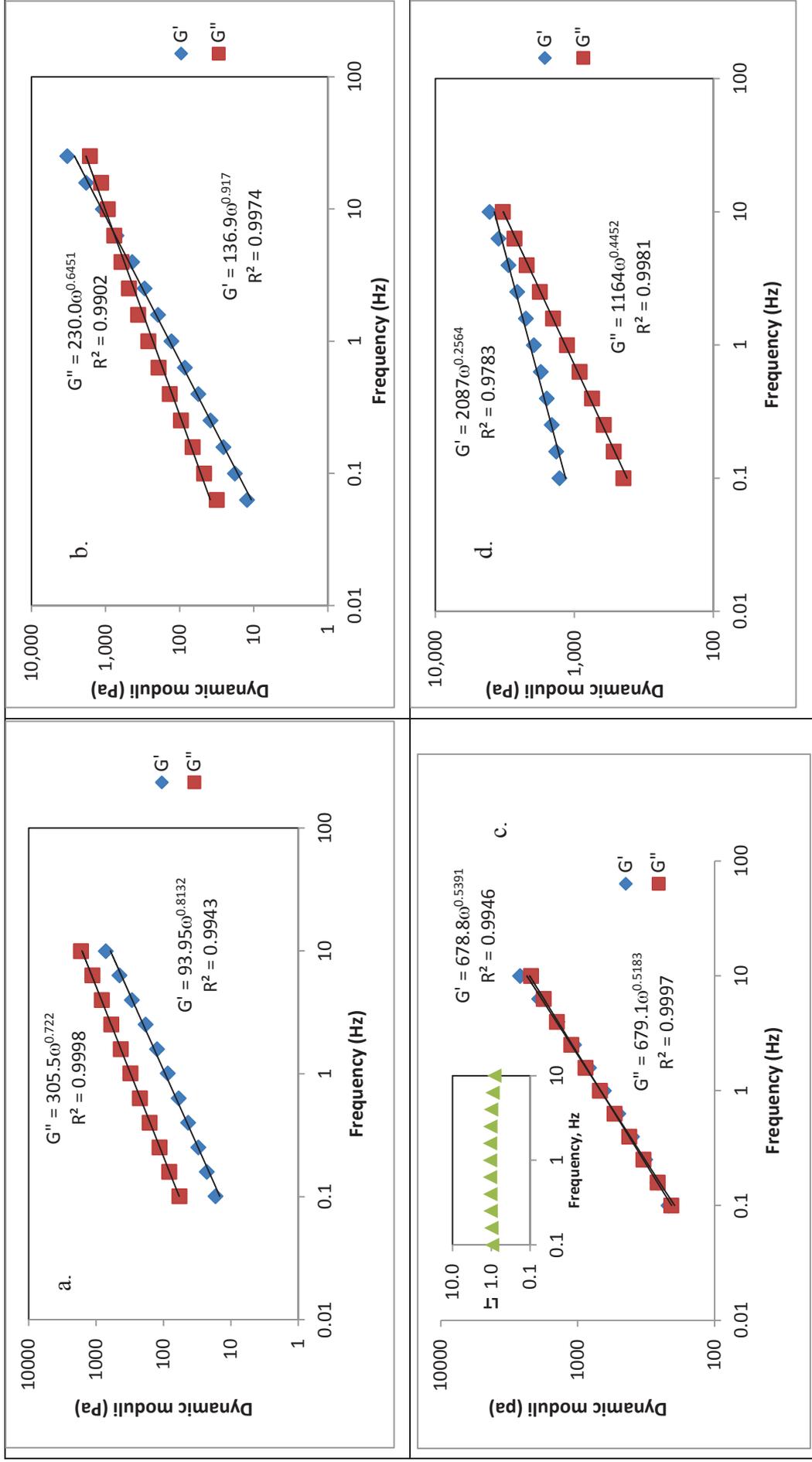


Figure 5.2 Frequency sweeps at 70°C on model Mozzarella cheeses subjected to varied amounts of shear work; a. 8.8, b. 26.3, c. 58.2, d. 73.7 kJ/kg. Model Mozzarella cheeses were manufactured in the Blentech cooker at 150 rpm screw speed (Day 1, Table 4.1) and 70 °C.

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) (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.

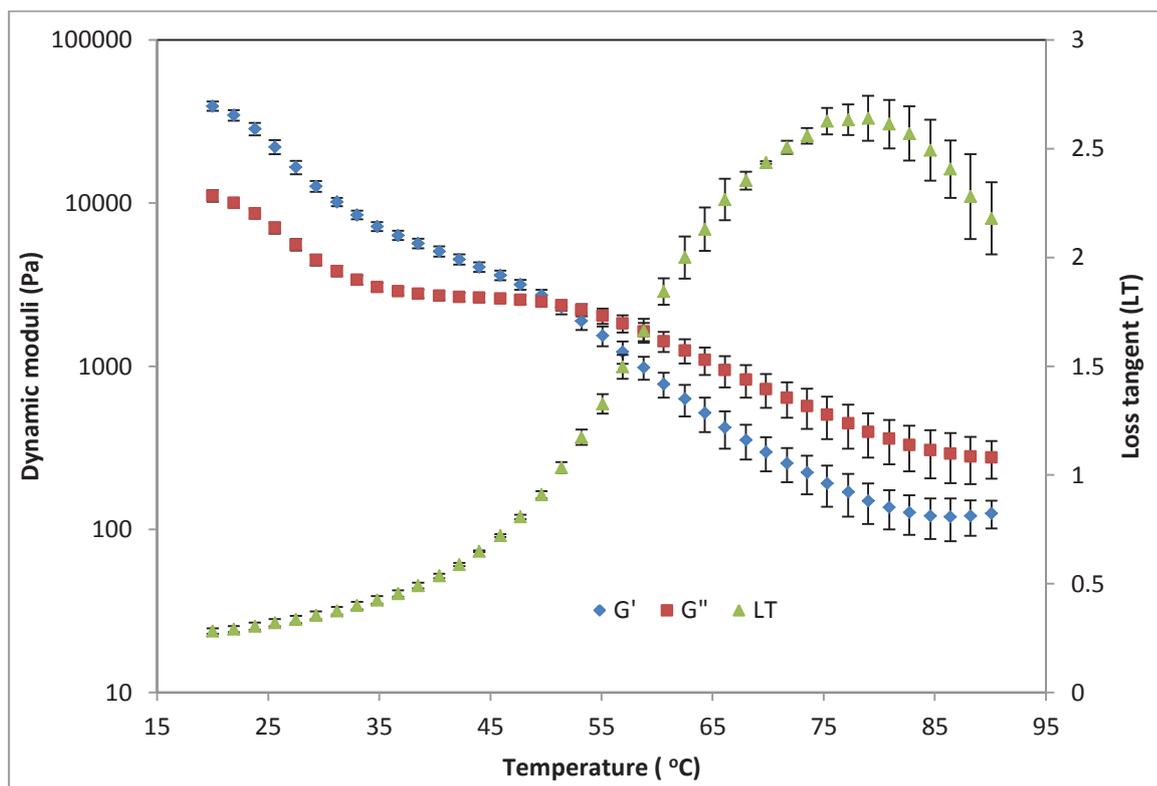


Figure 5.3 Temperature sweeps on model Mozzarella cheeses subjected to 5.0 kJ/kg of shear work. Model Mozzarella cheeses were manufactured in the Blentech cooker at 50 rpm screw speed (Day 1, Table 4.1) for 780 s and 70°C. Error bars represent one standard deviation (3 samples).

5.3.3. 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°C. Repeated temperature sweeps on one sample having 5.0 kJ/kg of shear work input were conducted first (Fig. 5.3). Both G' and G'' decreased with temperature rise throughout the test temperature range. LT continuously increased during heating, reached a peak at about 79°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°C and 50-65°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 °C was missing due to absence of fat. In the temperature range 20-50°C, the cheese behaved like a viscoelastic solid as $G' > G''$ and $LT < 1$. For the temperatures above 52°C, it acted like a viscoelastic liquid as $G'' > G'$ and $LT > 1$. The crossover temperature where $G'' = G'$ was at ~51.4°C (Fig. 5.3) 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°C, LT increased sharply until it reached LT_{\max} of 2.64 at ~79 °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 °C.

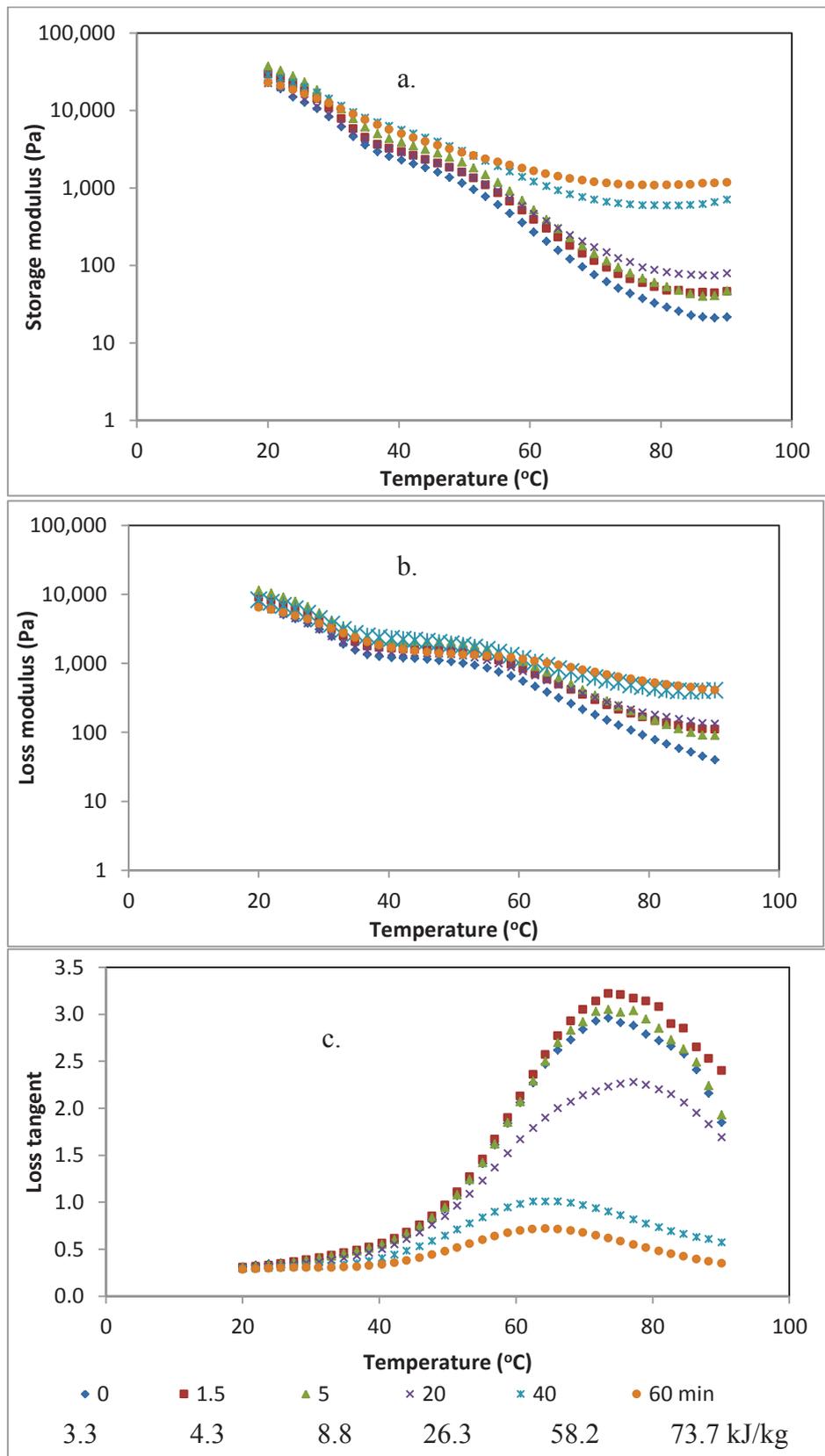


Figure 5.4 Temperature sweeps of model Mozzarella cheeses subjected to varied amounts of shear work. a. G' ; b. G'' ; c. Loss Tangent. Model Mozzarella cheeses were manufactured in the Blentech cooker at 150 rpm screw speed (Day 1, Table 4.1) and 70°C. Times and corresponding shear work inputs are given at the bottom of the figure.

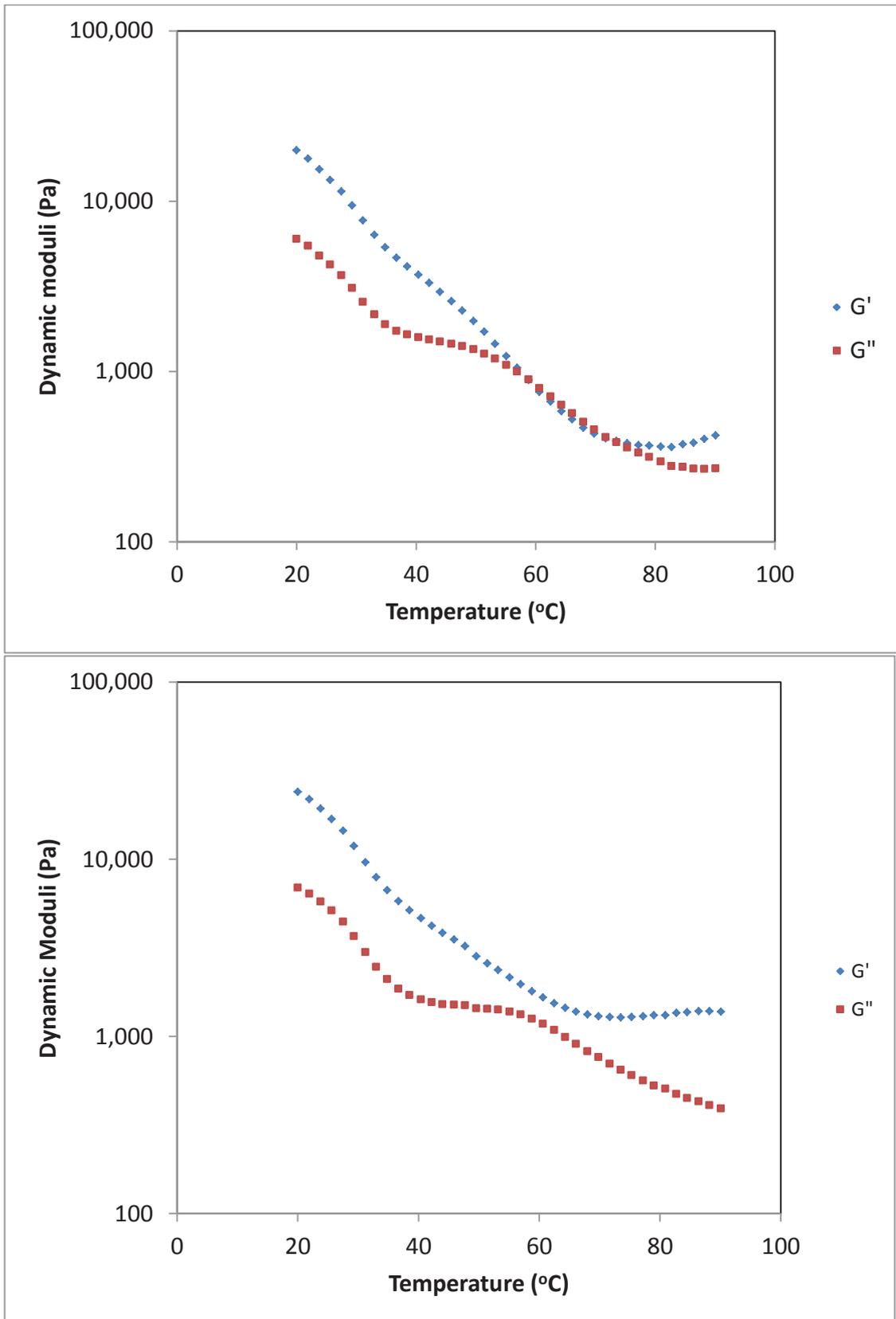


Figure 5.5 Temperature sweeps on model Mozzarella cheeses subjected to a. 58.2 b.73.7 kJ/kg shear work. Model Mozzarella cheeses were manufactured in the Blentech cooker at 150 rpm screw speed (Day 1, Table 4.1) and 70°C.

The effect of shear work on viscoelastic properties is clearly evident (Fig. 5.4) particularly in the temperature range 30-80 °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°C. Cheeses with lower shear work inputs, 3.3-26.3 kJ/kg, did not differ much in G' and G'' at ~70°C, suggesting minor changes to macroscopic structure in this shear work range. Higher shear work inputs (>50 kJ/kg) showed much higher values of both G' and G'' at temperatures of 60°C and above. Cheese with a shear work input of 58.2 kJ/kg showed overlap of G' and G'' values in the range 60-75°C (Fig. 5.5a). This corresponds well with the behaviour exhibited in the frequency sweep at 70°C (Fig. 5.2c), 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 °C in the high shear environment of the Blentech. At a still higher level of shear work input (73.7 kJ/kg), G' values were significantly higher than at lower shear work levels at all temperatures (Fig. 5.4). G' was also higher than G'' at all temperatures and no crossover temperature was observed (Fig. 5.5b). 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. 5.6). Slightly more consistent curves were found when both parameters were plotted against shear work.

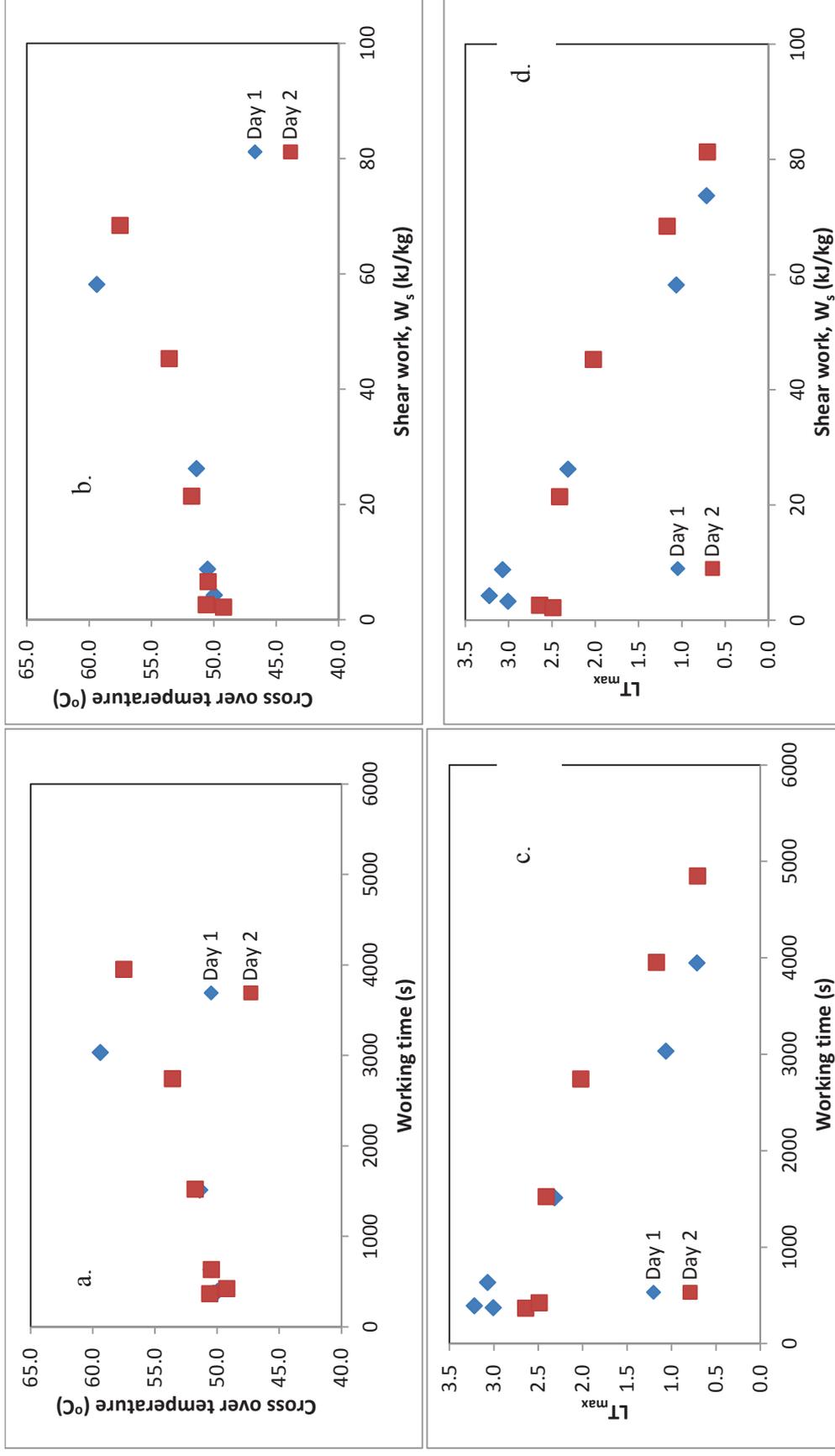


Figure 5.6 G'-G'' crossover temperature (a,b) and LT_{max} (c,d) of model mozzarella cheeses versus time (a,c) and shear work (b,d). Model Mozzarella cheeses were manufactured in the Blentech cooker at 150 rpm screw speed and 70°C (Table 4.1).

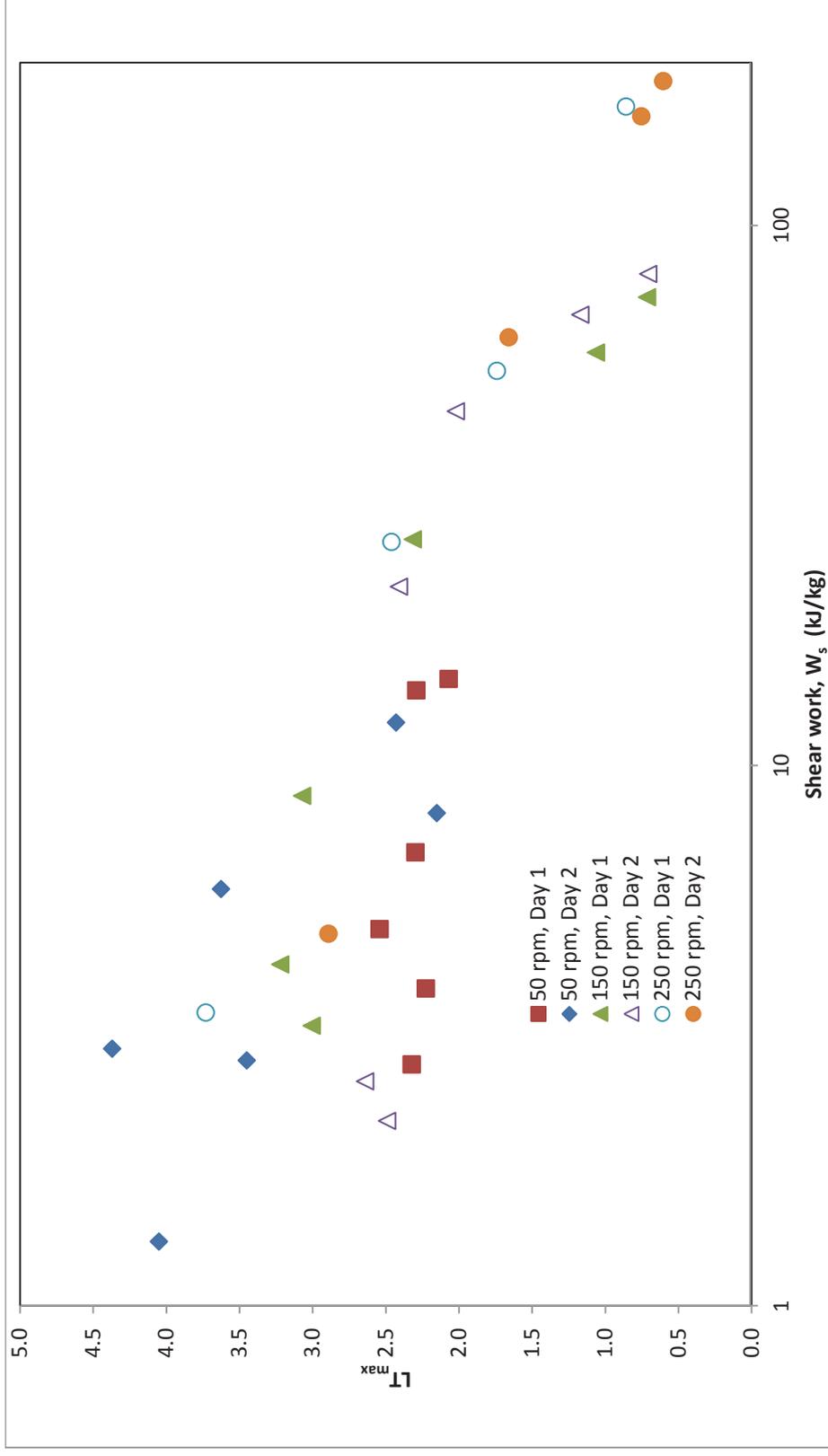


Figure 5.7 Effect of shear work on LT_{max} of model Mozzarella cheeses. Model Mozzarella cheeses were manufactured in the Blentech cooker at 50, 150 and 250 rpm screw speeds and 70°C (Table 4.1).

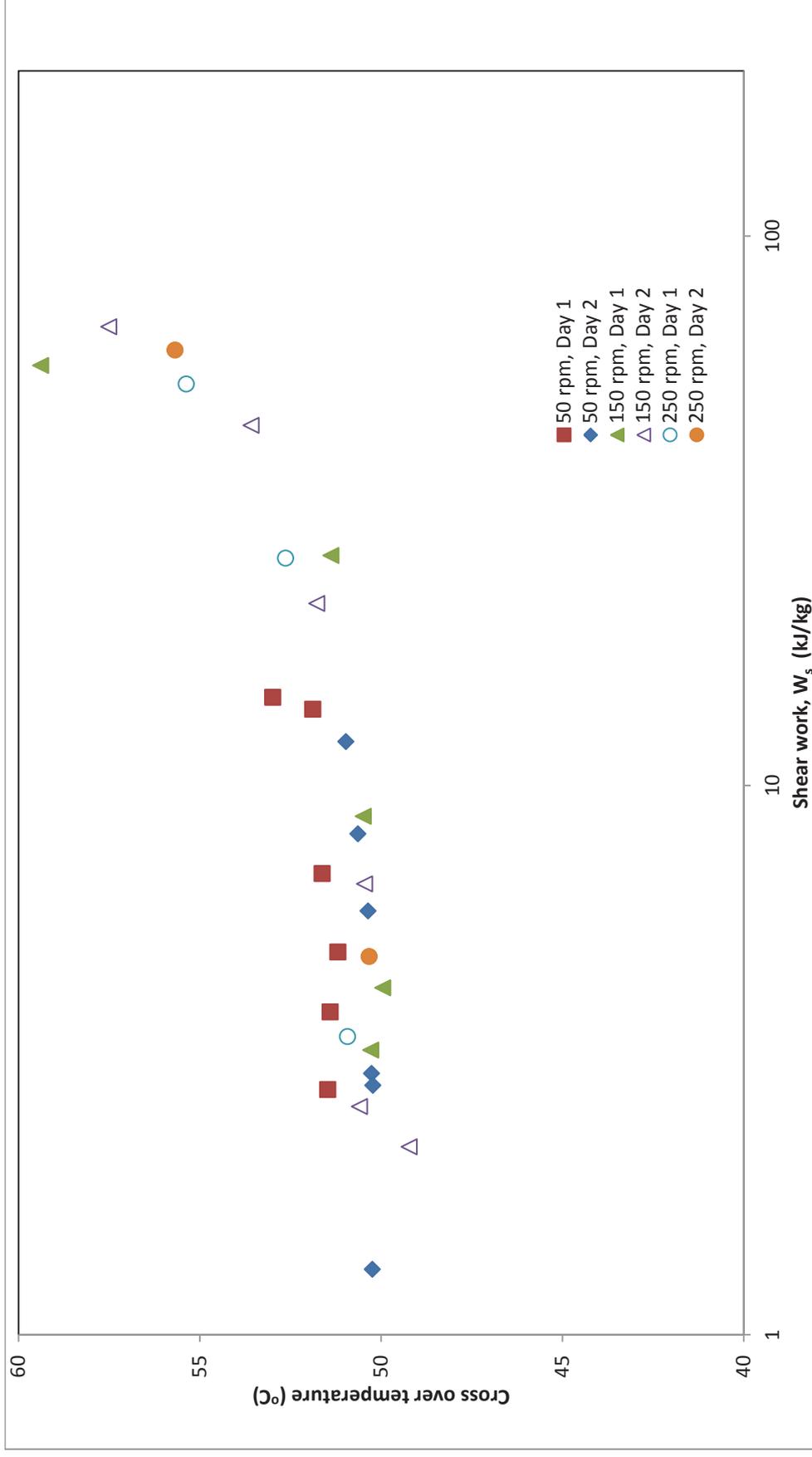


Figure 5.8 Effect of shear work on G' - G'' crossover temperature of model Mozzarella cheeses. Model Mozzarella cheeses were manufactured in the Blentech cooker at 50, 150 and 250 rpm screw speeds and 70°C.

Steady shear rheology and melt functionality data also showed better reproducibility when plotted against shear work (Chapter 4). 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 Chapter 4.

LT_{\max} and crossover temperature data for all 32 samples of full-fat cheese without TSC were plotted versus shear work (Figs. 5.7, 5.8). 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 $\sim 60^\circ\text{C}$ with shear work increase from 3.3 to ~ 60 kJ/kg. Crossover temperature is an indicator of the softening point of the cheese matrix (Gunasekaran & Ak, 2003).

In Chapter 4, it was shown 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.

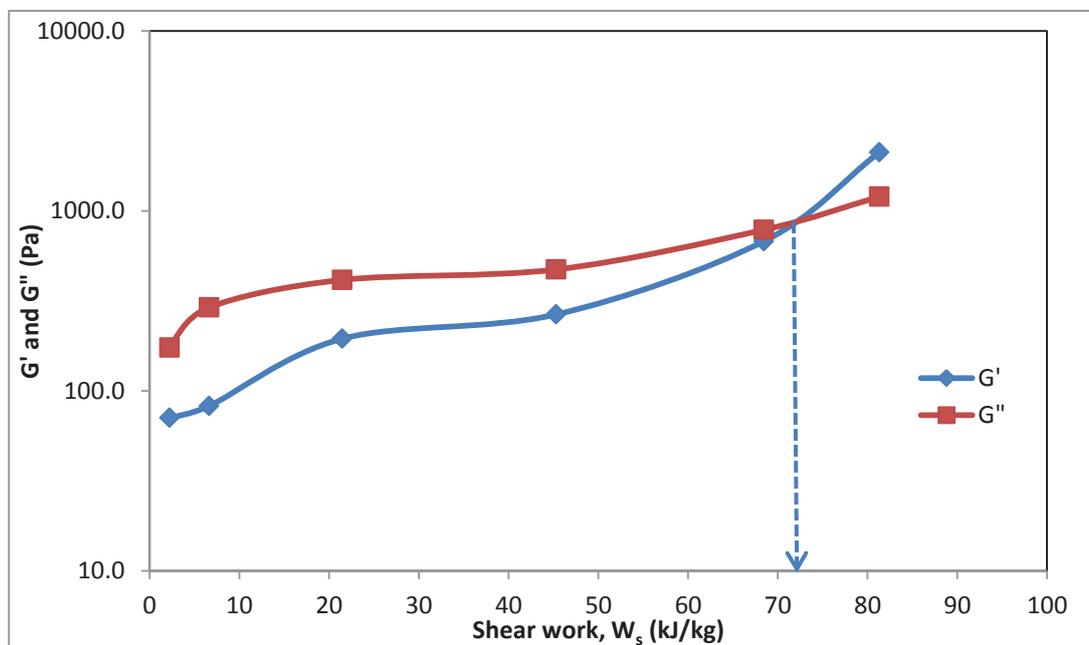


Figure 5.9 Effect of shear work on G' and G'' at 70°C for model Mozzarella cheeses. Model Mozzarella cheeses were manufactured in the Blentech cooker at 150 rpm screw speed (Day 2, Table 4.1) and 70°C . Dashed arrow indicates shear work at the transition state.

In an attempt to gain further insight into this transition, G' and G'' at 70°C were plotted against shear work for the day 2 runs at 150 rpm (Fig. 5.9). 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). The maximum in the torque-time curve occurred at 66.3 kJ/kg 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 5.1). 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 at 150 rpm screw speed was in the range 54-60 kJ/kg. This matches very well with the transition behaviour observed in the frequency sweeps at 58.2 kJ/kg shear work input (Fig. 5.2c) 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, 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 (Chapter 4).

Table 5.1 Shear work input at crossover of G' and G'' and at peak torque.

RPM	Day	Crossover $G'-G''$ * kJ/kg	Peak Torque* kJ/kg
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.

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. 5.10). For non-fat cheese crossover temperature increased from 50.1 to 55.7°C with increase in shear work from 4.4 to 128.1 kJ/kg. 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. 5.9 did not occur till 128 kJ/kg (Data not shown).

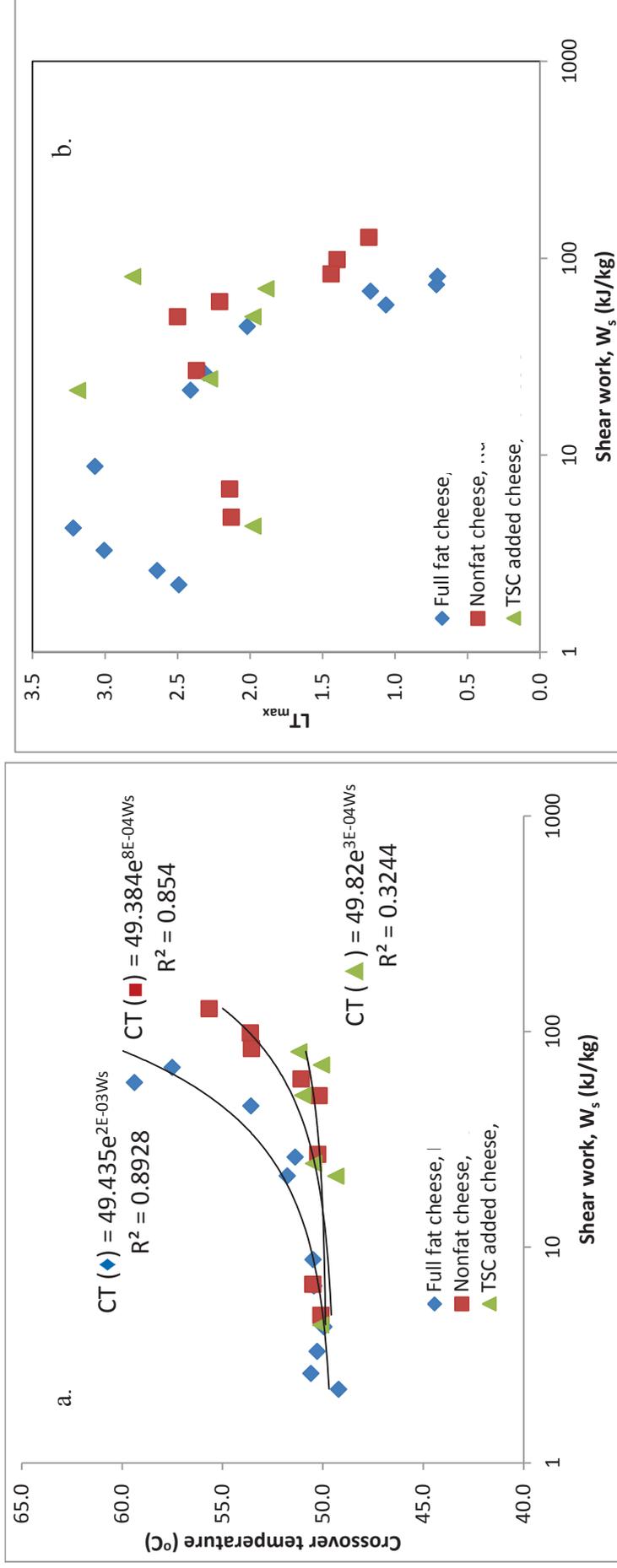


Figure 5.10 Effect of shear work on (a) $G' - G''$ crossover temperature and (b) LT_{max} of model Mozzarella cheeses - full fat, nonfat and with TSC added. Model Mozzarella cheeses were manufactured in the Blentech cooker at 150 rpm screw speed and 70°C (Table 4.1).

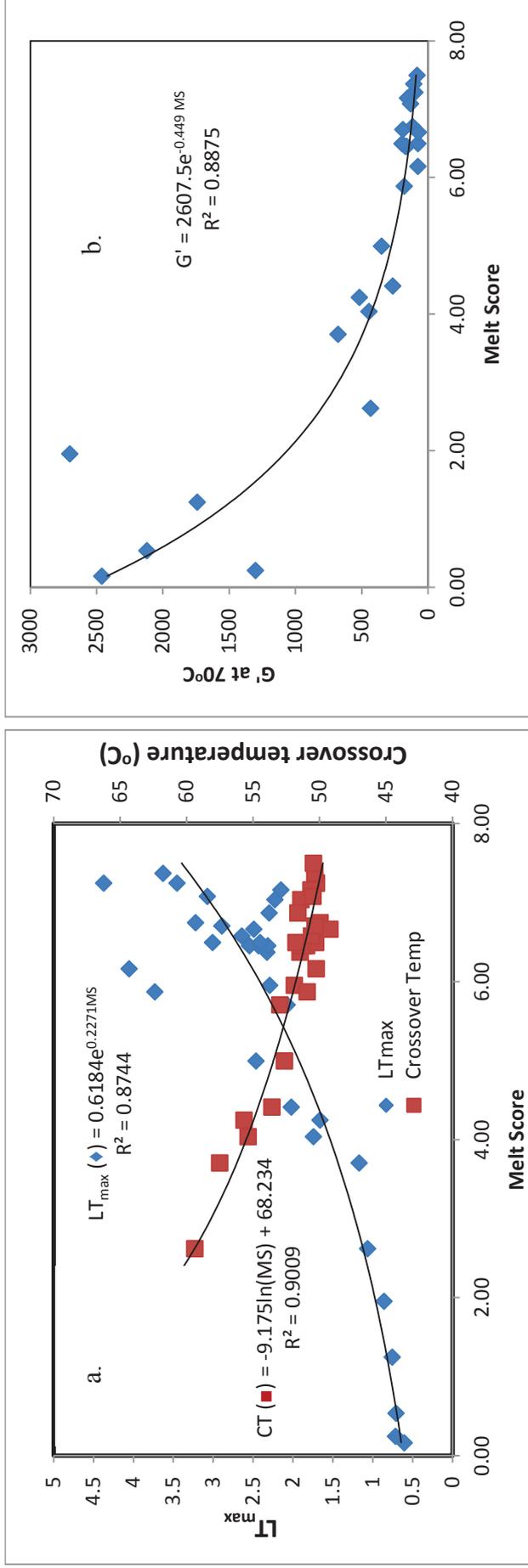


Figure 5.11 Correlation of LT_{max} and $G'-G''$ crossover temperature (a) and G' at 70°C (b) with melt score for the model Mozzarella cheeses. Model Mozzarella cheeses were manufactured in the Blentech cooker at 50, 150 and 250 rpm screw speeds and 70°C (Table 4.1).

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. 5.10), G' or G'' in the shear work input range 2-80 kJ/kg. 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) (Chapter 4).

5.3.4. 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. 5.11a). 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°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. 5.3). 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 °C was also found to correlate negatively ($R^2 = 0.89$) with melt score (Fig. 5.11b).

5.4. 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. 5.1, 5.2, 5.9). During working at 70 °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 °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. 5.2). 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. 5.2c), the overlap of the G' and G'' curves from 55 to 75°C in temperature sweeps at 58.2 kJ/kg (Fig. 5.5a) and the crossover of G' and G'' curves as a function of shear work (Fig. 5.9). The shear work values at the peak in Blentech torque-time curves (Chapter 4) correspond reasonably well to the shear work values at this critical point for structural transition (Table 5.1).

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. 5.7, 5.8). 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 5.1). The most likely explanation for this behaviour is the viscoelastic nature of the material (Chapter 4). 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. 5.2a, 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 °C changed significantly with shear work in the low shear work regime (2-10 kJ/kg) (Fig. 5.9). However, other rheological properties such as K , n , apparent viscosity at 0.01 s^{-1} , melt score (Chapter 4) and $G'-G''$ crossover temperature (Fig. 5.8) 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. 5.11). 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. 5.11).

The results indicate that protein-protein interactions strengthen as the cheese is sheared at 70 °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 in Chapter 4 and by 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. 5.10).

A schematic model is proposed in Fig. 5.12 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, Fig. 5.2b), the frequency behaviour indicates that the shear induced protein structures appear to interact with each other through physical entanglements (Fig. 5.12a). 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 (Chapter 3). 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.

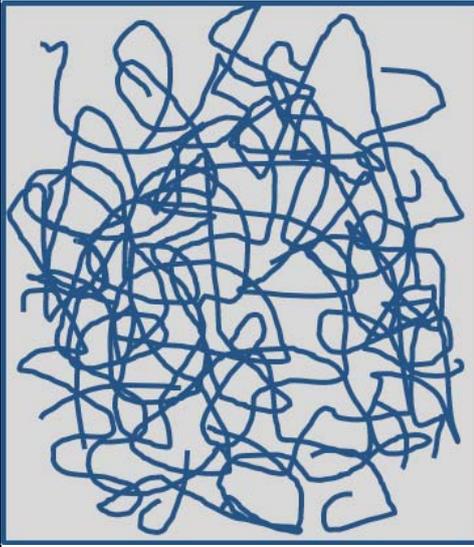
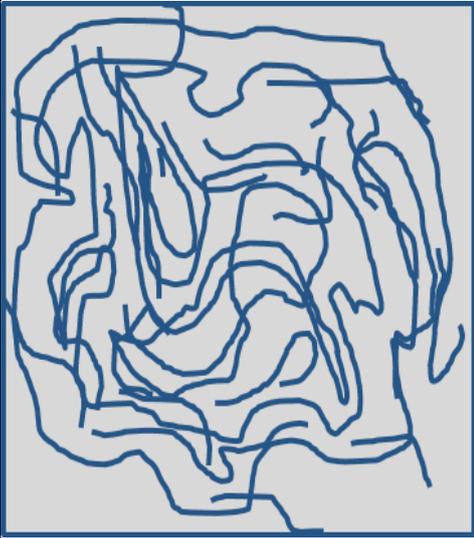
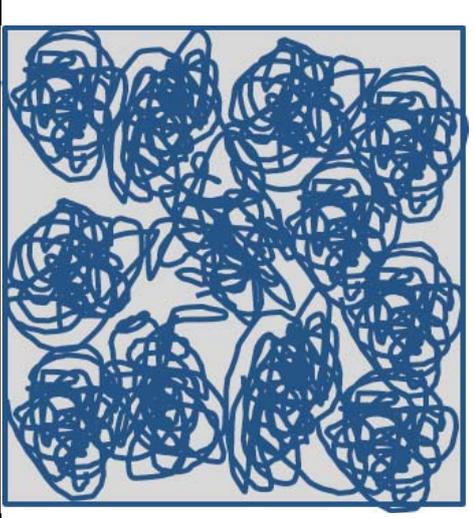
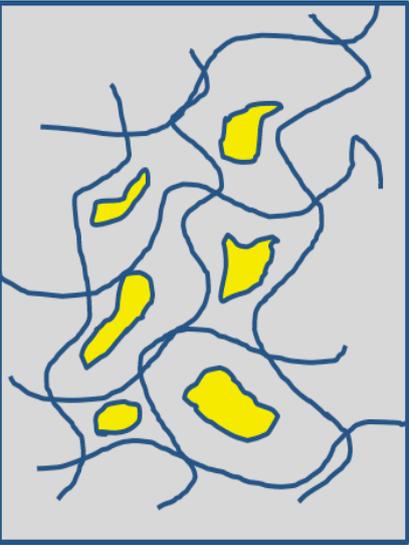
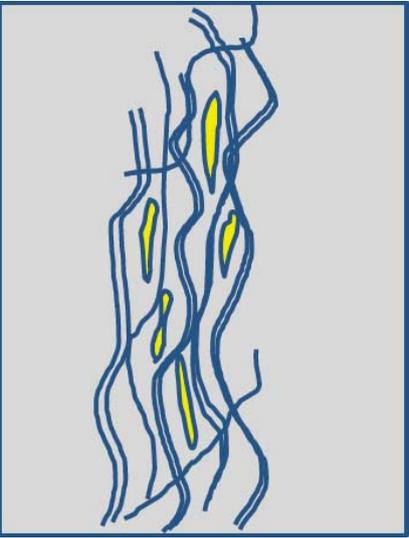
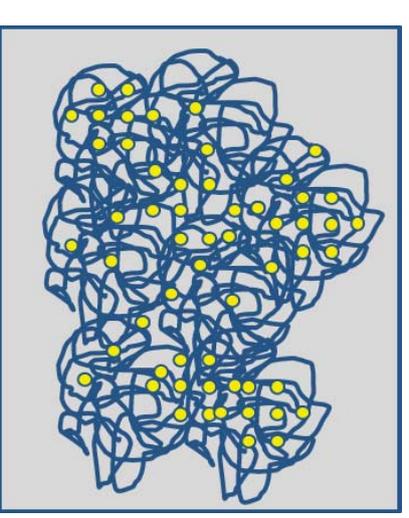
<p>Continuous phase – nonfat cheese</p>			
<p>Full fat cheese</p>			
	<p>a. Entangled polymer type behaviour at low shear work <30 kJ/kg</p>	<p>b. Structure at viscoelastic transition (Critical) point e.g. at Shear work ~58 kJ/kg using 150 rpm screw speed</p>	<p>c. Grossly overworked structure - aggregated casein particles, shear work levels >70 kJ/kg</p>

Figure 5.12 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 colour indicates fat particles. (Not to scale.).

Behaviour typical of entangled polymers is exhibited (Fig. 5.2b) 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) 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. 5.12b). Excessive shear work levels (>70 kJ/kg) 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. 5.12c). 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. 5.12 will be reported in Chapter 6 in this thesis.

5.5. 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.

6.0 Changes in creep behaviour and microstructure of model Mozzarella cheese during working

Chapter Summary

The effect of shear work input on the microstructure, fat particle size and creep behavior of sheared model Mozzarella type cheeses was studied. Cheese samples were prepared in a twin screw cooker at 70 °C by mixing protein and fat phases together with different amounts of shear work input. Major changes in cheese structure were observed while working at 150 rpm and 250 rpm screw speeds. Confocal microstructures plus macroscopic observations showed systematic changes in structure with increased shear work inputs with unmixed buttery liquid observed at <5 kJ/kg, typical Mozzarella type microstructures (elongated fat-serum channels) at 6-15 kJ/kg and homogeneously distributed, small size fat droplets at >58 kJ/kg. At very high shear work inputs, > 75 kJ/kg, striations or anisotropy in the microstructures had disappeared and small micro-cracks were evident. Volume-weighted mean fat particle size decreased with shear work input and particle size distributions also changed. A 4-element Burger's model was found adequate for fitting the creep data of model cheese at 70 °C but a 6-element model was required at 20 °C. As shear work input increased retarded compliance decreased and zero shear viscosity increased indicating the more elastic behavior of the cheeses with higher shear work input. It is believed that changes in the protein matrix are the main reason for increased elastic behavior.

6.1. Introduction

The process of Mozzarella cheese manufacture includes a hot-water (60-85 °C) stretching and working step that is normally carried out with single or twin screw cheese cookers. In this process step proteins in the cheese curd form into large protein strands resembling fibers and fat-serum pools are distributed within this fibrous network (McMahon, Fife, & Oberg, 1999). The presence of the fat-serum channels helps to deliver the desired melt functionality for pizza applications. Numerous studies have been conducted in the recent past using twin screw cookers for the manufacture of imitation cheese (Noronha, O'Riordan, & O'Sullivan, 2008; El-Bakry, Duggan, O'Riordan & O'Sullivan, 2010a, b), process cheese (Glenn, Daubert, Farkas & Stefanski, 2003; Kapoor, Lehtola & Metzger, 2004) and Mozzarella cheese (Mulvaney,

Rong, Barbano & Yun, 1997; McMahon et al., 1999; Yu & Gunasekaran, 2005; Chapter 4,b). These studies focussed mainly on the effect of processing or formulation on melt functionality.

Chapter 4 studied the steady shear rheology of model Mozzarella cheeses manufactured in a twin screw Blentech cooker with shear work input as a major variable. Steady shear viscosity increased exponentially with shear work input indicating strong work thickening. Very high shear work inputs led to macroscopic structural breakdown of the cheese network with disappearance of the fibrous structure, loss of stretch, serum syneresis and decrease in melt functionality. These phenomena were attributed mainly to an increase in the strength of protein-protein interactions. Steady shear viscosity was negatively correlated with melt functionality. Chapter 5 studied the oscillatory rheology of the same set of model Mozzarella cheeses. Frequency sweeps indicated that the cheese transformed from a viscoelastic liquid to a viscoelastic solid upon working at 70 °C. A critical stage indicating viscoelastic transition during processing was identified at a shear work input of 58.2 kJ/kg at 150 rpm. Mulvaney et al. (1997) also emphasised that the viscoelastic properties of Mozzarella cheese were influenced by the thermomechanical treatment given in a stretcher-cooker.

Stress relaxation and creep-recovery are common tests used for exploring transient viscoelastic behavior of many materials (Mezger, 2011). Both tests are used to study time dependent rheology in the linear viscoelastic region and both apply mechanical models e.g. Kelvin-Voigt for creep behavior. Many studies have been conducted on Mozzarella type cheeses and similar casein-based materials using creep-recovery and stress relaxation methods (Subramanian, Muthukumarappan & Gunasekaran, 2003; Muliawan & Hatzikiriakos, 2007; Manski, van der Zalm, van der Goot & Boom, 2008; Olivares, Zorrilla & Rubiolo, 2009; Bähler, Nägele, Weiss & Hinrichs, 2015). Creep tests are more common for cheese rheology, as they are easier to perform, can describe material behavior more practically and are the best way of obtaining zero-shear viscosity. Creep-recovery tests can also be used to study the internal structure of Mozzarella cheese and physicochemical changes with temperature and during ripening (Olivares et al., 2009).

The current study focuses on microstructural changes in model Mozzarella cheeses with varying shear work input using confocal laser scanning microscopy, fat particle size

analysis and creep-recovery behavior as tools. Nonfat cheese was included in the study to observe microstructural changes occurring in the absence of fat.

6.2. Materials and Methods

6.2.1. Materials

The materials used are outlined in section 4.2.1.

6.2.2. Manufacture of model Mozzarella cheeses

The manufacture of the model Mozzarella cheese is outlined in section 4.2.2. Shear work input was calculated as in section 4.2.3.

6.2.3. Fat particle size distribution

The fat particle size distribution of cheeses was obtained by disrupting the cheese matrix with chelating solution A (Walstra, 1965) and measuring size distributions using light scattering on the Mastersizer 2000 (Malvern Instruments, Worcestershire, UK). The experimental protocol suggested by Lee, Anema and Klostermeyer (2004) was used with some modifications. A representative sample was collected from at least three different locations in the cheese. Approximately 0.5 g cheese was added to 50 ml solution A and mixed by gentle swirling action to minimize shear effects. Solution A contained 0.375% w/w EDTA and 0.125% v/v Tween 20 at pH 10. Cheese samples were held for 16 h after solution A addition. Particle size measurements were performed at room temperature (21°C). Refractive indices were taken as 1.33 for the deionised water dispersant and 1.46 for milk fat. Particle size data were reported as average volume weighted diameter ($D_{4,3}$).

6.2.4. Confocal scanning laser microscopy (CSLM)

Confocal microscopy was used to determine the microstructure of cheese samples. Slabs of ~12 x 4 mm were cut from the cheese samples in the longitudinal fibre direction using a sharp razor blade and were then transferred to a stud holder with polyethylene glycol on the surface. Samples were frozen at -20 °C and sectioned into 50 µm slices on a cryo-microtome. Slices were immediately transferred to glass slides, stained with 0.4% Nile red and 0.2% fast green (made in citifluor to minimise photobleaching) and covered with a coverslip. The sectioned samples were stored at 4

°C for at least 48 h before imaging to ensure uniform dye uptake. Confocal images were taken using a Zeiss LSM 510 META confocal microscope (Carl Zeiss AG, Oberkochen, Germany) with excitation wavelengths of 488 nm and 633 nm. Images were taken 15 μm below the cheese surface.

6.2.5. Transient viscoelastic measurements (Creep)

The transient viscoelastic behavior of model Mozzarella cheese was studied by conducting creep and recovery tests at 20 °C or 70 °C. Creep tests were performed 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). Disc-shaped cheese samples of 20 mm diameter and ~ 2 mm thickness were prepared and equilibrated to test temperature as previously described in Chapter 3 except that a 1 N normal force was used to define the measurement gap and also to ensure good contact with the upper rotating plate. A 25 Pa shear stress was applied for 1001 s and then removed. The cheese was allowed to recover its strain for 3000 s. The resultant strain was measured as a function of time during the creep and recovery phases. The applied shear stress (25 Pa) was confirmed to be well within the linear viscoelastic limit using dynamic rheological tests.

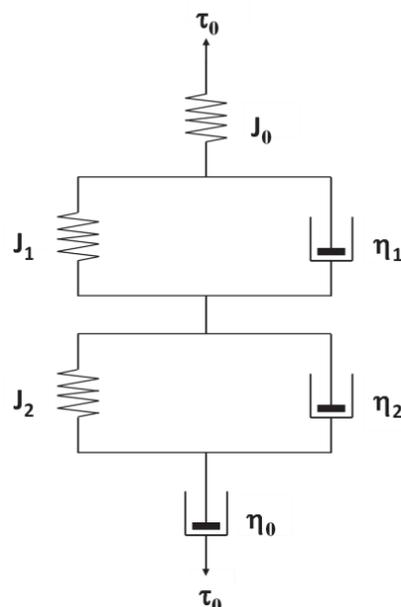


Figure 6.1 Six-element linear viscoelastic mechanical model used for describing creep and recovery behaviour of model Mozzarella cheeses. The model parameters are defined in section 6.2.5.1

6.2.5.1. Kelvin-Voigt model

Creep behaviour can be represented by a series of mechanical spring and dashpot elements. Four and six element Kelvin-Voigt models (also known as Burgers models) were fitted to the experimental creep data. The six element model comprises a Maxwell element (spring and dashpot in series) in series with two Kelvin elements (spring and dashpot in parallel) (Fig. 6.1). The Maxwell element adds the instantaneous compliance (spring) and zero shear viscosity controlling permanent deformation (dashpot). The creep phase was analysed to determine fit parameters and both the creep and recovery phases were then predicted with these fit parameters. Data are presented in the form of shear creep compliance (J) as outlined in Steffe (1996):

During the creep phase

$$J = f(t) = \frac{\gamma(t)}{\tau_0} \quad (6.1)$$

$$\gamma(t) = \frac{\tau_0}{G_0} + \frac{\tau_0}{G_1} \left(1 - e^{-\frac{t}{\lambda_1}}\right) + \frac{\tau_0}{G_2} \left(1 - e^{-\frac{t}{\lambda_2}}\right) + \frac{\tau_0}{\eta_0} \cdot t \quad (6.2)$$

$$J(t) = J_0 + J_1 \left(1 - e^{-\frac{t}{\lambda_1}}\right) + J_2 \left(1 - e^{-\frac{t}{\lambda_2}}\right) + \frac{1}{\eta_0} \cdot t \quad (6.3)$$

During the recovery phase

$$\gamma(t) = \gamma_{max} - \frac{\tau_0}{G_0} - \frac{\tau_0}{G_1} \left(1 - e^{-\frac{t}{\lambda_1}}\right) - \frac{\tau_0}{G_2} \left(1 - e^{-\frac{t}{\lambda_2}}\right) \quad (6.4)$$

Where

$\gamma(t)$ = Shear strain at time t

γ_{max} = Maximum strain attained during creep phase

τ_0 = Applied shear stress, Pa

G_0 = Instantaneous shear modulus, Pa

J_0 = Instantaneous shear compliance, Pa⁻¹

G_1 & G_2 = Viscoelastic moduli of two retarded elements, Pa

J_1 & J_2 = Retarded compliances, Pa⁻¹

η_0 = Zero-shear or Newtonian viscosity, Pa.s

λ_1 & λ_2 = retardation times of two retarded elements = $\frac{\eta_1}{G_1}$ and $\frac{\eta_2}{G_2}$, s

η_1 & η_2 = Shear viscosity in viscoelastic region, Pa.s

The parameters were obtained from the experimental curves in a stepwise fashion. G_0 was first calculated from 45 data points in the 0 - 0.85 s time range. η_0 was then calculated from 33 data points in the 420 – 975 s range. The final 4 parameters in equation 6.2 were obtained using the successive residual method in Excel. An alternative calculation method used non-linear regression in SigmaPlot (version 11.0) to obtain 5 of the parameters in equation 6.2 after η_0 had been determined as above and subtracted. The ‘5-parameter exponential rise to maximum’ model within the global curve fitting wizard was used. The alternative method gave similar values of the 5 parameters with goodness of fit $r^2 > 0.99$ (level of significance 5%) indicating that the 6 element model fitted the experimental data well. Relative recovery of strain at the end of the recovery step was also calculated (Patel, Dumlu, Vermeir, Lewille, Lesaffer, & Dewettinck, 2015).

$$\% \text{ Relative recovery, } \gamma_r = \frac{\gamma_{max} - \gamma_{end}}{\gamma_{max}} \cdot 100 \quad (6.5)$$

where γ_{end} is the strain at the end of the recovery phase.

6.3. Results

6.3.1. Microstructure of sheared model mozzarella cheeses

Confocal images of the model Mozzarella cheeses manufactured with varied shear work inputs are presented in Figs. 6.2, 6.3 & 6.4 for 50, 150 and 250 rpm screw speeds, respectively. Shear work induced microstructural changes at 50 rpm screw speed appear to be minor or subtle. However, major changes in both fat and protein phases were observed for 150 and 250 rpm screw speeds. In the initial stages of mixing (shear work typically < 5kJ/kg) milk fat can be seen in relatively large pools in the protein network (Fig.6.2, 1.3 & 2.9 kJ/kg and Fig. 6.4, 3.5 kJ/kg). At a macroscopic level at such low shear work values the cheese was runny, often with some buttery liquid present indicating that the cream was not yet well mixed into the structure (Chapter 4). Such large milk fat pools would lead to excessive fat leakage upon cheese melting.

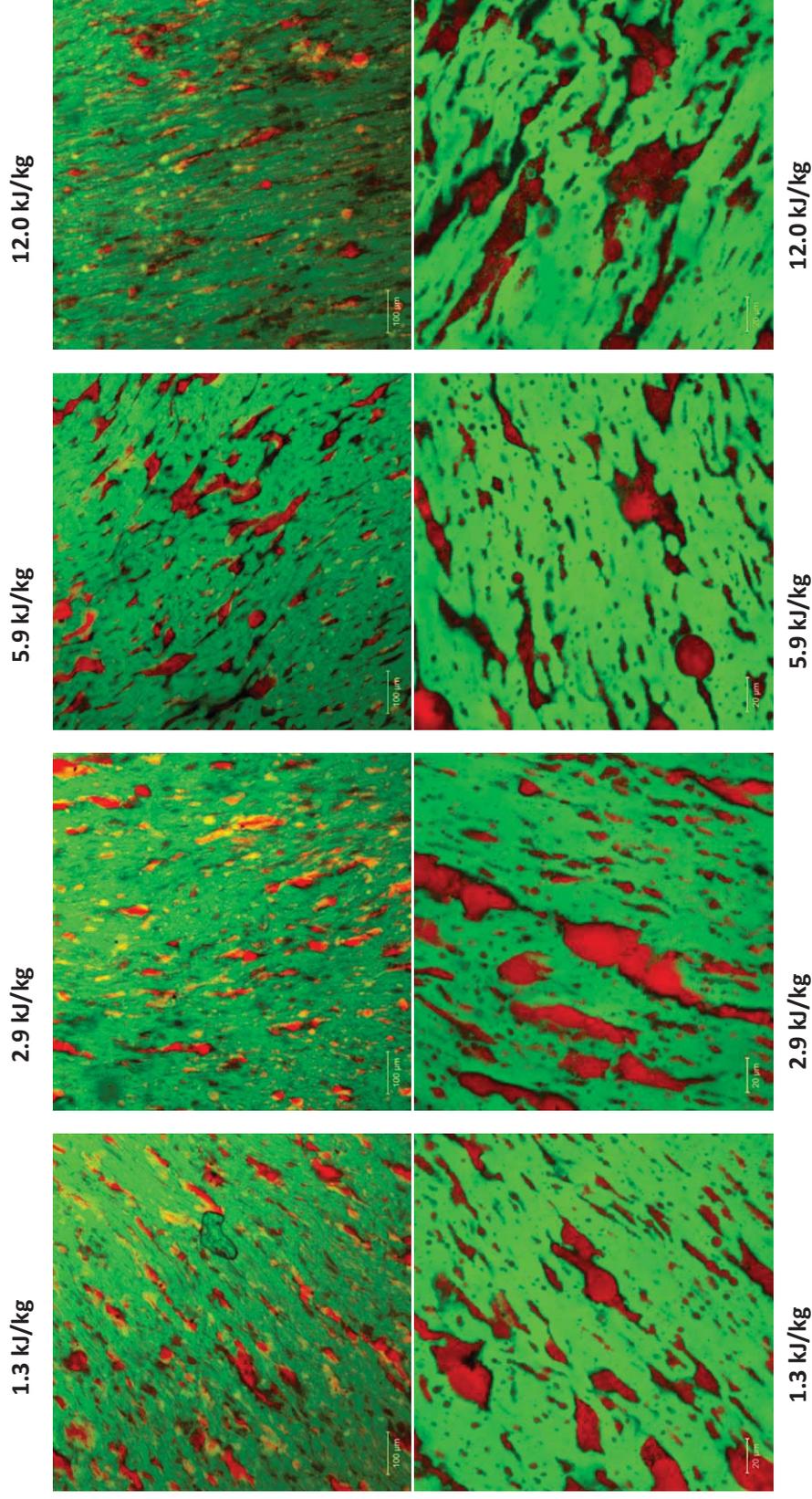


Figure 6.2 Confocal laser scanning microscopic images of model Mozzarella cheeses subjected to different amounts of shear work. Shear work inputs are noted on the micrographs. Model Mozzarella cheeses were manufactured in the Blentech cooker at 50 rpm screw speed (Day 2, Table 4.1) and 70°C. Red – fat, green – protein, black - air or water.

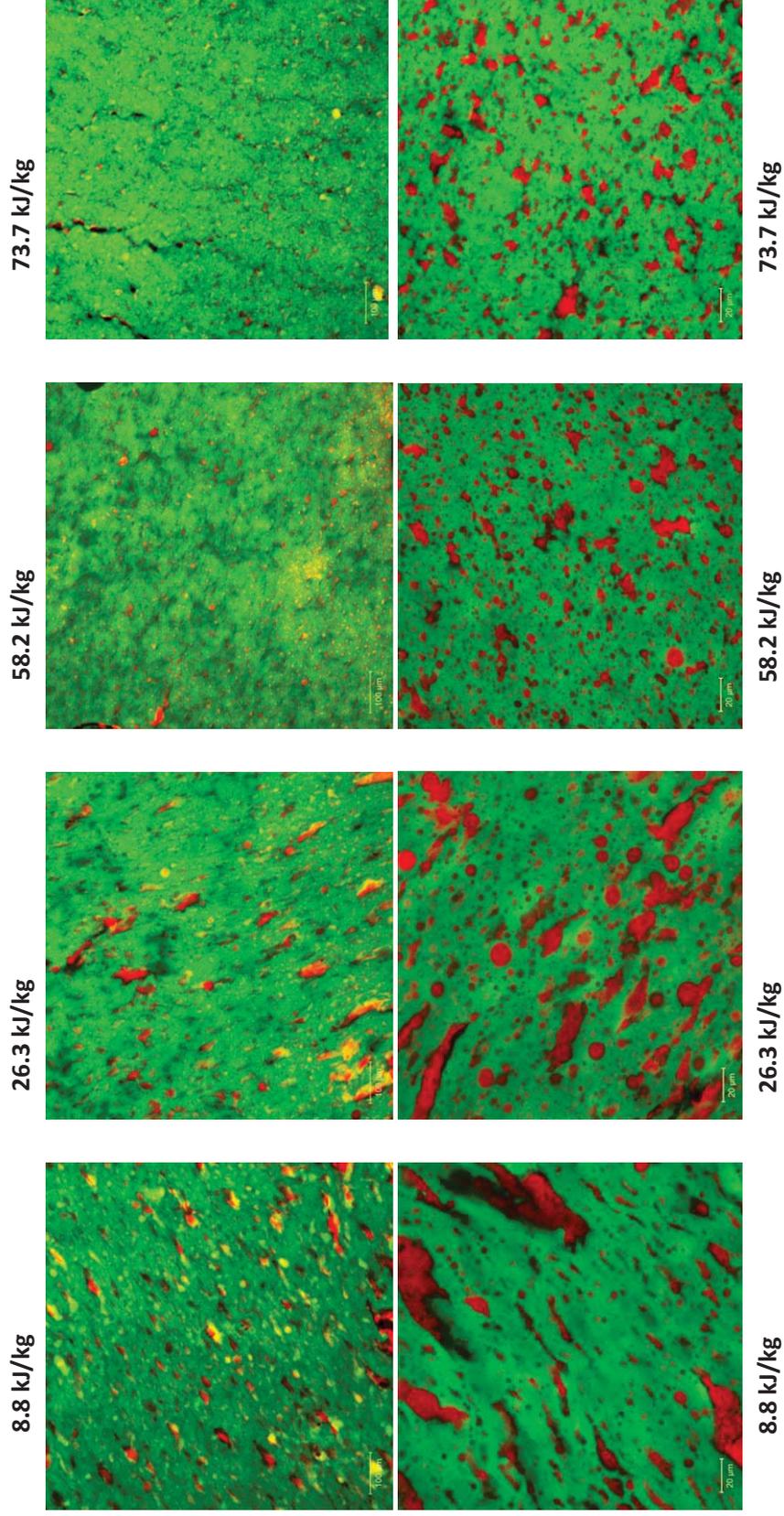
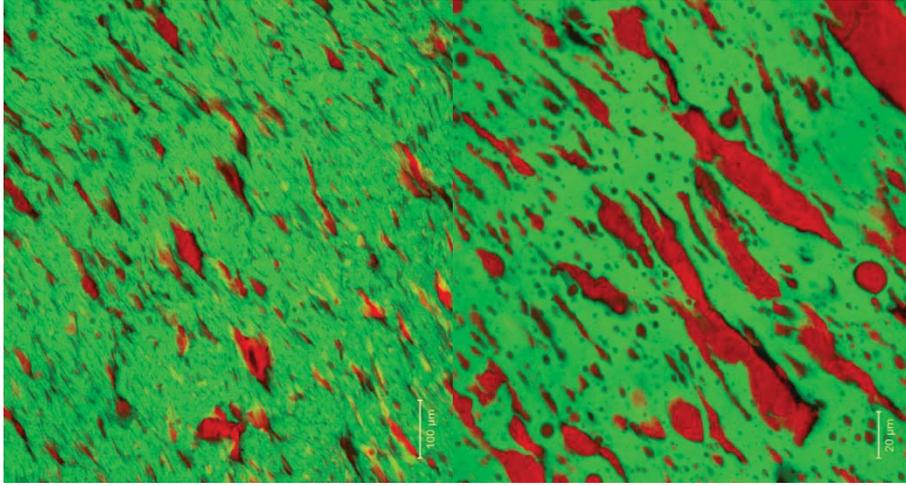
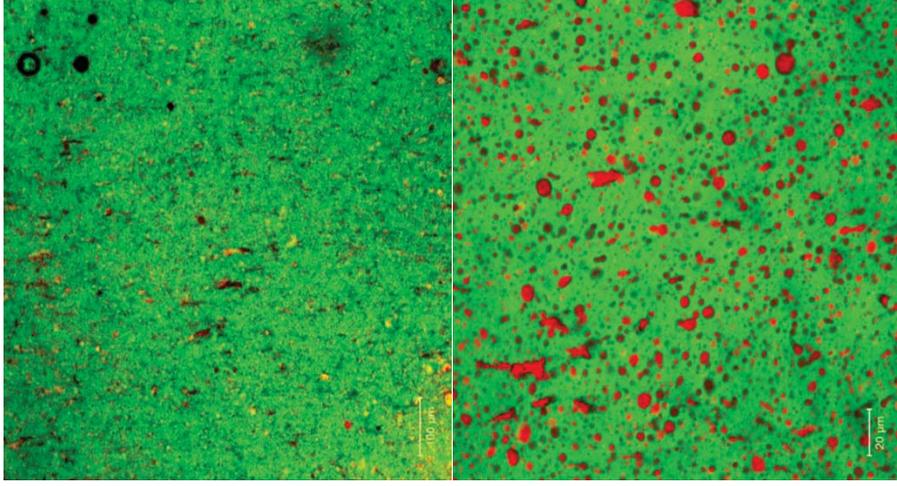


Figure 6.3 Confocal laser scanning microscopic images of model Mozzarella cheeses subjected to different amounts of shear work. Shear work inputs are noted on the micrographs. Model Mozzarella cheeses were manufactured in the Blentech cooker at 150 rpm screw speed (Day 1, Table 4.1) and 70°C. Red – fat, green – protein, black – air or water.

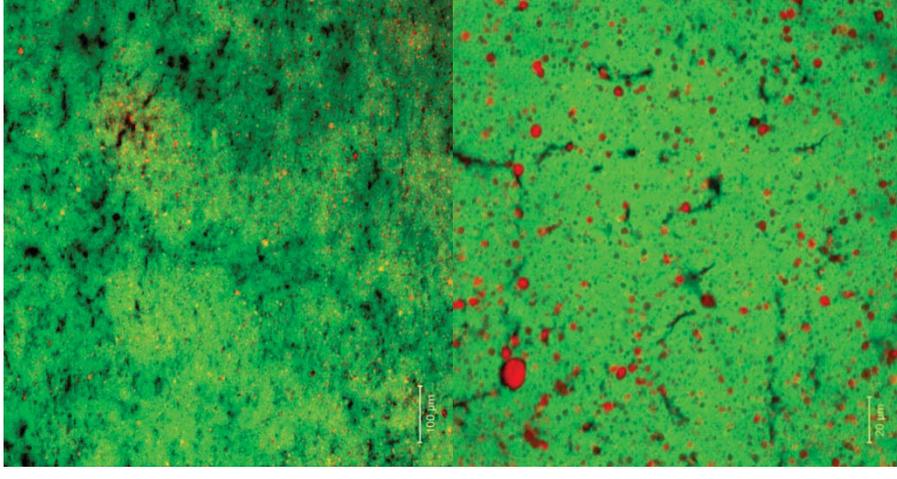
3.5 kJ/kg



53.9 kJ/kg



166.0 kJ/kg



3.5 kJ/kg

53.9 kJ/kg

166.0 kJ/kg

Figure 6.4 Confocal laser scanning microscopic images of model Mozzarella cheeses subjected to different amounts of shear work. Shear work inputs are noted on the micrographs. Model Mozzarella cheeses were manufactured in the Blentech cooker at 250 rpm screw speed (Day 1, Table 4.1) and 70°C. Red – fat, green – protein, black – air or water.

With further mixing (shear work 6-15 kJ/kg), a more typical mozzarella structure was observed (Fig. 6.2, 5.9 & 12.0 kJ/kg and Fig. 6.3, 8.8 kJ/kg). No unmixed creamy liquid was observed at a macroscopic level. For all microstructures at shear work <40 kJ/kg striated or anisotropic protein network structures were observed with fat dispersed in the protein mainly in the form of channels or elongated fat droplets. Hot water stretching and kneading action in the traditional manufacture of Mozzarella type cheeses also converts the casein mass into smooth, elongated and aligned microfibers in the direction of stretch with elongated fat channels between the fibers (McMahon et al., 1999; Oberg, McManus & McMahon, 1993).

Cheeses with shear work in the range 50-60 kJ/kg showed microstructures where elongated fat structures had virtually disappeared. The structures were isotropic and the fat globule size was now much smaller (Fig. 6.3, 58.2 kJ/kg and Fig. 6.4, 53.9 kJ/kg). At the highest shear work inputs microstructures showed a very fine dispersion of fat particles and an isotropic structure. Fine micro-cracks were also observed indicating a brittle material (Fig. 6.3, 73.7 kJ/kg and Fig. 6.4, 166 kJ/kg). These overworked cheeses no longer showed a fibrous macrostructure, were mechanically brittle and lacked stretch (Chapter 4).

6.3.2. Fat particle size distributions

Fat particle size distributions for all samples were either bimodal or trimodal distributions (Fig. 6.5a). The largest volumetric frequency peak near 35 μm and a smaller peak near 0.5 μm occurred for all 6 samples. The dominance of particles around 35 μm agrees with the confocal images. For the three samples at the highest shear work inputs a third peak in the range 2 – 4 μm is observed. At lower shear work values (<30 kJ/kg) the proportion of smaller particles was lower and the overall span of the distribution was larger. Cheese with the lowest shear work input (3.3 kJ/kg) showed a wide distribution with some very large particles (500 μm) that disappeared with further shear work input. Further increases in shear work input resulted in an increase in the proportion of smaller fat particle sizes and a narrowing in the width of the biggest peak (~35 μm). El-Bakry, Duggan, O’Riordan and O’Sullivan (2011) also reported narrowing of the fat globule size distribution upon mixing of imitation cheese in a twin screw cheese cooker.

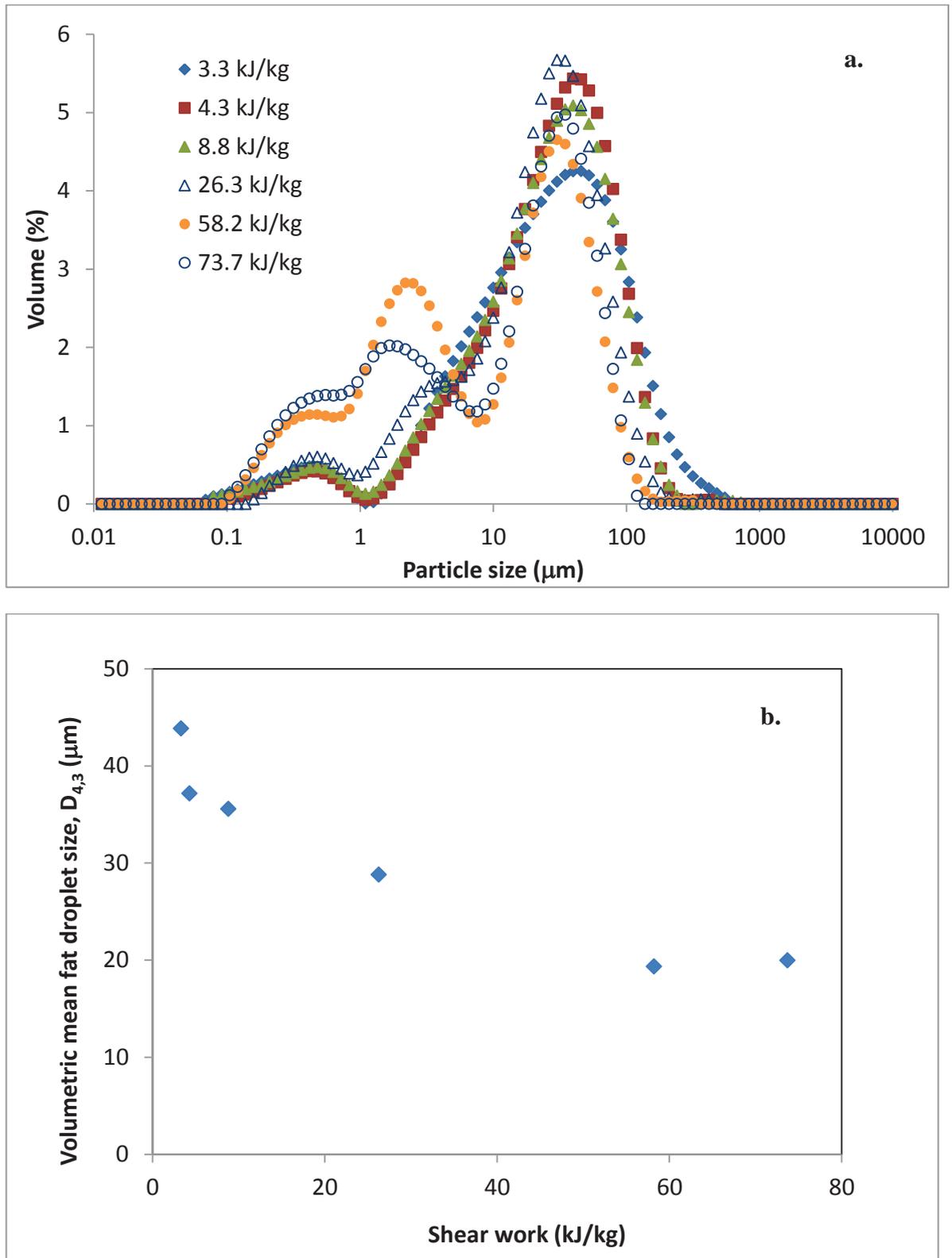


Figure 6.5 Effect of shear work on fat particle size of model Mozzarella cheese. a. Fat particle size distributions of model cheeses having varied shear work input; b. Volumetric mean fat particle size versus shear work input. Model Mozzarella cheeses were manufactured in the Blentech cooker at 150 rpm screw speed (Day 1, Table 4.1) and 70°C. Data represent the average of two measurements on the same sample.

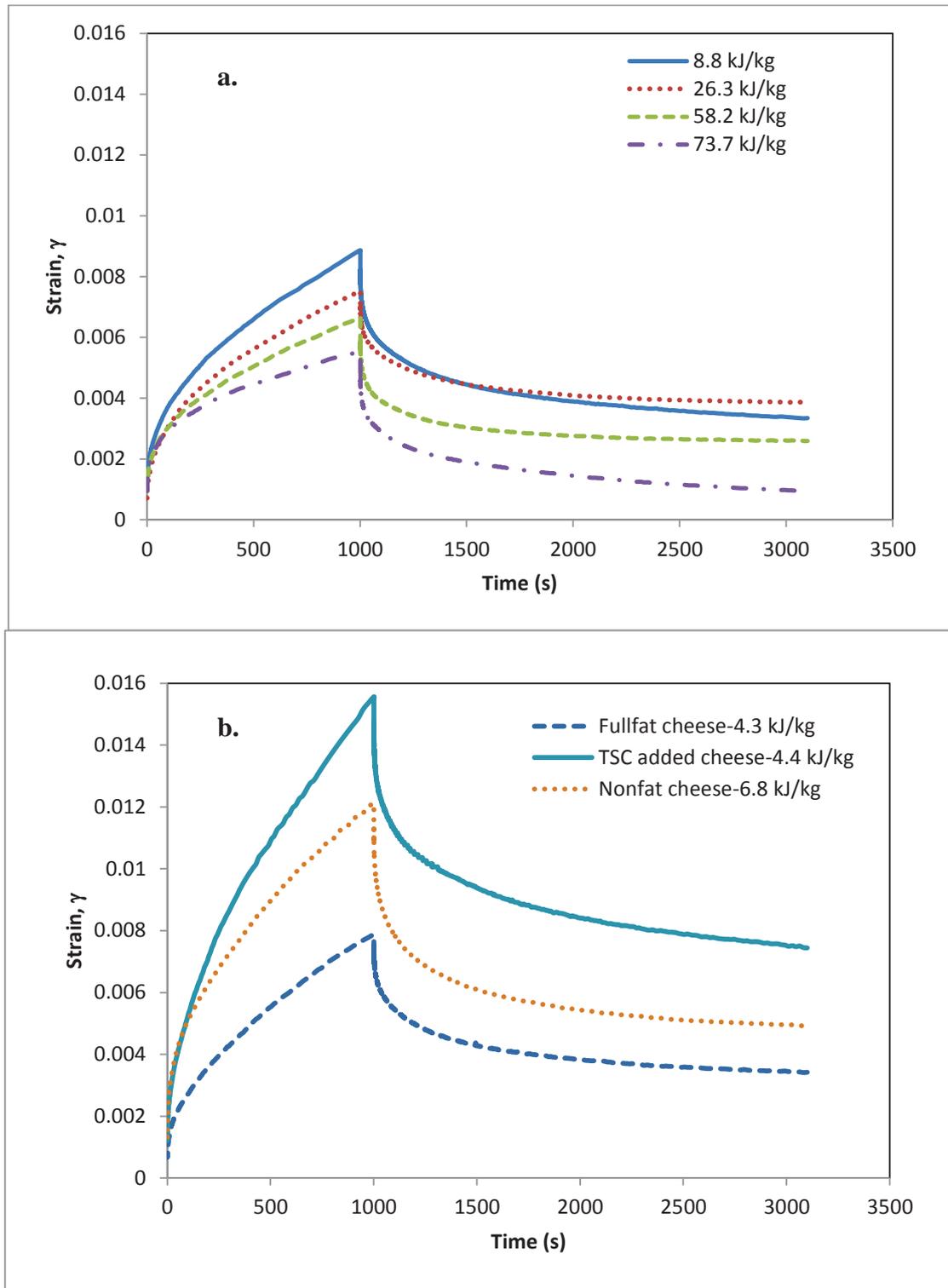


Figure 6.6 Creep and creep recovery curves of model Mozzarella cheeses at 20°C. Applied shear stress was 25 Pa (within the linear viscoelastic limit). a. Full fat cheeses having different shear work input; b. Full fat cheese, nonfat cheese and TSC added cheese with similar shear work input. Model Mozzarella cheeses were manufactured in the Blentech cooker at 150 rpm screw speed and 70°C (Table 4.1).

The presence of large particles (500 μm) and tiny particles ($< 1 \mu\text{m}$) was not evident on the confocal images indicating the practical limits of confocal imaging - limited sample selection and particle resolution $> 1 \mu\text{m}$.

Mean fat particle size ($d_{4,3}$) decreased with shear work input (Fig. 6.5b). The mean fat particle size decreased from 45 μm to 20 μm as shear work input increased from 3.3 to 74 kJ/kg.

6.3.3. *Transient viscoelastic properties*

Creep and recovery was used to study the transient viscoelastic nature of the model cheeses. Three distinct regions are visible in the creep phase of all the cheeses (Fig.6.6). These regions are instantaneous elastic deformation, viscoelastic or delayed elastic deformation and pure viscous creep. The strain during the first two regions (elastic and viscoelastic) is expected to be fully recovered whereas the strain from the last region (viscous flow) will result in permanent deformation. The relative contribution of the regions changed as shear work input increased. For all cheeses, a significant contribution of viscous flow to overall creep is observed as there was always residual permanent deformation even 3000 s after shear stress was removed.

Table 6.1 shows the maximum shear strain (γ_{max}), relative recovery of shear strain and fitted creep parameters using a 6-element Burger's model. γ_{max} decreased with shear work input. For shear work < 30 kJ/kg, γ_{max} was in the range of 0.0076-0.01. At the highest shear work input γ_{max} was 0.0051. Lower levels of γ_{max} indicate hardening of the material. γ_{max} was higher for nonfat cheese (0.011) and higher again for full fat cheese with TSC added (0.016). This indicates that nonfat cheese and TSC full fat cheese were softer than their full fat counterpart.

The extent of shear strain recovery should indicate the relative proportion of elastic components (instantaneous strain and delayed elasticity) in the cheeses. A high proportion of viscous creep will give a low relative recovery of shear strain. For shear work inputs in the range 3-58 kJ/kg, strain recovery was in the range of 57-65% (Fig. 6.6a and Table 6.1). However, for the excessively worked sample (74 kJ/kg) the recovery was much higher (85%). This indicates a significant decrease in the viscous flow component for excessively worked full fat cheese. This is expected given its much higher steady shear viscosity (Chapter 4). At similar shear work inputs (3-9 kJ/kg),

nonfat cheese exhibited a higher shear strain recovery than full fat cheese (Table 6.1). Fat at 20 °C is known to undergo plastic deformation contributing to permanent deformation. Nonfat cheese is therefore more elastic. Cheese with TSC added showed the lowest strain recovery of any of the cheeses. Chelation of salts such as calcium by TSC gives this cheese a lower steady shear viscosity (Chapter 4) so there is a higher viscous creep.

From the six elements of the Burger's model three represent elastic behavior, i.e. time-independent instantaneous compliance (J_0) and time dependent retarded compliances (J_1 and J_2). J_0 represents the Hookean spring element which is related to the undisturbed cheese structure consisting of a protein network and partially solidified fat (Olivares et al., 2009; Subramanian et al, 2003). The protein network in cheese is regarded as the major contributing factor to the elastic behavior. Higher values of J_0 indicate less rigidity meaning that the protein network is relatively free to rearrange between crosslinks (Olivares et al., 2009) and the material shows higher deformations. J_0 for the full fat model cheeses ($2.67-4.18 \times 10^{-5} \text{ Pa}^{-1}$) showed no definite trend with increase in shear work input (Table 6.1). J_0 for nonfat cheese was higher than that for full fat cheese indicating less rigidity.

J_1 and J_2 are the major components of the viscoelastic behaviour of model Mozzarella cheese (Subramanian et al., 2003). J_2 indicates the size of the fast viscoelastic deformations whereas J_1 indicates the size of the slower viscoelastic deformations. J_2 does not vary systematically with shear work. The decrease in J_1 with increasing shear work input indicates an increase of rigidity (Table 6.1). Values of both J_1 and J_2 were higher for nonfat cheese and cheese with TSC added than for full fat cheese suggesting a higher viscoelastic component to the response and a lower rigidity.

Retardation time (λ) is another important parameter in viscoelastic behaviour. It quantifies the delayed response to applied stress and can be linked to delayed elasticity (Mezger, 2011). λ_2 was in the range 3.3-5.1s and λ_1 in the range 129-193 s for all three types of model cheeses. No trends were evident for either λ_1 or λ_2 as a function of either shear work input or model cheese type. However, it was evident that J_1 decreased and η_1 increased with shear work input suggesting an increase in both elastic and viscous behaviour. Retardation time is inversely related to network elasticity.

Table 6.1 Fitted creep parameters obtained for model Mozzarella cheeses using a 6-element Burgers model*.

Cheese Type	Shear work (kJ/kg)	Maximum shear creep (γ_{\max})	Relative recovery of shear strain (%)	J_0 (10^{-5} Pa $^{-1}$)	J_1 (10^{-5} Pa $^{-1}$)	η_1 (10^6 Pa.s)	λ_1 (s)	J_2 (10^{-5} Pa $^{-1}$)	η_2 (10^5 Pa.s)	λ_2 (s)	η_0 (10^6 Pa.s)
Full fat	3.3	0.0097	56.9	3.29	9.91	1.32	129.71	2.47	1.01	3.32	4.49
	4.3	0.0079	59.9	2.67	8.88	2.17	191.17	2.93	1.39	4.11	5.95
	8.8	0.0089	64.9	4.18	10.1	1.57	158.89	4.25	0.93	4.00	5.57
	26.3	0.0076	64.7	3.09	8.45	1.81	153.08	4.16	1.21	5.05	5.98
	58.2	0.0066	60.1	3.14	7.73	2.11	160.27	4.31	1.17	5.01	7.99
	73.7	0.0051	84.9	2.96	5.64	2.74	153.66	2.95	1.36	3.95	12.10
Nonfat	6.8	0.0113	70.3	5.32	12.0	1.61	193.03	6.46	0.69	4.52	4.74
TSC added											
full fat	4.4	0.0157	54.8	4.72	16.8	1.00	170.31	4.82	0.72	3.75	2.79

* 25 Pa of shear stress was applied at 20 °C for 1001 s. The recovery phase was 3000 s. The parameters are defined in section 6.2.5.1.

Therefore, more elastic material should have smaller retardation times, while softer materials tend to have longer retardation times. Maybe the changes in J_1 and η_1 are such that no significant changes in retardation time occur.

η_0 measured by the creep method corresponds to the zero shear viscosity as the shear rates in this region are very low (10^{-6} s^{-1}). η_0 increased exponentially with shear work input. Steady shear viscosity at higher shear rates also increased exponentially with shear work input (Chapter 4). The exponential increase in viscosity during mixing and working of model Mozzarella cheese is linked with the increased strength of the protein matrix either because of more protein-protein bonds or an increase in their strength.

The 6-element Burger's model with parameters calculated from the creep phase data fits experimental data in the creep phase very well (Fig. 6.7). These parameters also fit experimental data for the first 500 s of the recovery phase, but do not fit adequately in the later stages. The experimental data indicates a higher recovery of applied strain than the model predicts. Fitting a 6 element model to the data for just the recovery phase also gives good curve fits but with much longer time constants than those in Table 6.1, e.g. 50 s and 990 s for full fat cheese with 4.3 kJ/kg shear work input.

At 70 °C the creep curve (Fig. 6.8) for full fat cheese indicated that overall deformation was dominated by pure viscous flow causing a high amount of permanent deformation with only 48% strain recovery. A 4-element Burger's model was found to fit the experimental data well. The following creep function was obtained at 70 °C after applying 0.05 Pa shear stress.

$$J(t) = 1.25 \times 10^{-4} + 3.71 \times 10^{-2} \left(1 - e^{-\frac{t}{10.46}}\right) + \frac{1}{491} \cdot t \quad (6.6)$$

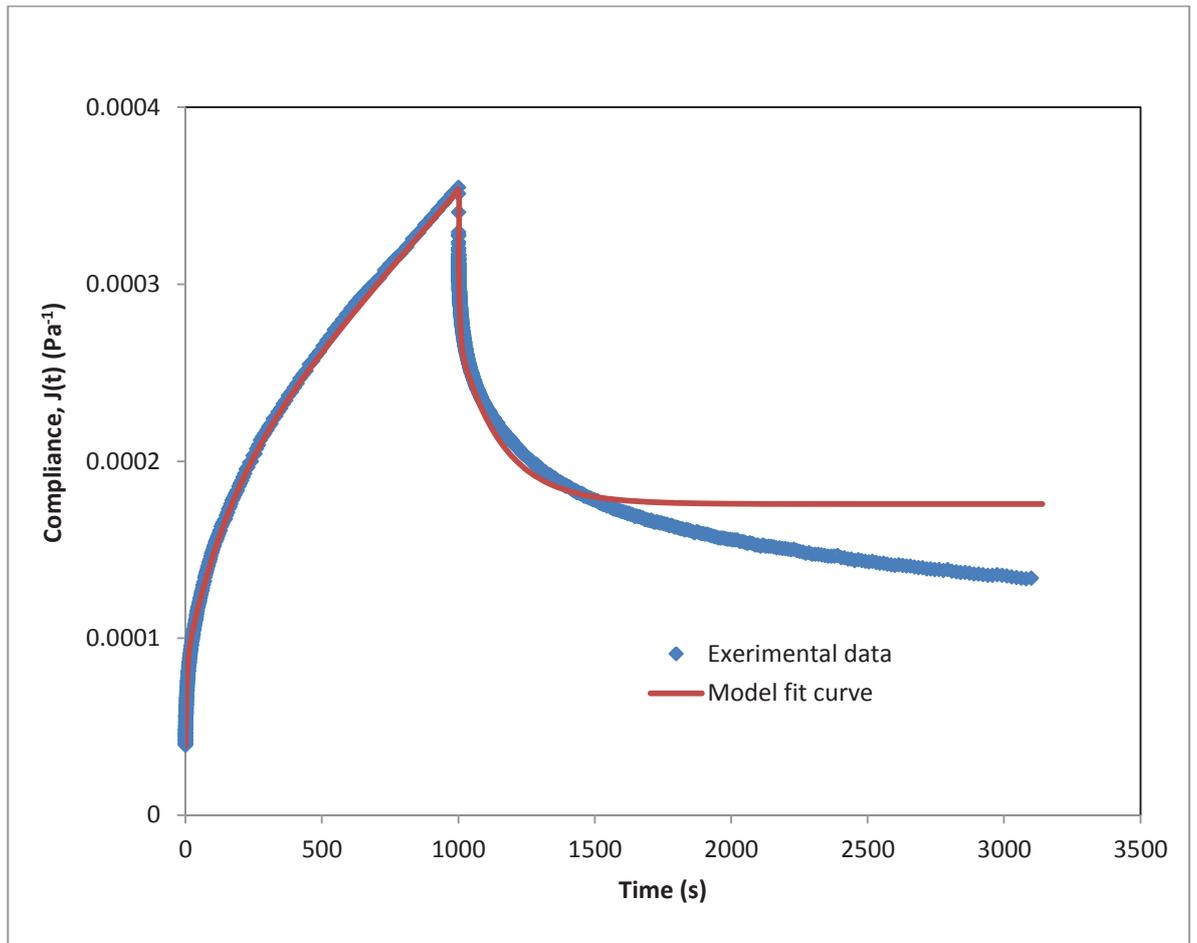


Figure 6.7 Creep and recovery compliance data and fitted curve of model Mozzarella cheese at 20°C. Applied shear stress was 25 Pa (within the linear viscoelastic limit). The fitted curve was obtained from predicted values of compliance using a 6-element Burger's model with parameters calculated for the creep phase only. The model cheese had 8.8 kJ/kg of shear work input and was manufactured in the Blentech cooker at 150 rpm screw speed and 70°C (Table 4.1).

Compared to data for the same sample at 20 °C J_0 is 3 times higher and J_1 is 366 times higher indicating a much less rigid structure. η_1 is 0.00018 times and η_0 is 0.000088 times that at 20 °C. At 70 °C both fat and protein phases are molten and therefore contribute significantly to viscous flow but the viscosity is much lower. Only one retardation time of 10.46 s is needed to represent the behavior at 70 °C.

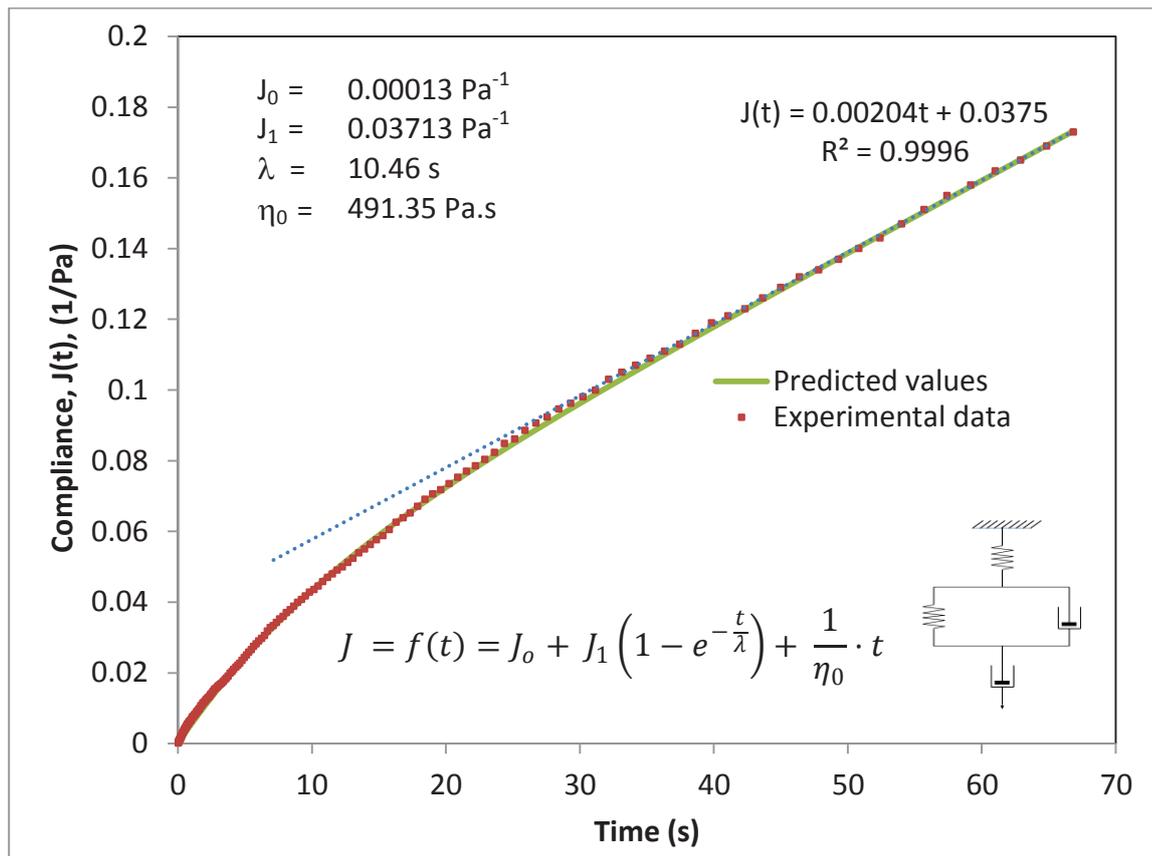


Figure 6.8 Creep compliance data and fitted curve of model Mozzarella cheese at 70°C. Applied shear stress was 0.05 Pa. (within the linear viscoelastic limit). The fitted curve (continuous line) used a 4-element Burger's model. Zero shear viscosity (η_0) was calculated from the linear regression line (dotted) in the later part of creep. The model cheese had 8.8 kJ/kg of shear work input and was manufactured in the Blentech cooker at 150 rpm screw speed and 70°C (Table 4.1).

6.4. Discussion

For any model cheese or imitation cheese where the fat and protein are added as separate phases mixing is a crucial part of structure development at both the macroscopic and microscopic levels. Because of the very high viscosity of the molten cheese and the low Reynolds number ($Re < 0.1$) the mixing will be laminar rather than turbulent. In laminar mixing of two phases of roughly similar volume the essential mechanism of structure development is layering, stretching and folding which leads to striated structures (Szalai, Alvarez, & Muzzio, 2004) such as those in Fig. 6.2 and also at low shear work in Figs. 6.3 & 6.4. This striation mechanism combined with the fiber forming properties of renneted casein at the right pH and calcium content leads to the formation of a typical Mozzarella structure with the right characteristics to have good

pizza functionality. In our experiments the layering, stretching and folding is generated by the mixing action of the twin augers in the Blentech cooker.

The presence of pockets that combine fat and serum in traditional Mozzarella type cheeses is well reported (Paulson, McMahon & Oberg, 1998; McMahon et al., 1999; Mizuno & Lucey, 2005; McMahon, Paulson & Oberg, 2005). The laminar mixing action of the augers creates similar, desirable striated protein structures with optimum sized fat-serum channels to give good melt functionality (Chapter 4). These striated structures are the basis for the mechanical and structural anisotropy observed in fat-protein networks (Cervantes, Lund, & Olson, 1983; Ak and Gunasekaran, 1997; Manski et al., 2008; Bast et al., 2015; Chapter 3).

Further mixing (shear work input >58 kJ/kg) resulted in striation break up, a more uniform distribution of fat within the protein network, the disappearance of anisotropic fiber structures (Figs 6.3 & 6.4), much finer fat particles (Fig. 6.5) and poor melt (Chapter 4). El-Bakry et al. (2011) also reported the disappearance of the microscopic fibrous character of imitation cheese with processing time and noted a honeycomb structure with prolonged working. Excessive working (>74 kJ/kg) eventually led to the breakdown of the protein matrix showing an aggregated macroscopic structure accompanied by loss of serum fluid (Chapter 4). Confocal images indicated a brittle material with microcracks present (Figs. 6.3 & 6.4). Excessive protein-protein interactions mediated by calcium ions can be related to the formation of these aggregated structures and the expulsion of serum (McMahon et al., 1999; Chapter 5).

The very large fat particles (500 μm) observed at low shear work input disappeared at higher shear work values (Fig. 6.5a). This rapid reduction of fat particle size suggests effective dispersive mixing in the initial working phases. Excessive working led to the occurrence of many more submicron fat particles (Fig. 6.5a). The fat particle size distribution after working molten cheese in twin screw cookers is expected to result from a dynamic equilibrium between particle break up by shear and particle growth by coalescence. The results indicate that particle break up by shear is dominant as $d_{4,3}$ continuously decreased with increasing shear work input (Fig. 6.5b). Coalescence may be increasing at high shear work levels as the curve flattens (Fig. 6.5b).

The changes in creep and recovery behavior at 20 °C of full fat model cheese with increasing shear work input broadly agree with the changes reported in steady shear

viscosity (Chapter 4) and oscillatory rheology (Chapter 5). Steady shear viscosity increased exponentially with shear work input (Chapter 4) and η_0 also increased exponentially with shear work input (Table 6.1). Frequency sweeps indicated a more elastic, solid-like structure with increasing shear work input (Chapter 5) and decreasing J_1 and increasing η_1 with increasing shear work input (Table 6.1) also indicate more elastic, solid-like behavior. The comparison between full fat cheese, nonfat cheese and TSC added cheese from the creep and recovery behaviour also agrees with previous work. At a similar shear work level full fat cheese is harder (lower γ_{max} , Table 6.1) and more rigid (lower J_0 , Table 6.1) than the other two cheeses and had lower frequency dependence indicating a harder, more solid-like structure (Chapter 5). η_0 was lowest for TSC added cheese whereas full fat and nonfat cheeses had similar η_0 . Full fat cheese and nonfat cheeses had similar steady shear viscosities but that for TSC added cheese was lower (Chapter 4).

The retardation times (λ_1 & λ_2) are time constants for the viscoelastic changes in the Kelvin elements in the model (Fig. 6.1). Retardation times are useful in the design of cheese forming devices such as block formers or extrusion processes. A useful rule of thumb is that the timescale of deformation must be longer than the retardation time if changes in shape are to be permanent. When processing at 70 °C holding of a new shape for at least 11 s is therefore necessary for a permanent shape change. At 20 °C two retardation times were needed to model the behaviour (Table 6.1). For all cheeses J_1 was 2 to 3 times J_2 so the longer retardation time λ_1 is more important to permanent shape change than λ_2 . A holding time of 150 – 200 s is therefore needed for a permanent shape change at 20 °C .

The retardation times reported here are the same order of magnitude as those reported for a cheese like material, shear structured and transglutaminase cross-linked 30 % calcium caseinate in the presence of palm fat, at 20 °C (Manski et al., 2008). Their 6-element Burgers model gave retardation times of 8-11 s and 200-260 s compared to our values of 3-5 s and 130-193 s.

Chapter 5 proposed a schematic model to describe structural changes in model Mozzarella cheeses as they were progressively sheared. The model showed the fat phase changing from large, elongated particles at low shear work input (< 30 kJ/kg), to smaller elongated fat particles at 58 kJ/kg, to small, spherical fat particles at > 70 kJ/kg.

The changes in fat particle size distribution and fat microstructure reported here add further experimental support for the model.

6.5. Conclusions

Shear work input affected the microstructure, fat particle size and creep behavior of model Mozzarella cheeses. The microstructure changed from an anisotropic structure with aligned fat-serum channels typical of pasta-filata type cheeses at low shear work to an isotropic structure with small fat globules and some micro-cracks with excessive shear work inputs. The number of large fat particles decreased rapidly in the initial phases of working suggesting effective dispersive mixing. A 6-element Burger's model was needed to adequately fit the creep data of model cheese at 20 °C but a 4-element model was satisfactory at 70 °C. As shear work input increased maximum shear creep decreased, retarded compliance decreased and zero shear viscosity increased indicating the more elastic behavior of the cheeses with higher shear work input.

7.0 Strain hardening and anisotropy in sheared model Mozzarella cheeses

Chapter Summary

Tensile fracture properties of model Mozzarella cheeses were studied with varying amounts of shear work input (3.3-73.7 kJ/kg). Cheeses were elongated by manual rolling at 65 °C followed by tensile testing at 21 °C on dumbbell-shaped samples cut both parallel and perpendicular to the rolling direction. Strain hardening parameters were estimated from stress-strain curves using three different methods. Fracture stress and strain for longitudinal samples did not vary significantly with shear work input up to 26.3 kJ/kg then decreased dramatically at 58.2 kJ/kg. Longitudinal samples with shear work input <30 kJ/kg, demonstrated significant strain hardening by all three estimation methods. At shear work inputs <30 kJ/kg strong anisotropy was observed in both fracture stress and strain. After a shear work input of 58.2 kJ/kg anisotropy and strain hardening were absent. Perpendicular samples did not show strain hardening at any level of shear work input. The study concluded that significant changes in fracture properties, anisotropy and strain hardening were caused by changes in the molecular interaction of proteins upon excessive shear work.

7.1. Introduction

Hot water stretching and kneading forms an essential step in the traditional manufacture of Mozzarella cheese. This process step causes the proteins to flow giving a plastic appearance and forming a fibrous protein network aligned in the direction of stretching (McMahon, Fife & Oberg, 1999). The fibrous structure is visible on a macroscopic level (Oberg, McManus & McMahon, 1993; Chapter 4). Chapters 4, 5 and 6 studied the effect of shear work input during this stretching and working step on the rheology and microstructure of model Mozzarella cheeses manufactured in a twin screw Blentech cooker at 70 °C. Shear work inputs were extended well beyond normal manufacturing limits to exaggerate any changes in the cheese caused by working. The range of rheological methods included steady shear viscosity, strain sweeps, frequency sweeps, temperature sweeps and creep behaviour. With increase in shear work input cheeses showed increases in steady shear viscosity and storage modulus and frequency sweeps at 70 °C showed a change from viscoelastic liquid to viscoelastic solid. These changes

all indicate work thickening of the cheese. Very high shear work inputs (>70 kJ/kg) led to macroscopic structural breakdown of the cheese network with disappearance of the fibrous structure, loss of stretch, serum syneresis and decrease in melt functionality. Microstructures of the overworked cheeses indicated disappearance of the fibrous character and the creation of a homogeneous structure with a fine dispersion of fat particles in a brittle protein network (Chapter 6). The observed phenomena were attributed mainly to an increase in the strength of protein-protein interactions with prolonged working.

Bast et al. (2015-Appendix 1 in this thesis) developed a tensile testing method to quantitate the anisotropy and strain hardening of Mozzarella cheese. The method involved deliberate elongation of cheese at 60 °C by manual rolling on a cooled metal surface. Commercial Mozzarella cheeses showed strong anisotropy for both fracture stress and strain after elongation and also showed significant strain hardening in the longitudinal or fibre direction. The study indicated that tensile testing was a good method to explore anisotropy and strain hardening because fracture location and mode of failure were clearly visible. Other studies on strain hardening in dairy protein systems explored fine stranded whey protein isolate gels (Lowe, Foegeding & Daubert, 2003), weak β -lactoglobulin gels (Pouzot, Nicolai, Benyahia & Durand, 2006) and gels formed by acidifying transglutaminase cross-linked casein (Rohm, Ullrich, Schmidt, Lobner & Jaros, 2014).

Rheological properties, microstructure and extent of anisotropy are all closely related to the functional characteristics of Mozzarella cheese for pizza application such as meltability, stretchability, elasticity, oiling-off and blister formation (Kindstedt & Fox, 1993; Olivares et al., 2009).

Strain hardening is well explored in gluten networks because it is important to attain optimum baking performance of bread dough by aiding holding capacity and stability of gas bubbles in the bread (Peighambardoust, van der Goot, van Vliet, Hamer and Boom, 2006; Peressini, Peighambardoust, Hamer, Sensidoni, & van der Goot, 2008; Kokelaar, van Vliet & Prins, 1996; Van Vliet, Janssen, Bloksma & Walstra, 1992; Van Vliet, 2008). The effect of working on tensile fracture properties and strain hardening of flour dough has also been studied. Peighambardoust et al. (2006) and Peressini et al. (2008)

observed a decrease in strain hardening upon prolonged working of flour doughs and attributed this to breakdown in the gluten network structure.

The objectives of this chapter are: 1. To measure the tensile fracture properties and anisotropy of model Mozzarella cheeses with varied shear work inputs (3.3-73.7 kJ/kg) to complement the other rheological tools that were used in earlier chapters; 2. To explore whether the model Mozzarella cheeses strain harden and to see the effect of shear work input on this strain hardening and 3. To apply to Mozzarella cheese a wider range of strain hardening measures as used for flour doughs.

7.2. Materials and Methods

7.2.1. Materials

The materials used are outlined in section 4.2.1.

7.2.2. Manufacture of model Mozzarella cheeses

The manufacture of the model Mozzarella cheese is outlined in section 4.2.2. Shear work input was calculated as in section 4.2.3. only cheese manufactured at 150 rpm were tested in this chapter.

7.2.3. Sample preparation for Tensile testing of model Mozzarella cheeses

Cheese samples were prepared for tensile testing using the method of Bast et al. (2015) with some variations. Cheese samples (~300 g) were melted by placing in a 65 °C water bath for about 2 h. Melted cheese was manually elongated on a cooled (4 °C) aluminium plate using a granite rolling pin (4 °C). Aluminium strips were attached to the plate to achieve a sheet thickness of 3-4 mm. Rolling was performed for 120 s at 10 rolls min⁻¹. Dumbbell-shaped samples were cut in both longitudinal (n=8) and perpendicular (n=9) orientations (Bast et al., 2015). Samples were kept at 21 °C for at least 1 h before tensile testing. Each rolling experiment was performed twice.

7.2.4. Tensile testing and data analysis

Tensile testing on elongated cheese samples was performed on a TA.XT2plus Texture Analyser (Stable Micro Systems Ltd., Godalming, UK) at 21 °C. Cross head speed was 2 mm s⁻¹ and trigger force was 0.01 N. The initial dimensions of the central section of each sample were measured using vernier callipers.

Force-displacement data were converted into true stress (σ) versus Hencky strain (ϵ) (Bast et al., 2015). The anisotropy ratio, R , for fracture stress was calculated as σ_L/σ_P where σ_L and σ_P are the fracture stresses in longitudinal and perpendicular directions, and similarly for fracture strain.

7.2.5. Strain hardening parameters

Strain hardening properties were calculated only for longitudinal samples as perpendicular samples showed no strain hardening. An empirical equation suggested by Hollomon (Kokelaar et al., 1996; van Vliet, 2008) provided two strain hardening parameters in uniaxial extension.

$$\sigma = K_{SH} \epsilon_H^{n_{SH}} \quad (7.1)$$

where K_{SH} is the strength coefficient (Pa) and n_{SH} is strain hardening index (SHI). $n_{SH} > 1$ indicates strain hardening behaviour. Equation (7.1) was normally fitted to stress-strain data over the strain range 0.4 to 0.05 before fracture. Good fits ($R^2 \sim 0.98-0.99$) were obtained over this strain range.

Strain hardening is directly observed as an increase in the slope of the true stress-Hencky strain curve with increasing strain. A strain hardening ratio was therefore calculated (Bast et al., 2015)

$$\text{Strain hardening ratio (SHR)} = \frac{\text{Maximum Modulus near fracture}}{\text{Initial modulus}} \quad (7.2)$$

Initial modulus was obtained by linear regression of each stress-strain curve in the strain range 0.01-0.25.

Strain hardening provides stability against uneven distribution of stress and incipient localised thinning allowing much larger extensions. Strain hardening allows the material to resist further thinning by locally increasing resistance to further deformation (Dobraszczyk & Vincent, 1999). According to the Considère criterion for necking stability in uniaxial extension

$$\frac{d\sigma}{d\epsilon} = \sigma \quad (7.3)$$

Or for strain hardening

$$\frac{d \ln \sigma}{d \varepsilon} > 1 \quad (7.4)$$

Therefore, another useful parameter for characterising strain hardening is $d \ln \sigma / d \varepsilon$ (van Vliet et al., 1992, van Vliet, 2008). Peighambardoust et al. (2006) called this differential apparent strain hardening (ASH).

7.2.6. Microscopy

Confocal scanning laser microscopy (CSLM) was done on a Zeiss LSM 510 META confocal microscope (Carl Zeiss AG, Oberkochen, Germany) with the method of described in Chapter 6. Cheese slabs (~12 x 4 mm) were frozen at -20 °C and sectioned into 50 µm slices on a cryo-microtome. Slices were immediately transferred to glass slides, stained with 0.4% Nile red and 0.2% fast green and covered with a coverslip. Samples were kept at 4 °C for at least 48 h before imaging to allow uniform uptake of dyes.

Because nonfat cheese was translucent, microstructure could be studied using transmission light microscopy on an Olympus BX60 (Olympus Optical Co. Ltd, Tokyo, Japan). A 1 mm slice (12 x 12 mm) of nonfat cheese was prepared using a sharp razor blade. Images were captured by a CCD camera (Axio Cam HRc, Carl Zeiss, Hallbergmoos, Germany).

7.2.7. Rheological measurements

Rheological measurements were conducted 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) using the method described in Chapter 3 for steady shear rheology and Chapter 5 for frequency sweeps. Disc shaped samples 20 mm diameter and ~ 2-3 mm thick were cut using a cork borer and wire cutter. Cheese discs were held at 70 °C for 2 min to ensure isothermal conditions. The perimeter of cheese discs was covered with a ring of soybean oil to prevent moisture loss. Flow curves were obtained at 70 °C and a power law model fitted to the data to obtain consistency coefficient K and flow behaviour index, n. Shear rates were applied with measurement times as follows: 60 s at 0.01 s⁻¹, 6.25 s at 0.1 s⁻¹, 0.5 s at 1 s⁻¹, 0.05 s at 10 s⁻¹, 0.05 s at 100 s⁻¹, 0.05 s at 200 s⁻¹. Frequency sweeps applied frequencies in descending order at 20 °C. Rheological measurements were conducted in triplicate.

7.2.8. Statistical analysis

Descriptive statistics, non-linear regression and ANOVA analysis were conducted on the data using SPSS software (version 20). Significant differences ($P < 0.05$) in the results were analysed using single factor ANOVA and the Duncan post hoc test to compare means.

7.3. Results

7.3.1. Tensile fracture properties of sheared model Mozzarella cheese

Both longitudinal and perpendicular samples exhibited non-linear stress/strain behavior (Fig. 7.1). At low strains ($\epsilon < 0.25$) both longitudinal and perpendicular samples behaved in a linear manner with similar values of initial modulus (~ 116 - 126 kPa). At small deformations, Hookean behaviour is expected in food materials. However, at higher strain levels ($\epsilon > 0.25$) nonlinear behaviour was observed. Longitudinal samples demonstrated strain hardening with a significant increase in tensile modulus. Further measures to quantify strain hardening are explored in section 7.3.2. Perpendicular samples exhibited slight strain softening. Perpendicular samples fractured at much lower strain.

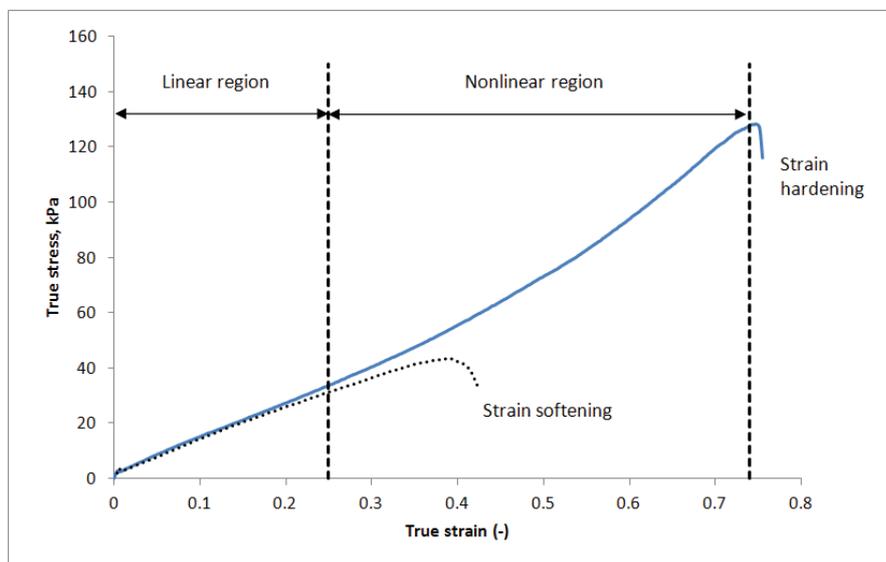


Figure 7.1 Typical stress-strain curves for model Mozzarella cheese samples in longitudinal (smooth line) and perpendicular (dotted line) orientations indicating strain hardening and strain softening respectively. Longitudinal samples show linear and nonlinear regions before tensile fracture. Model Mozzarella cheeses were prepared in the Blentech cooker with 3.3 kJ/kg of shear work at 70 °C using 150 rpm screw speed (Table 4.1).

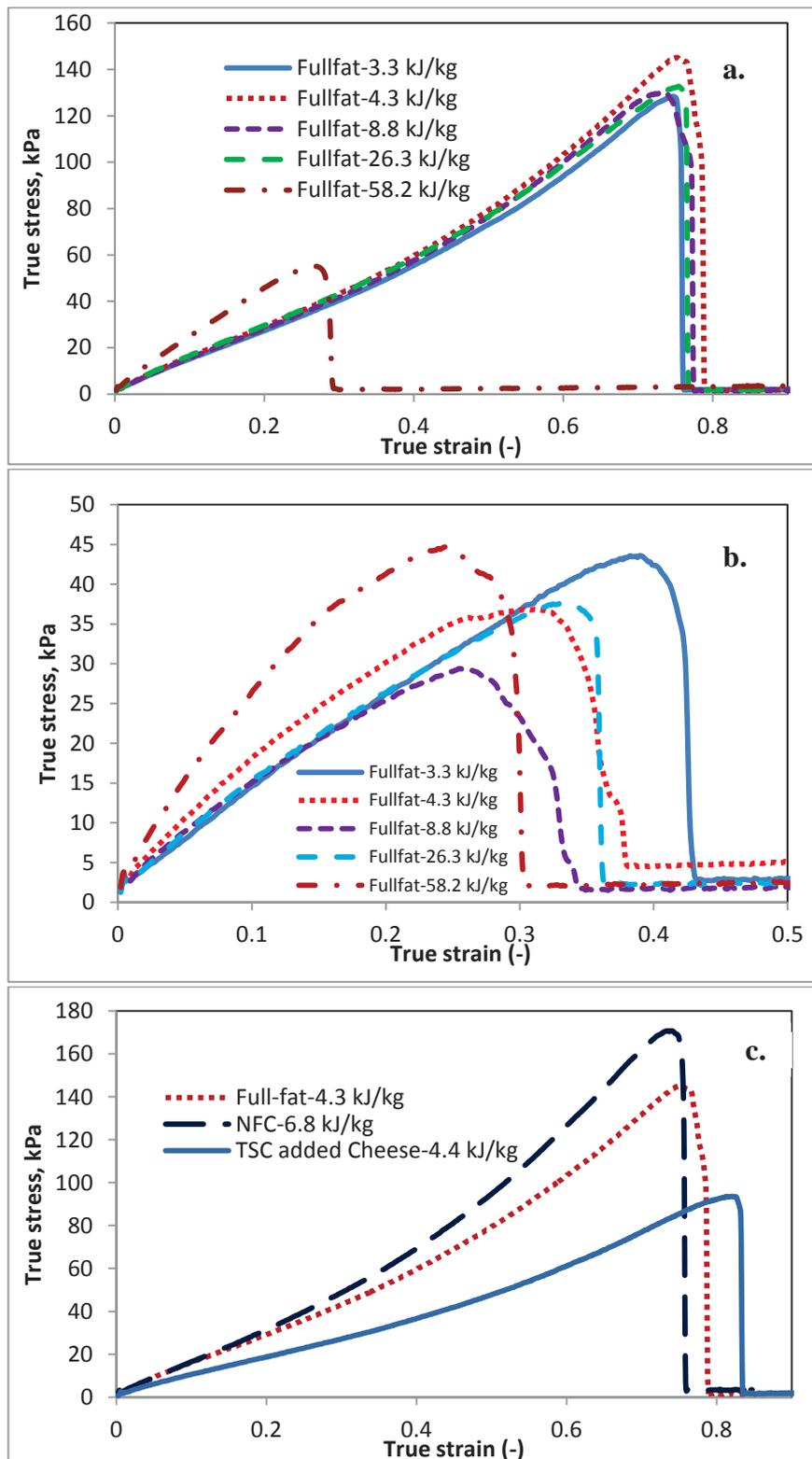


Figure 7.2 Stress-strain curves for model Mozzarella cheeses. Cheeses with varied amounts of shear work were cut in both longitudinal (a) and perpendicular (b) orientations. Full fat, nonfat and TSC added cheeses are compared at a similar shear work level in longitudinal orientation (c). Model Mozzarella cheeses were prepared in the Blentech cooker at 70 °C using 150 rpm screw speed. Typical curves were chosen from n=16 longitudinal samples and n=18 perpendicular samples (Table 4.1).

Table 7.1 Effects of shear work on tensile fracture properties of model Mozzarella cheeses.

	Shear work kJ/kg	Fracture stress (kPa)			Fracture strain (-)		
		Longitudinal	Perpendicular	Anisotropy Ratio	Longitudinal	Perpendicular	Anisotropy Ratio
Full fat cheese	3.3	121.0 ± 27.0 ^a	40.2 ± 14.5 ^{bc}	3.01	0.75 ± 0.07 ^{ac}	0.37 ± 0.09 ^d	2.0
	4.3	115.7 ± 22.1 ^a	32.3 ± 12.1 ^{bf}	3.59	0.75 ± 0.09 ^a	0.34 ± 0.10 ^{de}	2.2
	8.8	125.5 ± 16.4 ^a	27.9 ± 8.2 ^{bf}	4.49	0.74 ± 0.06 ^{ac}	0.29 ± 0.09 ^e	2.5
	26.3	128.6 ± 21.5 ^a	30.6 ± 12.2 ^{bf}	4.20	0.77 ± 0.07 ^a	0.30 ± 0.09 ^e	2.6
	58.2	44.4 ± 14.6 ^{bc}	41.3 ± 11.2 ^{bc}	1.07	0.21 ± 0.05 ^b	0.22 ± 0.06 ^b	0.9
Nonfat cheese	6.8	159.2 ± 33.5 ^c	47.7 ± 17.7 ^e	3.34	0.68 ± 0.14 ^c	0.34 ± 0.07 ^{de}	2.0
Nonfat cheese – constant strain rate	6.8	140.8 ± 42.8 ^c	40.1 ± 8.1 ^e	3.5	0.58 ± 0.10 ^c	0.31 ± 0.05 ^{de}	1.9
TSC added full fat cheese	4.4	78.5 ± 19.2 ^d	21.9 ± 3.9 ^f	3.58	0.70 ± 0.09 ^{ac}	0.29 ± 0.04 ^e	2.5

Values are means with standard deviations from n=16 longitudinal samples and n=18 perpendicular samples (n=4 for constant strain rate experiment). Means for the same parameter, e.g. fracture stress, with different superscript letters are significantly different (P<0.05).

Table 7.2 Effect of shear work on strain hardening properties of model Mozzarella cheeses.

Sample	Shear work input (kJ/kg)	Initial modulus (kPa)	Maximum modulus (kPa)	Strain hardening ratio	Apparent strain hardening, $d\ln\sigma/d\epsilon_H$	Strength coefficient, K_H (Pa)	Strain hardening index, n_{SH}
Full fat cheese	3.3	129.3 ± 10.1 ^a	235.5 ± 42.3 ^a	1.81 ± 0.24 ^a	2.60 ± 0.15 ^a	184.9 ± 20.9 ^{ab}	1.33 ± 0.05 ^a
	4.3	128.7 ± 17.7 ^a	219.7 ± 34.3 ^a	1.79 ± 0.26 ^a	2.57 ± 0.16 ^a	171.8 ± 29.4 ^{ab}	1.32 ± 0.05 ^a
	8.8	141.9 ± 14.8 ^a	231.5 ± 28.3 ^a	1.65 ± 0.27 ^a	2.52 ± 0.13 ^a	189.5 ± 13.2 ^b	1.30 ± 0.05 ^a
	26.3	142.1 ± 21.1 ^a	238.7 ± 40.9 ^a	1.72 ± 0.28 ^a	2.51 ± 0.10 ^a	181.2 ± 22.3 ^{ab}	1.31 ± 0.05 ^a
	58.2	248.4 ± 59.7 ^b	188.9 ± 48.1 ^b	0.76 ± 0.07 ^b	-	3.6 ± 1.3 ^c	0.49 ± 0.03 ^b
Nonfat cheese	6.8	174.0 ± 27.9 ^c	314.0 ± 58.9 ^c	1.76 ± 0.37 ^a	2.84 ± 0.09 ^b	273.5 ± 38.7 ^d	1.35 ± 0.08 ^a
Nonfat cheese - constant strain rate	6.8	198.1 ± 13.4 ^e	334.2 ± 9.3 ^c	2.03 ± 0.59 ^a	3.55 ± 0.20 ^d	367.0 ± 6.7 ^f	1.42 ± 0.01 ^d
TSC added full fat cheese	4.4	104.9 ± 6.0 ^d	148.3 ± 27.0 ^d	1.41 ± 0.23 ^c	2.39 ± 0.09 ^c	125.1 ± 14.5 ^e	1.25 ± 0.07 ^c

Values are means with standard deviations from n=16 longitudinal samples (n=4 for constant strain rate experiment). Means within a column with different superscript letters are significantly different (P<0.05).

For full fat cheese (Fig. 7.2a, b) all samples at shear work levels ≤ 26.3 kJ/kg produced similar stress-strain curves. At smaller strains ($\epsilon < 0.25$), initial modulus of full fat cheese increased from 129.31 kPa to 248 kPa with shear work (3.3 to 58.2 kJ/kg) indicating the creation of a stiffer structure upon working. Longitudinal and perpendicular samples indicated strain hardening and strain weakening behaviour respectively. At 58.2 kJ/kg both longitudinal and perpendicular samples showed strain weakening behaviour and had a low fracture strain of about 0.3. When comparing longitudinal samples of the 3 model cheeses (Fig. 7.2c) the order of both initial stiffness and extent of non-linear behaviour was nonfat > full fat > TSC added full fat cheese.

Longitudinal samples of full fat cheese indicated no significant difference in either fracture stress or fracture strain with shear work input up to 26.3 kJ/kg (Table 7.1), followed by a dramatic decrease in both fracture stress and strain at 58.2 kJ/kg. At lower shear work input (≤ 26.3 kJ/kg) all longitudinal samples exhibited similar material failure behaviour indicating similar structure and strength. The decrease ($P < 0.05$) in fracture stress with increase in shear work suggests that the cheese matrix has lower strength after prolonged working. Similar observations were made from tensile testing of dough systems subjected to different levels of working (Peighambardoust et al., 2006). The initial tensile modulus of the cheese increased dramatically from 142.1 kPa at 26.3 kJ/kg to 248.4 kPa at 58.2 kJ/kg. Fracture stress did not change significantly with shear work input for perpendicular samples (Table 7.1) whereas fracture strain decreased significantly as shear work increased. Fracture strain usually regarded as an indicator of structural arrangement, so a decrease in fracture strain with increasing shear work for perpendicular samples indicates significant differences in structure, e.g. more inherent weaknesses in the structure causing crack initiation, propagation and fracture (Fig. 7.2b). The large percentage variations in fracture strain for perpendicular samples (Table 7.1) probably arise from the random occurrence of such structural weaknesses or imperfections. At low shear work inputs of 3.3-26.3 kJ/kg for the full fat cheeses, longitudinal samples had higher values for both fracture stress ($\sigma_f = 115-128$ kPa) and fracture strain ($\epsilon_f = 0.74-0.77$) than the perpendicular samples ($\sigma_f = 27-40$ kPa; $\epsilon_f = 0.29-0.37$). The anisotropy index was higher for fracture stress (3.0-4.5) than for fracture strain (2-2.6). Both indicated significant anisotropy ($P < 0.05$) in fracture properties. At a shear work input of 58.2 kJ/kg anisotropy had disappeared for both fracture stress and fracture strain (Table 7.1).

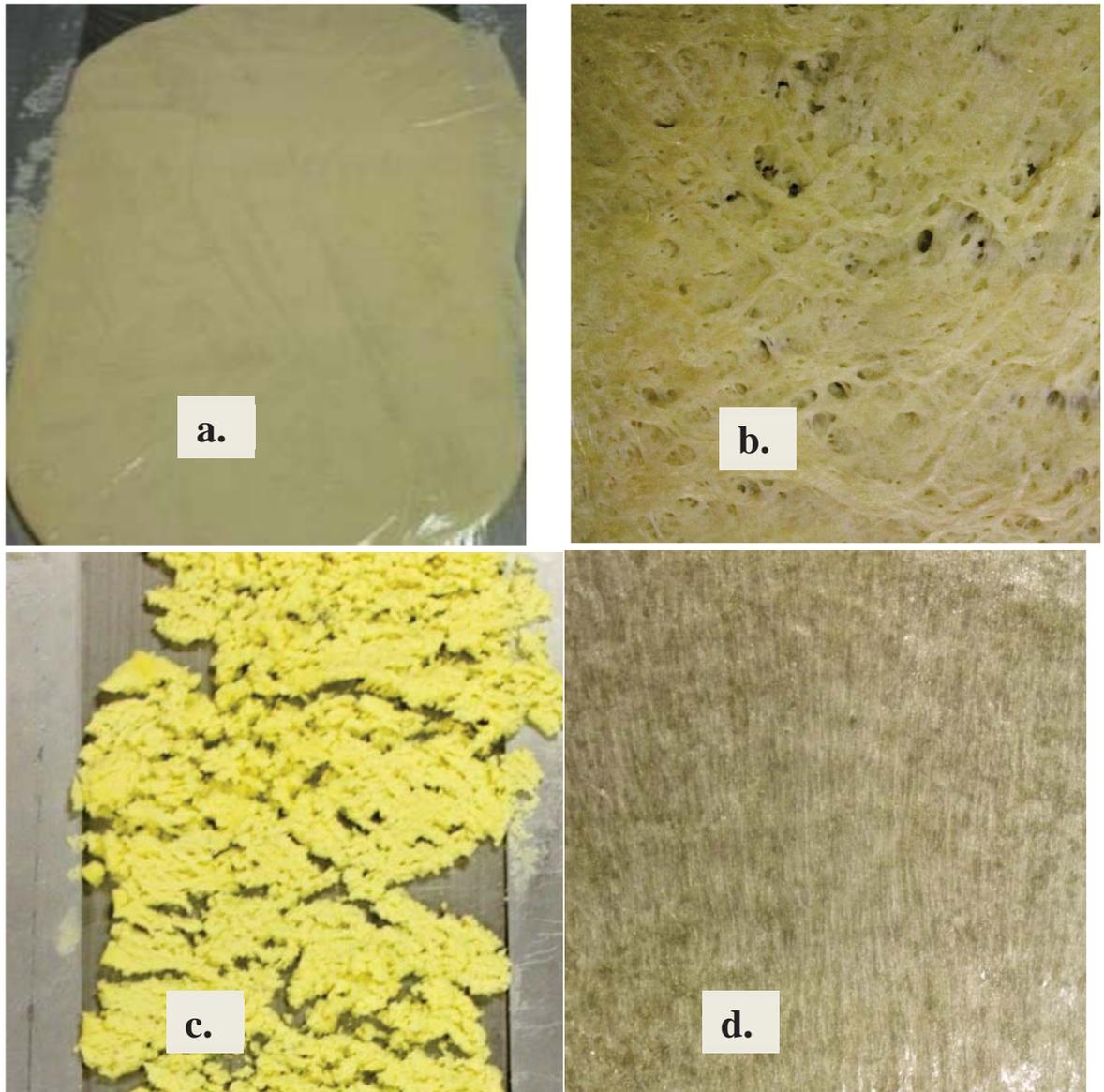


Figure 7.3 Visual appearance of elongated model Mozzarella cheeses. Cheese samples were prepared in the Blentech cooker with varied amounts of shear work input at 70 °C and 150 rpm; full fat cheese a. 8.8, b. 58.2, c. 73.7 kJ/kg, nonfat cheese d. 6.8 kJ/kg (Table 4.1).

The fracture stress for nonfat cheese was ~33 % higher ($P < 0.05$) than that for full fat cheese with similar shear work input for longitudinal samples and ~59 % higher ($P < 0.05$) for perpendicular samples (Table. 7.1). Nonfat cheese has a 56 % higher protein content (Table 4.1) so more structural protein elements are expected in nonfat cheese per unit cross sectional area, giving rise to higher values of fracture stress. TSC added full fat cheese had ~32% lower ($P < 0.05$) fracture stress than full fat cheese for both longitudinal and perpendicular samples.

The different cheeses behaved very differently during the elongation process (Fig. 7.3) and this had an impact on their tensile fracture behaviour. At low and moderate shear work inputs the cheese elongated well giving a smooth, homogeneous cheese layer. At 58.2 kJ/kg the cheese did not flow well giving a heterogeneous cheese layer with a number of weak spots (Fig. 7.3b). With excessive working (73.7 kJ/kg), rolling could not be conducted satisfactorily as even at 65 °C the cheese was brittle, there was no continuous flowing mass and the resulting cheese layer was highly heterogeneous (Fig. 7.3c). Representative dumbbell tensile samples could not be cut from the cheese sheet.

7.3.2. Strain hardening of model Mozzarella cheeses

Strain hardening parameters for samples cut in longitudinal orientation are presented in Table 7.2. SHI was the least variable parameter (coefficient of variation ~4%) followed by ASH (~7%) and then SHR (~13%). The SHR method was the most variable and is the ratio of two moduli. ASH, K_H and SHI are obtained from fits to the whole non-linear portion of the fracture curve and are probably a better indicator of strain hardening. For the full fat cheeses with shear work input up to 26.3 kJ/kg SHR varied from 1.65 to 1.81, ASH from 2.51 to 2.60 and SHI from 1.30 to 1.33. For all three parameters values greater than 1 indicate strain hardening (Bast et al., 2015 for SHR; Peighambardoust et al., 2006 for ASH; van Vliet et al., 1992, van Vliet, 2008 for SHI). All three parameters therefore indicate significant strain hardening. At a shear work input of 58.2 kJ/kg strain softening was observed with both SHR (0.76) and SHI (0.49) being less than 1. A decrease ($P < 0.05$) in strain hardening with an increase in shear work input from 26.3 kJ/kg to 58.2 kJ/kg suggests weakening of the cheese matrix or a higher prevalence of fracture initiating cracks with progressive working. Peighambardoust et al. (2006) also reported a reduction in ASH values with progressive mixing of bread dough. Zheng et al. (2000), Gras et al. (2000) and Peressini et al. (2008) concluded that over-mixing led to diminished tensile fracture properties of bread dough under extension tests.

The nonfat cheese and TSC added cheese samples also showed significant strain hardening for all 3 parameters (Table 7.2). ASH and SHI values were significantly higher ($P < 0.05$) for nonfat cheese (2.84 and 1.35) and lower ($P < 0.05$) for TSC added cheese (2.39 and 1.25) than for full fat cheese with similar shear work input (Table 7.2). Table 7.2 also presents results for fracture tests performed on nonfat cheese at constant strain rate rather than constant crosshead speed. The TA.XT2plus was programmed to

increase crosshead speed with time in order to maintain a constant strain rate of 0.2 s^{-1} . This indicated an even higher extent of strain hardening with ASH and SHI both significantly higher than at constant crosshead speed.

7.3.3. *Structural anisotropy in model cheeses*

Model cheese samples before elongation exhibited microstructural anisotropy with orientation of fat in one direction (Fig. 7.4a1, a2). Tensile testing of these samples during preliminary studies, however, showed no significant anisotropy for either fracture stress or fracture strain. Melting and elongation of the same cheese sample similarly indicated microstructural anisotropy with the fat channels somewhat larger (Fig. 7.4b1, b2, b3). After melting and elongation the structure shows globular fat in some regions (Fig. 7.4b3) and coalesced elongated fat particles in other regions (Fig. 7.4b2). This microstructural alignment of fat particles is obviously a major contributor to anisotropy in tensile fracture properties. Nonfat cheese samples also indicated microstructural alignment of the protein structure by transmission light microscopy (Fig. 7.4c). A simple photograph of nonfat cheese macrostructure also suggested orientation in the direction of rolling (Fig. 7.3d). Similar structural orientation has been reported previously at various length scales for nonfat cheeses (Mizuno & Lucey, 2005) supporting our observations.

7.3.4. *Small strain oscillatory shear rheology of model cheeses*

Mechanical spectra at $20 \text{ }^{\circ}\text{C}$ of full fat, nonfat and TSC added cheeses in the form of frequency sweeps are shown in Fig. 7.5. All three cheeses exhibited viscoelastic solid behavior ($G' > G''$) with low and similar frequency dependence (slope, $n \sim 0.16 - 0.18$) suggesting the presence of a physically stable network. The n values are consistent with those reported for casein gels and fat-filled casein gels (Zhou & Mulvaney, 1998). Storage moduli for full fat cheese were higher than those for nonfat cheese and TSC added cheese, probably because of the contribution from solid fat at $20 \text{ }^{\circ}\text{C}$ (Zhou & Mulvaney, 1998). The storage modulus of milkfat ($G_f' = 292 \text{ kPa}$) was higher than that of the cheese matrix ($G_m' = 164 \text{ kPa}$) at $20 \text{ }^{\circ}\text{C}$ (Yang, Rogers, Berry & Foegeding, 2011), so fat would be expected to reinforce the matrix.

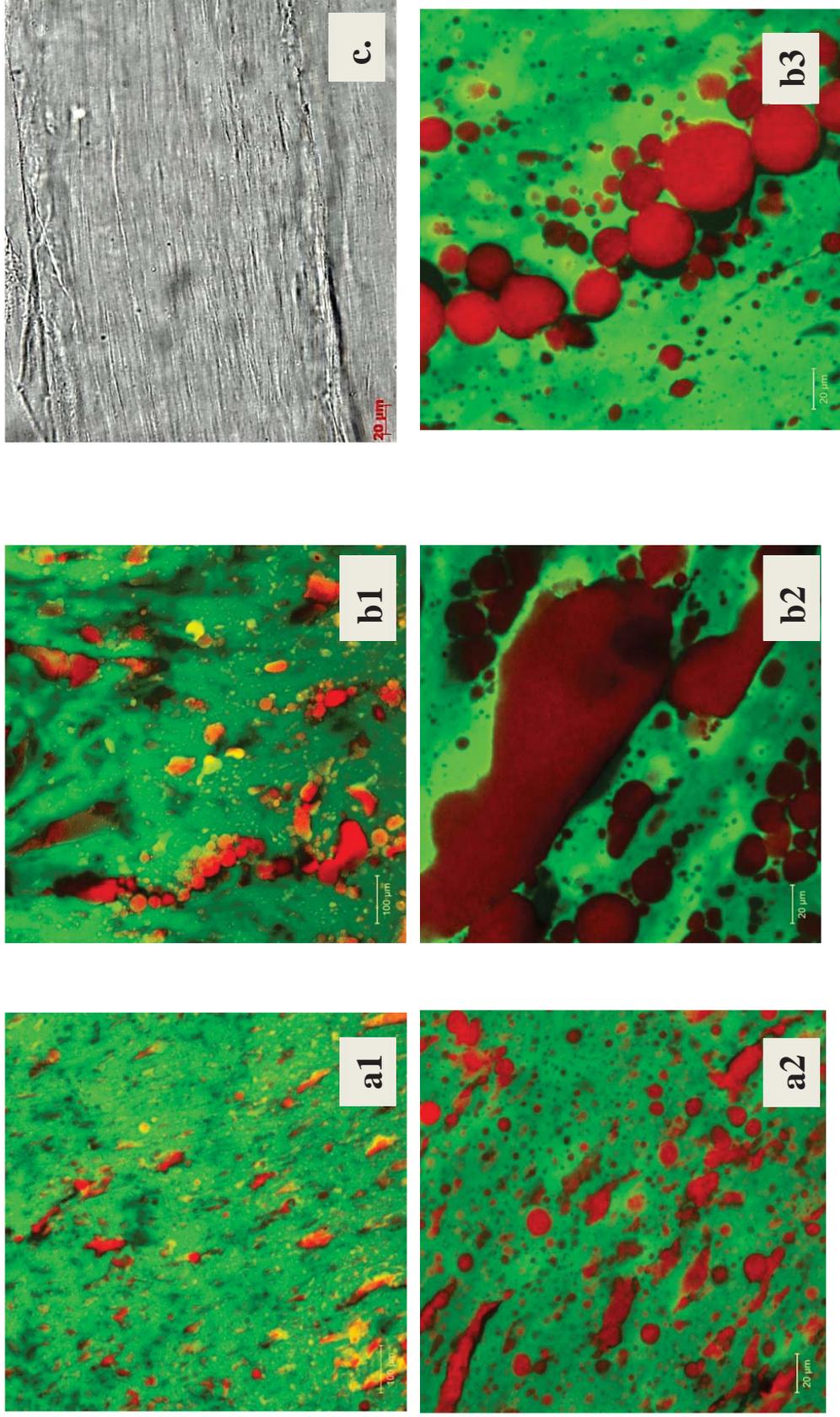


Figure 7.4 Microstructures of model Mozzarella cheeses; CSLM images of normal (a1,a2) and elongated (b1,b2,b3) full fat Mozzarella cheeses after 26.3 kJ/kg of shear work input; Transmission light microscopy (LM) image of nonfat Mozzarella cheese (c) after 6.8 kJ/kg of shear work input. Cheese samples were prepared in the Blentech cooker at 70 °C and 150 rpm (Table 4.1). Red - fat, green - protein and black - air/water.

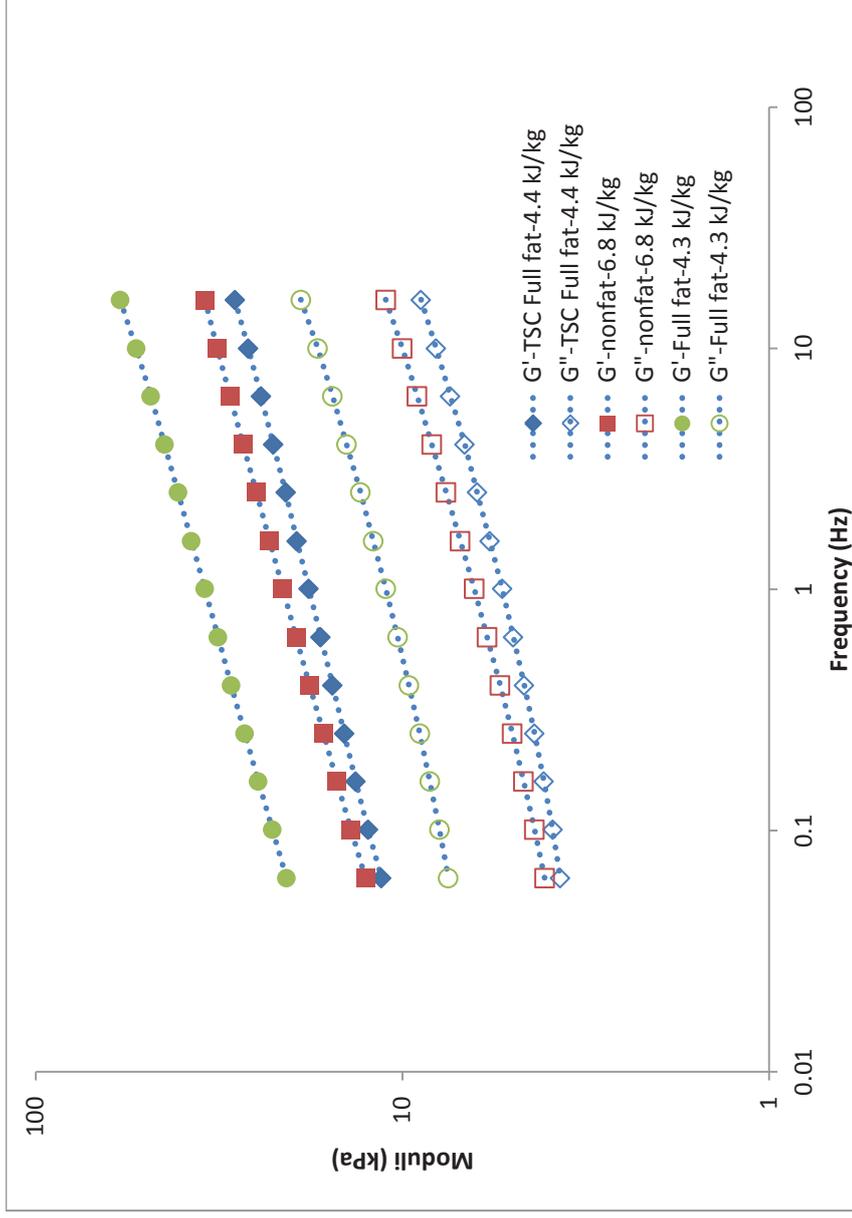


Figure 7.5 Storage moduli (closed symbols) and loss moduli (open symbols) of model Mozzarella cheeses obtained from frequency sweeps at 20 °C on full fat, nonfat and TSC added cheeses by giving 4.3, 6.8 and 4.4 kJ/kg of shear work input respectively at 70 °C and 150 rpm (Table 4.1).

7.3.5. Steady shear rheology of elongated model cheeses

In Chapter 4, it was noted that the manual rolling process caused huge work thickening in as little as 18 s (Bast et al., 2015) whereas shear in the Blentech caused almost no work thickening after 4000 s at 50 rpm. It was interesting to know whether the work thickening from rolling was only evident in tensile fracture properties or whether it also caused changes in steady shear viscosity. Steady shear flow curves at 70 °C on rolled cheese samples (Table 7.3) indicate that the flow behavior index decreased slightly after rolling indicating more shear thinning. Consistency coefficient and apparent viscosity at 0.01 s⁻¹ increased by 1.43 and 1.52 times respectively (P<0.05) after elongation. The increase in shear viscosity indicates significant work thickening, but much less than the work thickening indicated by tensile fracture properties with fracture stress increased by 5.7 times parallel to the fibres and by 2.1 times perpendicular to the fibres (Bast et al., 2015). The type of deformation is quite different in the two cases though. Tensile fracture measures the strength of a material while pulling in one direction whereas steady shear rheology measures resistance to shear flow.

Table 7.3 Effect of rolling on steady shear rheology of model Mozzarella cheese* at 70°C.

	Normal cheese	Rolled cheese
Consistency coefficient, K (Pa.s ⁿ)	131.2 ± 10.2 ^a	188.2 ± 20.2 ^b
Flow behaviour index, n	0.73 ± 0.01 ^a	0.72 ± 0.01 ^b
Apparent viscosity at 0.01 s ⁻¹ , Pa.s	449.3 ± 8.3 ^a	682.7 ± 76.3 ^b

* Cheese was prepared by giving 26.3 kJ/kg of shear work input at 150 rpm and 70 °C
Values are means with standard deviations from 4 repetitions. Means within a row with different superscript letters are significantly different (P<0.05)

7.4. Discussion

The anisotropy index range for fracture stress σ_f of 3.01-4.49 (Table 7.1) for the elongated model cheeses was similar to the value for an elongated commercial Mozzarella cheese (3.0) but less than that for string cheese (6.0) (Bast et al, 2015) or fibrous fat-calcium caseinate materials (highest 14.2) (Manski et al., 2008). Possible reasons for the higher degree of anisotropy in other reports could be the higher protein to moisture ratio for string cheese (0.59 compared to 0.39), the presence of transglutaminase cross-linking enzyme in the material of Manski et al. (2008) and the

use of specific shearing processes that increased fibrous character in both the cases quoted.

To help explain anisotropy in full fat, elongated model Mozzarella cheese, a structural model is proposed (Fig. 7.6a). A continuous protein-gel contains a dispersed phase of fat particles elongated in the direction of rolling as observed by CSLM (Fig. 4a, b). At small strains (<0.25), the stress-strain curves for longitudinal and perpendicular samples are very similar because the main deformation is in the gel network, e.g. initial modulus 126 kPa for longitudinal and 116 kPa for perpendicular samples (Fig. 7.1). As strain increases perpendicular to the long axis of the fat particles, fracture is initiated at low strains because of the large amount of fat-protein interface in this orientation. We assume this interface is a structural weakness. This results in lower values of fracture stress and strain for the perpendicular orientation (Table 7.1). As strain increases parallel to the long axis of the fat particles, fracture occurs in the gel phase as there is much less fat-protein interface. Fracture is reached at higher strains and therefore higher fracture stress.

Nonfat cheese is also highly anisotropic (Table 7.1) and shows evidence of structural alignment at a macroscopic/visual scale (Fig. 7.3d) and a microscopic scale (Fig. 7.4c). Structural alignment at a microscopic scale was also observed by Mizuno and Lucey (2005). Shearing in the Blentech or elongation by rolling may be causing localised fracture or shear banding as observed in dough systems (Kieffer and Stein, 1999, Peighambardoust et al., 2006) resulting in reduced bond strength between fractured planes. Shear banded gluten structures led to a fibrous texture and were regarded as a major cause for structural anisotropy in sheared dough (Peighambardoust et al., 2006).

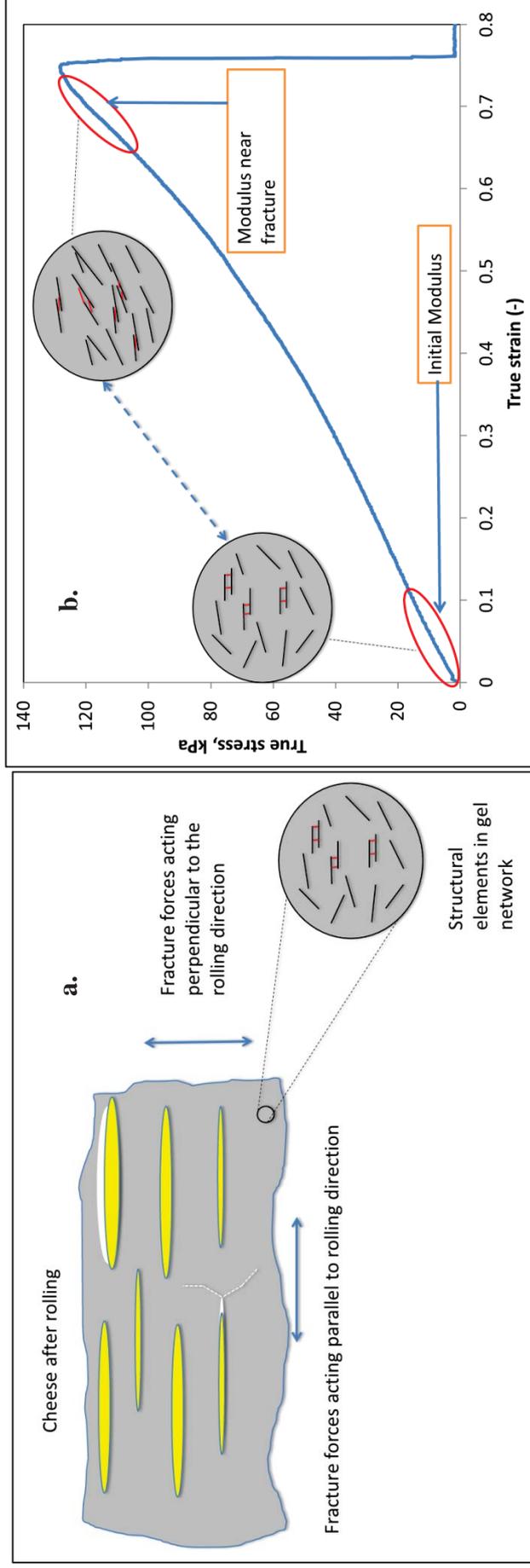


Figure 7.6 Schematic model explaining structural basis for anisotropy (a) and strain hardening (b) during tensile fracture of Model Mozzarella cheeses. Grey and yellow areas indicate gel phase and fat phase respectively. Further structural elements within the gel phase are presented in magnified grey circles.

Strain exerted in the perpendicular orientation may cause early fracture because of weak bonding between fibres (Taneya, Izutsu, Kimura & Shioya, 1992; Ak and Gunasekaran, 1997) while strain in the longitudinal orientation requires fracture of the fibres resulting in higher fracture strains and stresses. This explanation for nonfat cheese anisotropy must be combined with that in the paragraph above to get a more complete picture for full fat cheese, i.e. alignment of the protein structure is an important factor in explaining the anisotropy of full fat cheese. The observed elongation of the fat phase is probably just an indicator of what happening to the stronger protein phase.

Strain hardening in flour dough has been more widely studied (e.g. Kokelaar et al., 1996; van Vliet et al., 1992; van Vliet, 2008; Peighambardoust et al., 2006 and Peressini et al., 2008) than strain hardening of protein structures based on casein. Peighambardoust et al. (2006) reported that flour doughs strain hardened perpendicular to the fibers as well as parallel and that fracture strain was often higher for perpendicular samples than for longitudinal samples. Fracture stress usually showed no anisotropy apart from dough from one type of flour (Spring). The results of Manski et al. (2008) using sheared and transglutaminase cross-linked casein structures are more similar to those reported in this chapter in that strong anisotropy was observed and only longitudinal samples strain hardened, not perpendicular samples. A possible model to explain strain hardening suggests two casein structural elements – individual polymer molecules and elongated clusters of cross-linked, casein polymers (Fig. 7.6b). Crosslinked elements are assumed to be stiffer. After strain hardening late in the straining process three major changes are depicted – both elements are more aligned, the initial cross-links are more tightly bound and additional cross-linking has occurred. Bast et al. (2015) suggest some possible reasons for the two distinct regions on the stress-strain curve, linear initially then exponential or strain hardening.

The loss of both strain hardening and anisotropy of full fat cheese at high shear work input (58 kJ/kg) derives from changes in the microscopic structure of the material. The presence of fat and serum channels in Mozzarella-type cheeses is widely reported (Paulson, McMahon & Oberg, 1998; McMahon et al., 1999; Mizuno & Lucey, 2005; McMahon, Paulson & Oberg, 2005). Chapter 6 presented CSLM images of striated protein structures with aligned fat-serum channels at moderate shear work levels (3.3-25.3 kJ/kg) in model Mozzarella cheeses and attributed this to the laminar mixing action in the Blentech. These striated structures lead to mechanical and structural anisotropy in fat-protein networks at various length scales (Cervantes, Lund, & Olson, 1983; Ak and Gunasekaran, 1997; Manski et al., 2008; Bast et

al., 2015; Chapter 3). At high shear work inputs (>58 kJ/kg) the striated structure had disappeared and a macroscopically homogenous, isotropic cheese structure occurred with finely dispersed fat and no fibrous nature or stretch (Chapters 4, 6). This cheese showed no structural or mechanical anisotropy. Parallel behavior was reported for prolonged working of flour dough in a z-blade mixer (Peighamardoust et al., 2006). Decreases in tensile fracture stress, fracture strain and ASH were observed with progressive mixing, indicating weakening of the dough matrix. Strain hardening in flour dough depends on the amount and quality of gluten so the loss of ASH was attributed to extensive breakdown of the gluten network structures.

The higher values of fracture stress for nonfat cheese compared to full fat cheese are because the fat particles act as weak areas in the structure. Bast et al. (2015) demonstrated that the tensile strength of milkfat at 21 °C is very low. It is interesting that the strain hardening parameters for nonfat cheese were mostly not significantly different from those for full fat cheese in spite of the higher protein content and the absence of low strength fat in the structure. The addition of a calcium chelating salt (TSC) to the cheese would be expected to result in weaker but still flexible protein-protein bonds, leading to a weaker but still cohesive structure (Chapter 4). Fracture stress for TSC added cheese (78.5 kPa) was much lower ($P < 0.05$) than full fat cheese (115.7 kPa) and nonfat cheese (159.2 kPa) (Table 7.1) as expected but fracture strain was not significantly different. It is interesting that TSC addition reduced work thickening in the Blentech to a very low level (Chapter 4) but did not reduce strain hardening very much (Table 7.2). Cheese with TSC added still strain hardens very significantly by all three measures used. Maybe calcium is very important to the work thickening bonding mechanism but not so important to the strain hardening bonding mechanism. Lower values of initial modulus for TSC added cheese indicate significant changes in protein matrix upon calcium chelation causing lower stiffness.

It is surprising that steady shear viscosity increases by 52% after simple elongation of model Mozzarella cheese for 120 s whereas shearing in the Blentech at 50 rpm for 4000 s caused only small increases in steady shear viscosity (Chapter 4). The one dimensional elongational flow caused by rolling is apparently very effective at work thickening. One possible explanation of the results is that rolling causes elongation of the primary protein particles thus increasing viscosity because the particles with a higher aspect ratio occupy more hydrodynamic space.

One aspect of the results that is not well understood is that although the structure is macroscopically fibrous and the microstructure shows anisotropy there is no anisotropy in the stress-strain curves at strains below about 0.25 (Fig. 1). The anisotropy only develops at higher strains and is largely related to the fact that longitudinal samples strain harden at strains > 0.25 , whereas perpendicular samples begin to strain weaken and then fracture. Maybe the aligned structure has many more microcracks for initiation of fracture in the perpendicular orientation. These microcracks would open up as the cheese is strained leading to earlier fracture.

7.5. Conclusions

Both strain hardening and anisotropy were observed after elongation in sheared model Mozzarella cheeses at moderate levels of shear work (3.3-26.3 kJ/kg). Structural alignments of both protein and fat phases were regarded as major contributing factors to this behaviour. Strain hardening and anisotropic character were absent from a model cheese with prolonged working (>58 kJ/kg) because the structure was homogeneous and isotropic but also contained a number of weak spots. Anisotropy and strain hardening were also observed with nonfat cheese. This is attributed to the presence of macroscopic protein fibres in the direction of rolling even in the absence of fat. Schematic models are proposed to explain strain hardening and anisotropy in a full fat model Mozzarella cheese. The model consists of fat dispersed in a gel matrix having two structural elements, cross-linked and non-cross-linked caseins.

8.0 Extensional viscosity using lubricated squeezing flow and time-temperature superposition of sheared model Mozzarella cheeses.

Chapter Summary

LSF and TTS techniques were applied to sheared model Mozzarella cheeses. Transient extensional viscosity was determined using constant strain rate LSF method on the Anton Paar rheometer. Model cheeses exhibited strain rate thinning and strain hardening behaviour at lower strain rate ($\dot{\epsilon}_b = 0.001 \text{ s}^{-1}$). Equibiaxial extensional viscosity obtained at $\dot{\epsilon}_b = 0.001 \text{ s}^{-1}$ and $\epsilon_b = 0.4$ discriminated sample treatments well and it increased exponentially with shear work input supporting proposed work thickening model in Chapter 4. TTS on model cheese confirmed that experimental cheeses exhibited entangled polymer melt type behaviour at low shear work levels ($<10 \text{ kJ/kg}$), a structural transition at 58.2 kJ/kg and VE solid at higher shear work levels ($>70 \text{ kJ/kg}$). Further work is proposed to obtain the relaxation spectrum of cheese to predict its behaviour during processing and application.

8.1. Introduction

The manufacturing process of Mozzarella type cheese involves a working step that involves stretching and folding action which gives rise to the typical fibrous protein network structure. Cheese undergoes extensional deformation during the stretching action. During application of Mozzarella cheese on pizza, under baking conditions, blister formation also involves biaxial extensional deformation. Therefore, it is important to study the extensional rheology of cheese.

Lubricated squeezing flow (LSF) is a relatively simple technique to measure extensional viscosity as it doesn't need sophisticated instruments and is suitable for samples which can't hold their shape for long, e.g. molten Mozzarella. LSF can be performed on texture analysers in constant load or constant speed mode. In LSF strain rate changes with time and deformation, therefore it is difficult to separate time effects from the material response. Constant strain rate tests would overcome this problem. Huc, Mariette and Michon (2014) developed a constraint strain rate LSF method on an Anton Paar MCR 302 rheometer. They varied the vertical cross head speed exponentially to achieve constant biaxial strain rates.

Time-Temperature Superposition (TTS) is a useful technique to obtain master curves in an extended frequency range. Based on Boltzmann's superposition principle, total deformation

can be obtained by adding individual strains within the linear viscoelastic limit. Frequency sweeps are conducted at different temperatures. A reference temperature is chosen and frequencies are shifted on the horizontal axis to obtain alignment of dynamic moduli. Low temperature corresponds to higher frequencies and vice-versa. TTS helps in understanding molecular interactions taking place during melting of the cheese matrix, particularly of the protein network at high temperatures, as these reactions have different relaxation times, and thus are frequency dependent. In this work, the effect of shear work on extensional viscosity of model cheese was studied and also master curves for sheared model cheeses were obtained.

8.2. Materials and Methods

8.2.1. Materials

Model cheese samples were prepared in the pilot plant at FRDC using the detailed protocol given in section 4.2. Three versions of cheese samples, fullfat, nonfat and TSC added full fat cheese were prepared by mixing ingredients (protein gel, cream, water and salt) together and working in the Blentech cheese cooker at 70 °C and 150 rpm. Shear work ranged from 3.3 to 73.7 kJ/kg with different working times (375-2950 s) (Table 4.1). Samples were frozen after preparation and were thawed at 4 °C for at least three days for structure equilibrium before measurement.

8.2.2. Lubricated Squeezing flow (LSF)

Constant strain rate LSF was performed on model cheese at 45 °C using an MCR 302 rheometer (Anton Paar, Graz, Austria) as a texture analyser in compression mode (Huc et al., 2014). A non-sticky plastic film was glued to both upper (Diameter 25 mm) and lower smooth parallel plates and a medium viscosity mineral oil film was applied to the contact surfaces to ensure perfect slip conditions. The method was successful at 45°C but it did not work well (sensitivity issues) at higher temperatures as the cheese was relaxing faster at the timescale of deformation. Cheese samples were cut in 25 mm diameter discs at 5 °C using a cork borer and wire cutter. Samples were then placed between the rheometer plates and heated to 45 °C using the in-built Peltier heating system for both upper (H-PTD-200) and lower plates and left for 2 min to attain temperature equilibrium.

Normal force (F_N) was measured during squeezing. During constant speed LSF, biaxial strain rate increases with deformation. The moving head was driven at an exponentially

decreasing speed (dH/dt) maintaining constant biaxial strain rate $\dot{\epsilon}_b$ (s^{-1}) (Fig. 8.1). The following equations apply,

$$\dot{\epsilon}_b = -\frac{1}{2} \left[\frac{dH}{H.dt} \right] \quad (8.1)$$

$$H = H_0 e^{-2\dot{\epsilon}_b t} \quad (8.2)$$

H_0 is the initial height and H the height at time t .

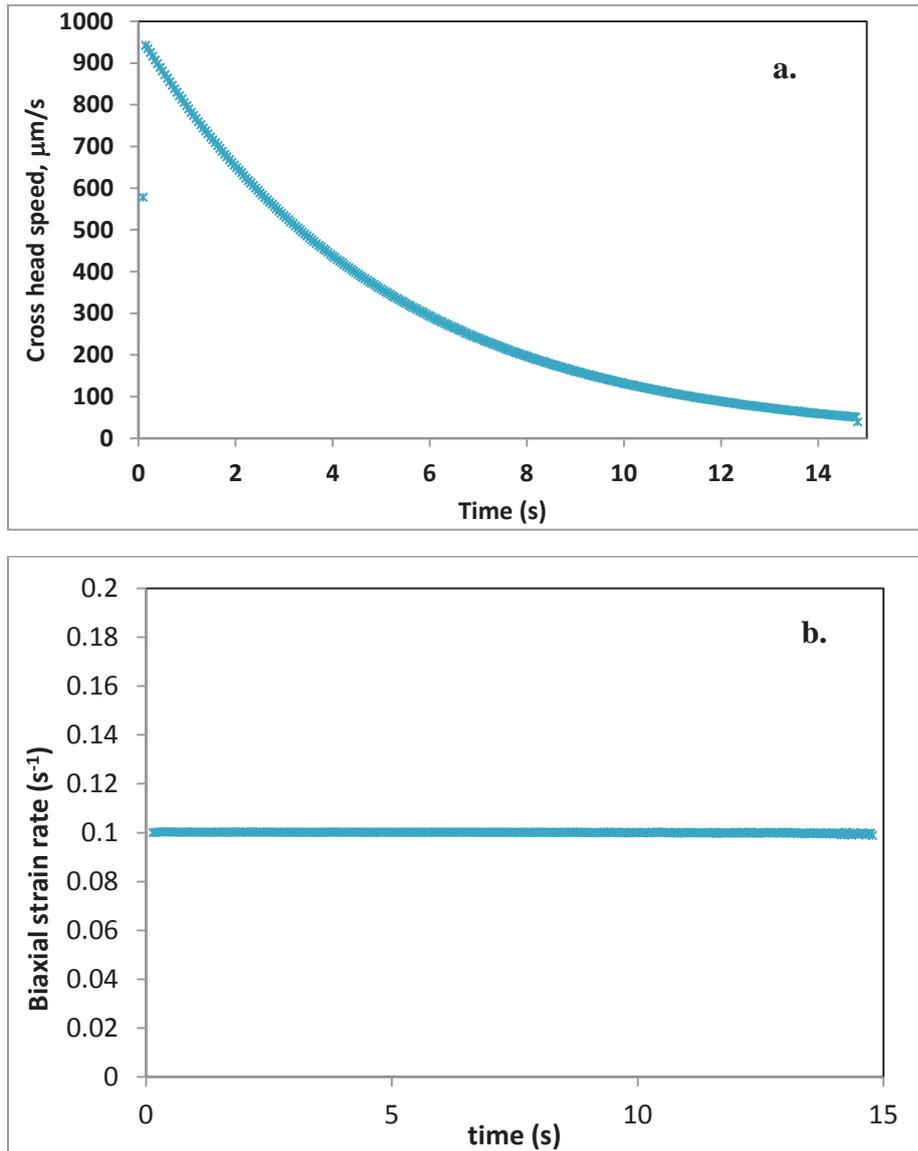


Figure 8.1 LSF experiments on MCR 302 using a. exponentially decreasing cross head speed; b. ensuring constant strain rate.

Biaxial elongational stress difference, σ_b and biaxial Hencky strain, ϵ_b were calculated from the following equations as given by Chatraei et al. (1981) and Campanella et al., (1987)

$$\sigma_b = \frac{F_N}{\pi R^2} \quad (8.3)$$

$$\varepsilon_b = -\frac{1}{2} \cdot \ln\left(\frac{H(t)}{H_0}\right) \quad (8.4)$$

Biaxial transient elongational viscosity

$$\eta_b = \frac{\sigma_b}{\dot{\varepsilon}_b} \quad (8.5)$$

8.2.3. Time-temperature superposition

For creation of master curves at a large range of frequencies, the time-temperature superposition principle was used. Frequency sweeps ($0.4\text{-}100 \text{ rad}\cdot\text{s}^{-1}$) within the linear viscoelastic limit were conducted on an MCR 301 (Anton Paar, Graz, Austria) using 20 mm serrated plate geometry applying 0.5% strain amplitude at different temperatures ($5\text{-}70 \text{ }^\circ\text{C}$) with $5 \text{ }^\circ\text{C}$ intervals. Data were captured with a logarithmic slope at 5 points per decade. Cheese samples (at $5 \text{ }^\circ\text{C}$) in disc shape (20 mm diameter and $\sim 3 \text{ mm}$ thickness) were placed between the plates by closing the gap till a 1 N normal force was attained (Chapter 3). Vegetable oil was spread around the periphery of the cheese disc in order to avoid moisture loss during experiments.

8.3. Results and Discussion

8.3.1. Extensional viscosity of sheared model Mozzarella cheese

A typical stress-strain curve for model Mozzarella cheese obtained using constant strain rate LSF is shown in Fig. 8.2. As shown in the figure, the cheese exhibited nonlinear behaviour concave downwards at smaller strains ($\varepsilon_b < 0.30$). At larger strains nonlinear behavior concave upwards was observed with some strain hardening as stress increased more than proportionately with strain. More scatter in the data was observed at higher strains ($\varepsilon_b > 1.0$) which may be attributed to thinning of the lubricating film. The evolution of equibiaxial extensional viscosity (also known as biaxial growth coefficient) is shown in Fig. 8.3 at three strain rates. Data suggest the transient nature of the material. Data obtained at three different strain rates coincide indicating reliability of the measurements. Estimated values of biaxial extensional viscosity in the linear viscoelastic region could be plotted using a relaxation spectrum to check fitting of the data. We couldn't perform this check as we hadn't determined the relaxation data for the samples. For viscoelastic materials, it is expected that

predicted extensional viscosity would reach a steady state value with time. If the measured values of extensional viscosity are higher than the predicted values at longer timescales (or larger strains), this indicates apparent strain hardening in LSF (Guadarrama-Medina, Shiu & Venerus, 2009).

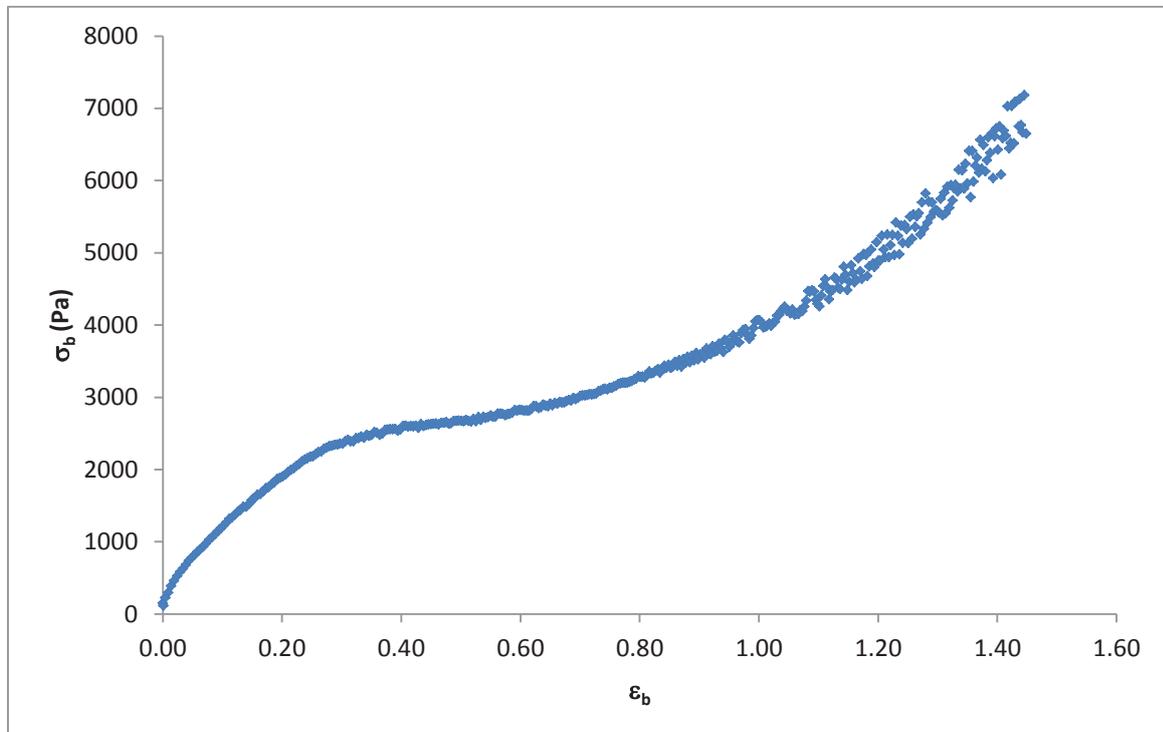


Figure 8.2 Example of stress (σ_b) - strain (ϵ_b) curve obtained for model cheese at 0.1 s^{-1} strain rate using constant strain rate LSF at $45 \text{ }^\circ\text{C}$.

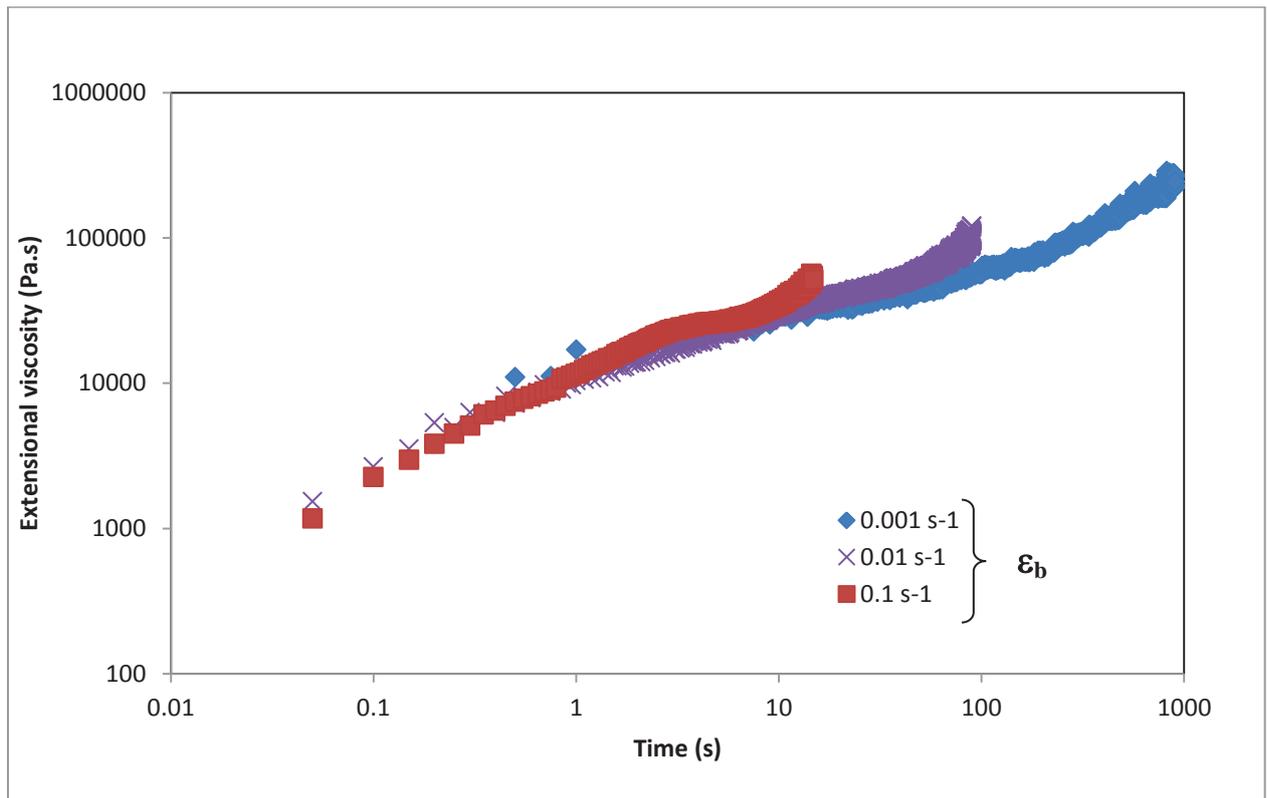


Figure 8.3 Extensional viscosity vs time curves of model Mozzarella cheese (8.8 kJ/kg) obtained in constant strain rate LSF at three different strain rates and at 45 °C.

Flow curves for equibiaxial extensional viscosity were prepared at various levels of strain (Fig. 8.4). A Power law model ($\eta_b = K \cdot \dot{\epsilon}^{n-1}$) was found suitable to fit the experimental data. Strain rate thinning behaviour was observed for extensional viscosity of model cheese samples obtained in the strain range 0.01-0.90. A slightly higher extent of strain rate thinning was observed at higher strains. Similar trends were observed for the samples produced at different shear work inputs. These results are consistent with other results reported for cheese (Ak & Gunasekaran, 1992; Rohm & Lederer, 1992; Huc et al., 2014).

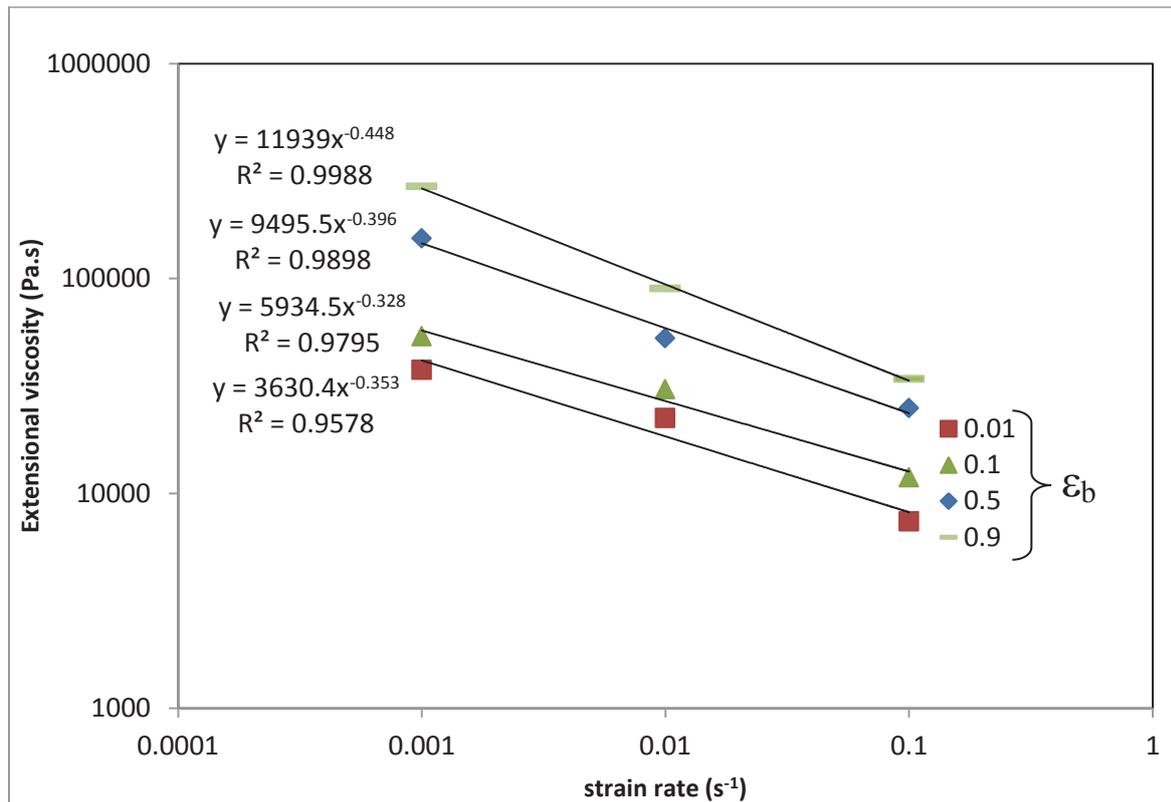


Figure 8.4 Iso-strain (biaxial) flow curves of model Mozzarella cheese (8.8 kJ/kg) exhibiting strain rate thinning behaviour at 45 °C.

When $\ln(\sigma_b)$ is plotted against strain (ϵ_b), a linear relationship is expected for viscoelastic materials (Fig. 8.5). The slope of the linear regression line indicates extent of strain hardening (Launay & Michon, 2008). All samples within the shear work input range 8.8-73.7 kJ/kg, exhibited strain hardening behaviour (slope = 2.1-2.3) at 0.001 s⁻¹ within the 0.05-0.8 strain range as per the criteria (slope >2.0) suggested by van Vliet (1992) for biaxial deformation. This implies structural changes in cheese during biaxial extension (Huc et al., 2014). The formation of dense protein regions during compression may be considered as one of the factors contributing to strain hardening (Ak & Gunasekaran, 1997). However, the slope values suggested by van Vliet (1992) referred to bubble stability in dough during proofing and baking and may be less relevant to Mozzarella-type cheese. No observable difference in strain hardening behaviour of cheese samples was observed with varied amounts of shear work treatment. This contrasts with the tensile strain hardening behavior in Chapter 7 where no strain hardening was observed at higher shear work inputs (58.2 kJ/kg).

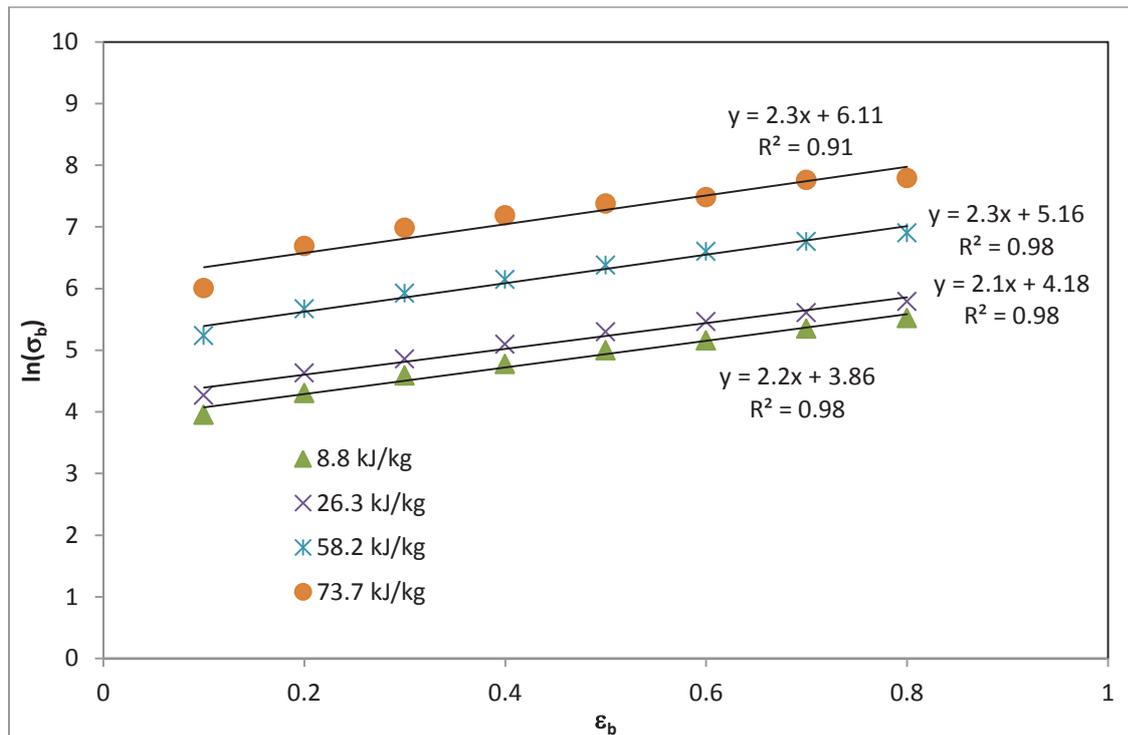


Figure 8.5 Strain hardening observed in model Mozzarella cheese during constant strain rate LSF experiments at 45 °C ($\dot{\epsilon}_b = 0.001 \text{ s}^{-1}$). The slope of the regression lines indicates extent of strain hardening.

At lower strain rates (0.001 s^{-1}) the LSF technique differentiated very well between shear work treatments with >10 fold increase in biaxial extensional viscosity upon increase in shear work from 8.8 kJ/kg to 73.7 kJ/kg (Fig. 8.6a). Extensional viscosity at 0.4 strain corresponding to 400 s was selected to compare the effect of shear work on model cheeses. An exponential increase in extensional viscosity was observed with shear work input, indicating work thickening behavior (Fig. 8.6b). This relationship validates the proposed work thickening model for steady shear rheology in Sections 4.3.2 and 4.4. The exponents for shear viscosity and extensional viscosity were the same i.e. $\sim 0.03 \text{ kg/kJ}$, showing good agreement on the shear work induced effects on rheology. The proposed mechanism of work thickening was discussed in detail in Chapter 4.

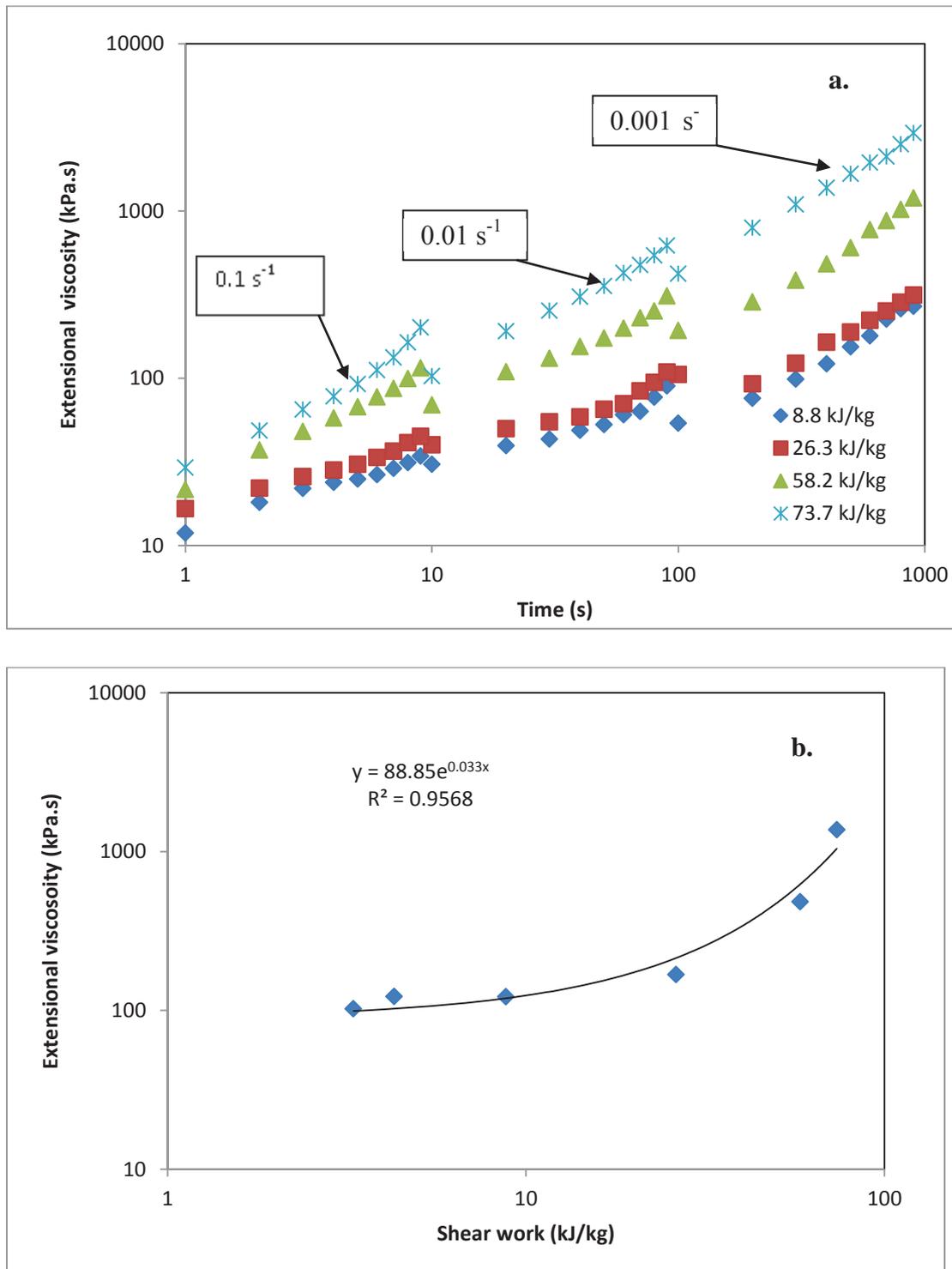


Figure 8.6 Effect of shear work on biaxial extensional viscosity of model cheese obtained using constant strain rate LSF at 45 °C; a. at three strain rates (0.1,0.01, 0.001 s⁻¹); b. at 0.001 s⁻¹ and 0.4 strain. Samples were prepared by working in the Blentech at 150 rpm (Day 1, Table 4.1) and at 70 °C.

8.3.2. Time temperature superposition (TTS)

Time-temperature superposition (TTS) was attempted on model cheeses in order to prepare a master curve expanding the frequency regime by conducting frequency sweep experiments at various temperatures and shifting the rheological data on the frequency axis at a selected reference temperature, i.e. 70°C. Rheological parameters obtained for model cheese in the temperature range 5-70°C were reduced to a reference temperature ($T_0=70^\circ\text{C}$) using the temperature dependent horizontal shift factor for frequency (a_T). Low temperature leads to elastic response of the material and thus corresponds to a higher frequency region. On the other hand, high temperature results in a more viscous response from the sample because of weaker molecular interactions and thus, corresponds to a lower frequency region.

Model cheeses having shear work 8.8 kJ/kg demonstrated viscoelastic (VE) solid behaviour ($G' > G''$) at higher frequencies which corresponds to lower temperature (below gel-sol transition temperature) (Fig. 8.7a). Similarly, at lower frequencies (at high temperature) model cheese behaved like a VE liquid ($G' < G''$). A cross over point ($\sim 135 \text{ rad.s}^{-1}$) in the master curve would indicate the gel/sol transition point which can also be linked with the corresponding temperature ($\sim 50\text{-}55^\circ\text{C}$) of cheese melting (Fig. 8.7a). This also indicates the entangled polymer network type rheological behaviour where weak physical interactions control the material response (Tunick, 2011).

Higher shear work input (58.2 kJ/kg) resulted in overlap of G' and G'' data in the lower frequency regime ($0.4\text{-}700 \text{ rad.s}^{-1}$) corresponding to $60\text{-}70^\circ\text{C}$ (Fig. 8.7b). In this region, G' and G'' both increased at a similar rate with frequency resulting in relatively less independence of LT with respect to frequency thus indicating a critical stage of transition (Winter & Chambon, 1986) at this level of shear work. A more detailed discussion has been given in Section 5.3.2 regarding the critical structural and rheological transition of model cheese induced by shear work. Excessive working (73.7 kJ/kg) led to a transition of material to a viscoelastic solid as observed in the master curve (Fig. 8.7c) with $G' > G''$ at all frequencies.

Master curves for nonfat cheese (6.8 kJ/kg) and TSC added full fat cheese (4.4 kJ/kg) are shown in Fig. 8.8. The cross over frequencies on the master curves are $\sim 400 \text{ rad/s}$ and $\sim 253 \text{ rad/s}$ for nonfat and full fat TSC added cheeses respectively.

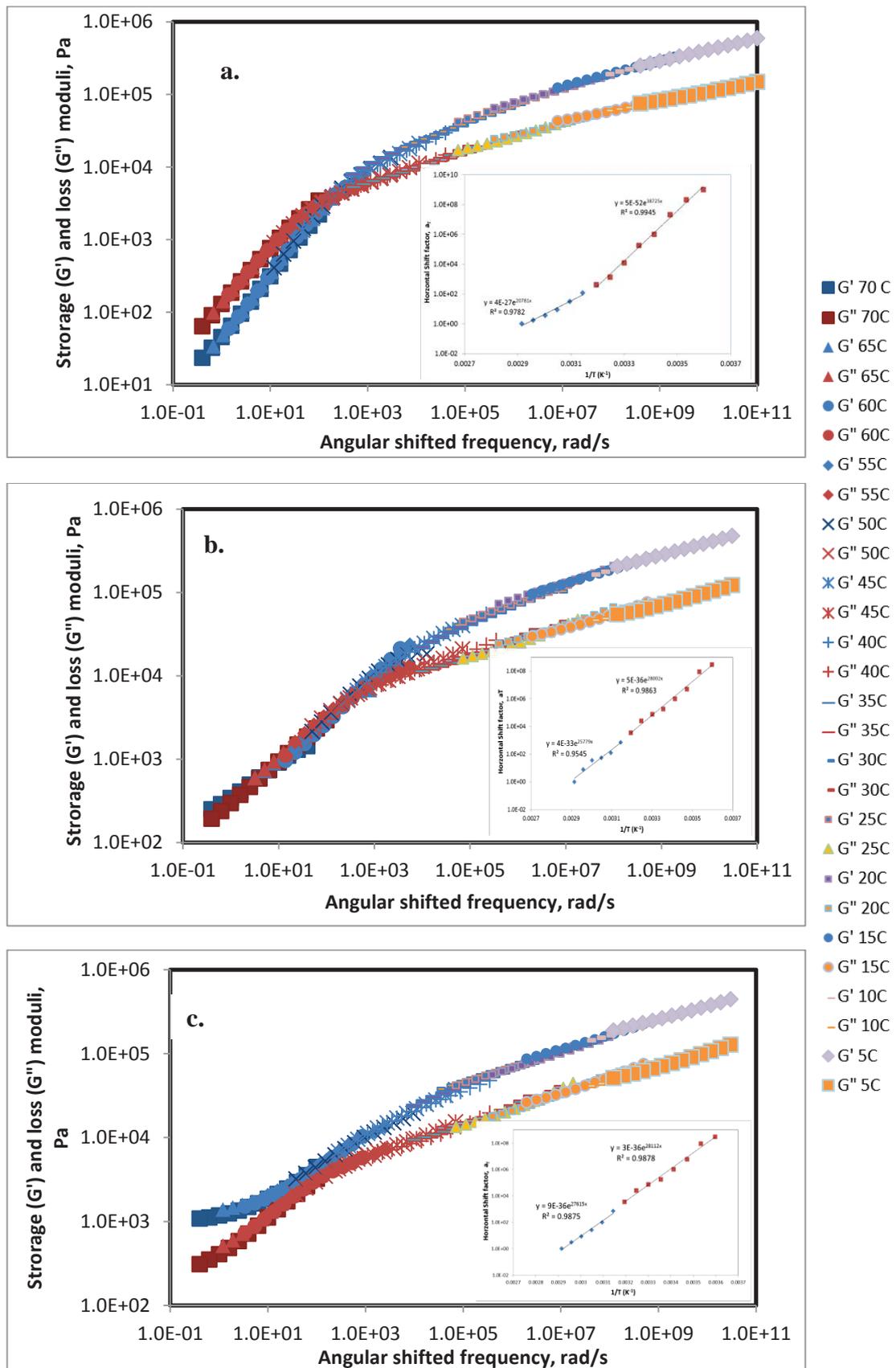


Figure 8.7 Master Curves of fullfat model Mozzarella cheeses at 70 °C (Reference temperature) having varied amount of shear work; a. 8.8 kJ/kg; b. 58. 2 kJ/kg; c. 73.7 kJ/kg. Inset pictures depict temperature dependence of horizontal (a_T) shift factors.

Both cheeses exhibited entangled polymer melt type behaviour. Nonfat cheese was more elastic and rigid than TSC added cheese. All cheeses above the cross over point demonstrated very low frequency dependence ($n < 0.20$) indicating a viscoelastic solid network resembling physical or cross-linked gel networks (Tunick, 2011).

Temperature induced changes in the protein phase could affect the rheology significantly. A major change at higher temperatures is the transfer of calcium from solution to an insoluble form which interacts with proteins (mainly casein) and may enhance protein-protein interactions (Lucey et al., 2003). Another change is increased hydrophobicity of caseins leading to expulsion of water or decreasing solubility. These phenomena make cheese a thermorheologically complex material resulting in deviations in the master curve (Udayarajan et al., 2007).

The horizontal frequency shift factor for model cheese would be expected to follow the Arrhenius equation

$$a_T = \text{Exp} \left[\frac{E_a}{R} \left(\frac{1}{T} - \frac{1}{T_{ref}} \right) \right] \quad (8.6)$$

Where E_a is activation energy (J.mol^{-1}), R is universal gas content ($8.314 \text{ J.mol}^{-1}.\text{K}^{-1}$), T is the measurement temperature in K , and T_{ref} is the reference temperature.

Analysis of shift factors can be helpful in determining whether relaxation times follow Arrhenius kinetics (Udayarajan et al., 2007).

Table 8.1 Activation energies for horizontal shift factors (a_T) used for master curves of model cheese.

Cheese type	Shear work (kJ/kg)	Activation energies for a_T (kJ.mol ⁻¹)		
		5-40 °C	45-70 °C	5-70 °C
Full fat cheese	8.8	321.9	172.7	265.7
	26.3	293.5	176.1	262.0
	58.2	232.8	214.3	229.9
	73.7	233.7	229.6	241.1
Nonfat cheese	6.8	374.4	182.0	360.6
TSC added full fat cheese	4.4	460.4	187.4	315.3

Shift factors for model cheese decreased with increase in the temperature of measurement. Viscosity also decreases with temperature; therefore a positive correlation

is expected between shift factor and viscosity. A distinct discontinuity in temperature dependence was observed for full fat cheese (8.8 kJ/kg), nonfat cheese (6.8 kJ/kg) and TSC added full fat cheese (4.4 kJ/kg) with different behaviour in two temperature regions, i.e. 5-40 and 45-70 °C. However, full fat cheeses with 58.2 and 73.7 kJ/kg of shear work input did not exhibit this discontinuity. From the plots between shift factor and $1/T$, the activation energies required for relaxation of cross links and entanglements (Kasapis & Sworn, 2000; Nickerson et al., 2004; Udayrajan et al., 2007) in model cheeses in these two distinctive regions are presented in Table 8.1. All cheeses exhibited higher activation energies (230-460 kJ.mol⁻¹) in the first region of heating (5-40 °C) than in the high temperature zone (45-70 °C). These values were consistent with previous reported work (Udayrajan et al, 2007).

A higher level of activation energy denotes a more rapid change in viscosity (or any other property) with temperature (Steffe, 1996). In other words it is a measure of the extent of temperature dependency of a property.

Activation energy increased with shear work input for the higher temperature range (45-70 °C) indicating more temperature dependence. This may be a further indication of increased protein-protein interactions at higher temperature (Udayarajan et al., 2007). Shift factors for TSC added cheese ($E_a=187.4$ kJ.mol⁻¹) and nonfat fat cheese ($E_a=182.0$ kJ.mol⁻¹) were slightly more temperature dependent than that for full fat cheese ($E_a=172.7$ kJ.mol⁻¹) in the higher temperature range (45-70 °C). Activation energies obtained in the temperature range of 5-70°C were consistent (229-265 kJ/kg) irrespective of shear work input. However, this is too broad a temperature range to generalise the activation energy for the melting process.

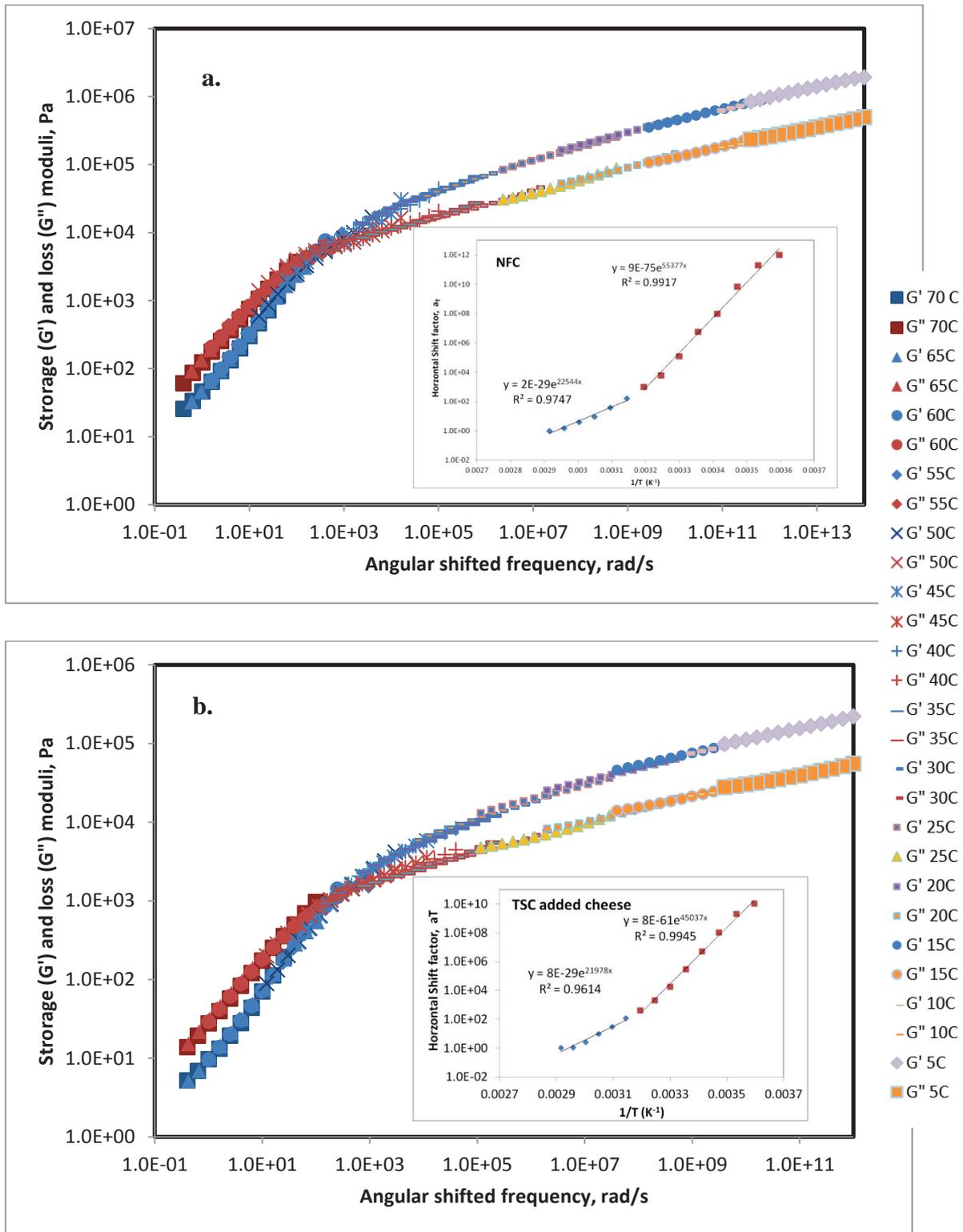


Figure 8.8 Master Curves of model Mozzarella cheeses at 70 °C (Reference temperature); a. nonfat cheese (6.8 kJ/kg); b. TSC added full fat cheese (4.4 kJ/kg). Inset pictures depict temperature dependence of horizontal (a_T) shift factors.

Further analysis of superimposed rheological parameters and temperature dependence of shift factors is required. Because of its wide applicability, TTS is recommended as a

methodology for future rheological measurement on model cheese. This could potentially be used to explain the temperature dependent rheological response of the cheese matrix due to changes in molecular level interactions, e.g. protein-protein, fat-protein and calcium-protein interactions.

8.4. Conclusions

LSF and TTS techniques were applied to model Mozzarella cheeses having varied amounts of shear treatment. Constant strain rate LSF experiments were successfully conducted on model cheeses on the Anton Paar rheometer. Strain rate thinning at all strains and strain hardening ($\dot{\epsilon}_b = 0.001 \text{ s}^{-1}$) were observed for model cheeses. Equibiaxial extensional viscosity ($\dot{\epsilon}_b = 0.001 \text{ s}^{-1}$ and $\epsilon_b = 0.4$) was found to distinguish well between Shear work inputs. An exponential relationship between extensional viscosity and shear work was found, supporting the proposed work thickening model in Chapter 4. TTS on model cheese confirmed that 58.2 kJ/kg was a structural transition point. All experimental cheeses exhibited entangled polymer melt type behaviour at low shear work levels (<10 kJ/kg). Higher shear work levels resulted in transition from VE liquid to VE solid material. A more thorough study is proposed to measure the relaxation spectrum of cheese to predict its behaviour during processing and application.

9.0 Overall discussion and conclusions

This study investigated the effect of shear work treatment on various rheological properties of model Mozzarella cheese and developed an understanding of their relationship with melt functionality. The overall goal was to find appropriate rheological methods to establish and validate links between the process and the application of model Mozzarella cheeses. The study focused on the development of suitable methods to measure rheology at temperatures appropriate to melt functionality in pizza application by producing experimental samples with a range of shear work treatment, conducting rheological studies on these samples and by finding correlations with melt functionality.

The core hypothesis of the project was that Mozzarella cheese work thickens during working in a twin screw cooker. If this hypothesis is true then this work thickening phenomenon is likely to also affect rheology, structure and melt functionality of cheeses. The main sub-hypothesis states that any unmelt of model cheese on a pizza is caused by overworking and some measure of rheology should be able to correlate with this unmelt. To test these hypotheses, a range of model Mozzarella cheeses were produced by giving varied amounts of shear work input using the method described in section 4.2.2. Samples were then subjected to a range of rheological tests (Chapter 4-8) and also to the Schreiber melt test. The rheological property that correlates best with melt score and also changes most with shear work would be useful as a process control tool for optimizing functionality during the production of model cheese.

Two possible criteria for selection of the best rheological property could be the extent of correlation with melt functionality and the sensitivity to melt score change. Table 9.1 shows a matrix of Pearson correlation coefficients for all rheological properties and melt score. It is evident from that table that all rheological properties correlate strongly ($r > 0.90$) with melt functionality. All rheological properties correlated negatively with melt score except LT_{max} , flow behaviour index n and fracture properties. Fig. 9.1 and Fig. 9.2 demonstrate the relationship of rheological properties with shear work and melt score, respectively. Rheological properties (RP) were normalized to a uniform scale by dividing by the minimum value of that property so that they can all be plotted together on one graph. This method of normalization was chosen as it would show which properties differentiated best between samples as shear work was varied. The increase

in some rheological properties of model cheese followed an exponential growth function with respect to shear work input (Fig. 9.1). This confirms the core hypothesis and supports the work thickening model proposed in section 4.4. Work thickening at the same time leads to a decrease in melt score (negative correlation, Fig. 9.2) confirming the main sub hypothesis. Work thickening is attributed to enhanced protein-protein interactions as discussed extensively in Chapters 4 and 5.

A comparison of rheological properties suggests that apparent viscosity (measured at 70 °C) appears to be most affected by changes in shear work or melt functionality, followed by G' at 70 °C, extensional viscosity and then consistency coefficient, K (Fig. 9.1 and Fig. 9.2). G' at 70 °C was more sensitive to melt functionality at lower levels of shear work (3.3-26 kJ/kg). For best process control of melt functionality in the lower shear work region G' at 70 °C would be the best rheological property. Measurement of apparent viscosity of viscoelastic materials in an online process has usually been much easier than G' . However, recent online sensors based on vibrational rheology would be able to measure oscillatory shear properties such as G' .

A structural model for changes in the cheese has been proposed in section 5.5 (Fig. 5.12). The model suggests that protein strands at lower levels of shear work inputs interact with each other through physical and other weak interactions, behaving like an entangled polymer network. At higher shear work levels (58.3 kJ/kg at 150 rpm), sufficient energy is delivered to the cheese to cause stronger interactions, e.g. chemical cross-links or calcium mediated interactions. This was identified as a critical stage during the working of model cheese. Further increases in shear work input (73.7 kJ/kg at 150 rpm), resulted in random aggregation of protein strands in the cheese matrix tightly embedding the fine fat particles, and resulting in macroscopic structural failure, loss of serum and simultaneous loss of melt and stretch properties of the model cheese. Studies on non-fat cheese and cheese with tri-sodium citrate added revealed that shear work induced changes were mainly governed by changes in the protein phase.

Table 9.1 Pearson correlation coefficients of Melt score and rheological properties of full fat model Mozzarella cheeses^a.

	Melt Score	K	n	η_{app}	LT _{max}	Cross over temperature	G'70	Fracture stress	Fracture strain	Initial Modulus	Extensional Viscosity
Melt Score	1	-.956**	.997**	-.902*	.956**	-.989**	-.934**	.980**	.985**	-.975**	-.940*
K		1	-.958**	.988**	-.872*	.999**	.997**	-.969**	-.986**	.993**	.999**
n			1	-.904*	.963**	-1.000**	-.939**	.969**	.987**	-.994**	-.944*
η_{app}				1	-.791	.997**	.994**	-.983**	-.995**	.992**	.993**
LT _{max}					1	-.963**	-.846*	.870	.904*	-.949*	-.850
Crossover temperature						1	.987**	-.965**	-.985**	.996**	1.000**
G'70							1	-.940*	-.962**	.985**	.999**
Fracture stress								1	.992**	-.959**	-.981*
Fracture strain									1	-.986**	-.985*
Initial Modulus										1	.995**
Extensional Viscosity											1

** . Correlation is significant at the 0.01 level (2-tailed).

* . Correlation is significant at the 0.05 level (2-tailed).

^aModel cheeses were prepared in twin screw Blentech cooker at 150 rpm and 70 °C (Table 4.1).

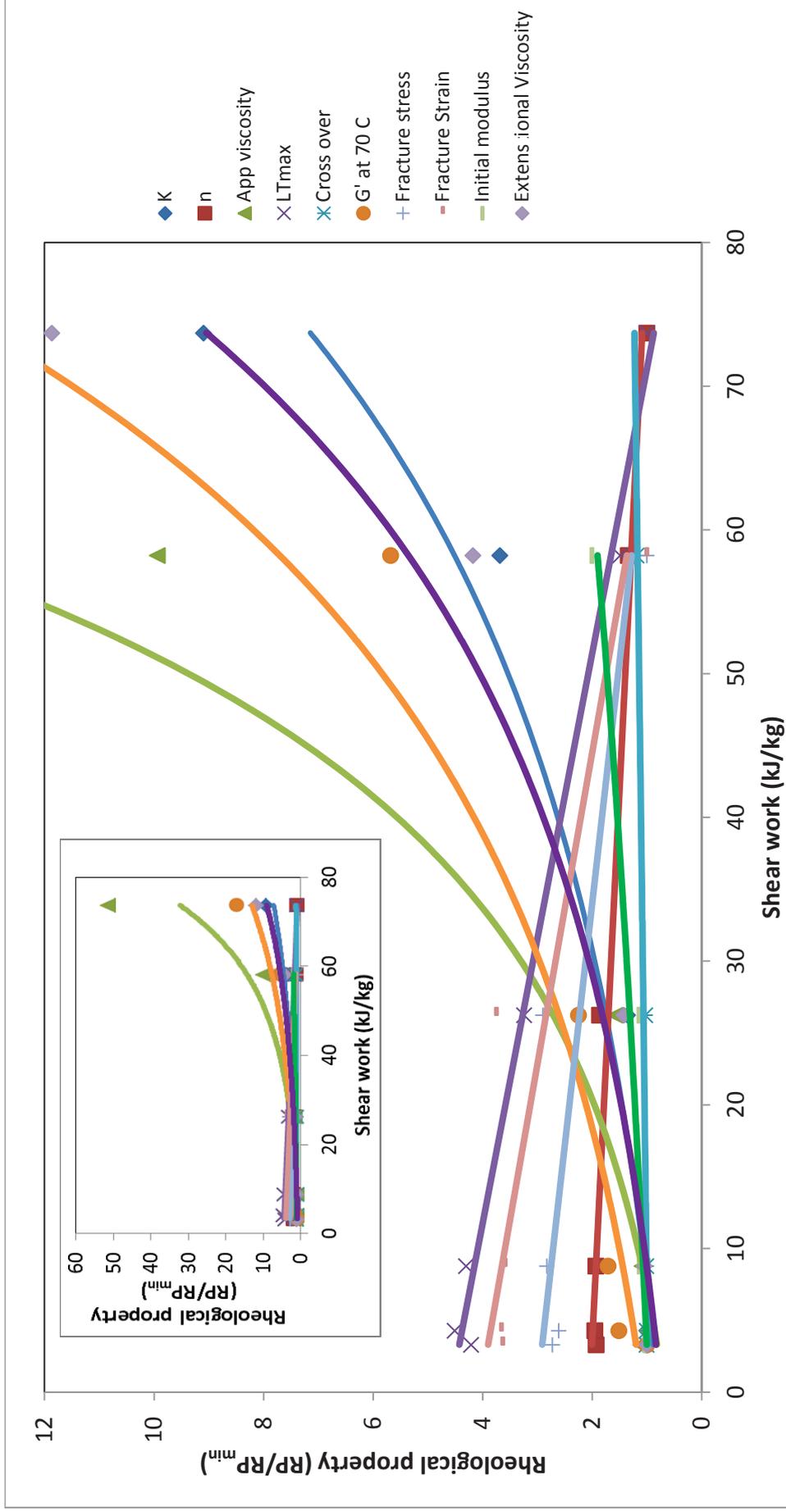


Figure 9.1 Shear work induced changes in rheological properties of model Mozzarella cheese

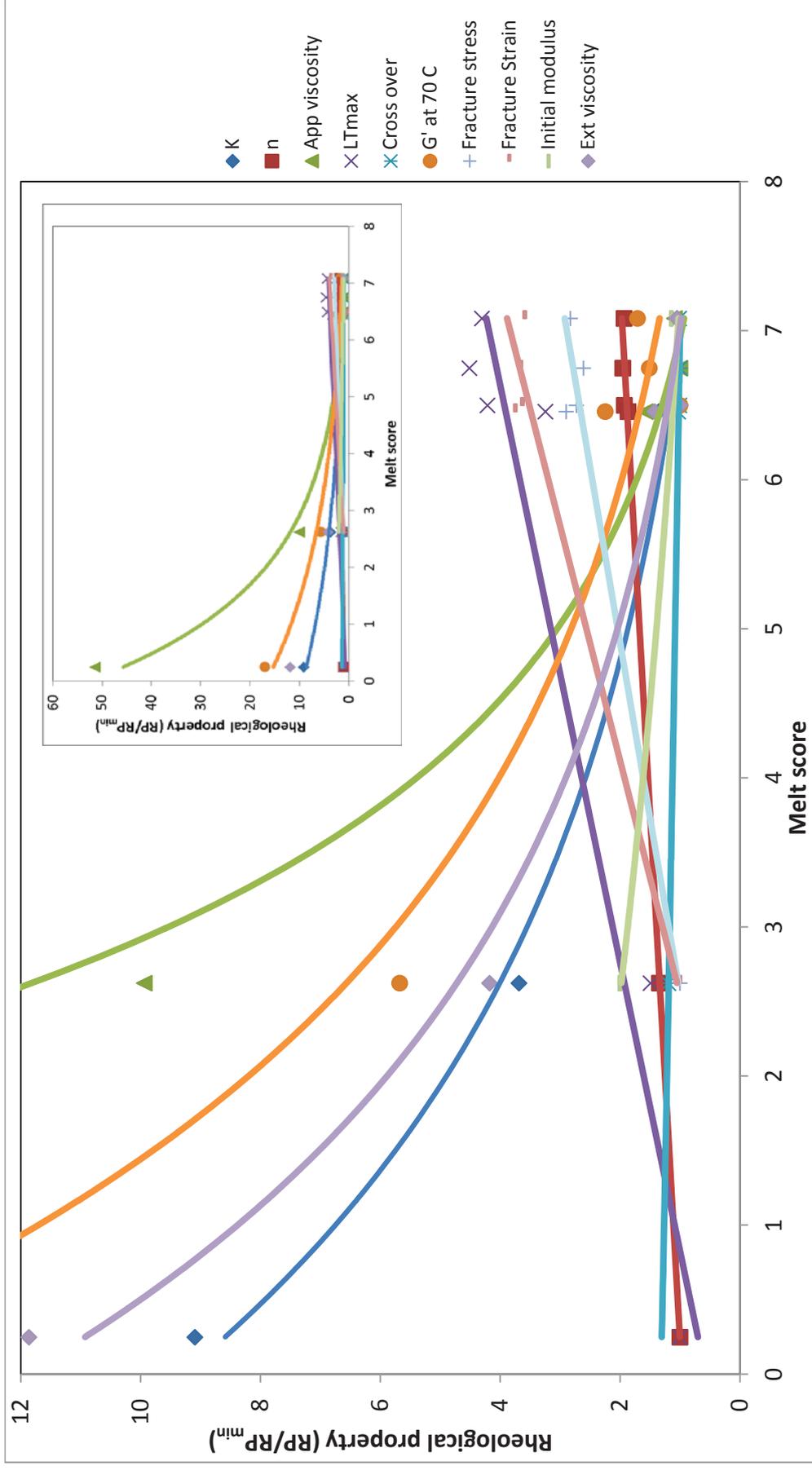


Figure 9.2 Changes in various rheological properties as a function of melt score.

Fig. 9.3 shows structure-process-property linkages discovered by the project and clearly indicates the possibility of using shear work and G' as tools in a process control loop for manufacturing model Mozzarella cheese. A limitation of the work is that the linkages have been tested for only one composition (Fat 23%, Protein 21% and Moisture 53%) and for only batch operation in a single device, i.e. Blentech. The best rheological properties that correlate well and change most with melt functionality are apparent viscosity and G' . Both properties could be used to establish a relationship with structural elements. We have selected G' at 70 °C as an example here because it is most sensitive in the lower shear work range. Fat particle size ($D_{4,3}$) was chosen as a structural parameter in this study as it was relatively to measure. However, network characteristics (image analysis of cryo-SEM/TEM micrographs or 3-D imaging and simulation studies or constitutive models) of the protein phase could also be considered as structural links but they are difficult to measure.

The linkage diagram follows a path that starts with a desired melt score (e.g. 5.5, Fig. 9.3a), reads the corresponding relative rheological property (RP) on the y-axis; then translates this to absolute RP. This then links to fat particle size ($D_{4,3}$) (Fig 9.3b). ~ 30 μm in this case, and then to the process variable (shear work) (Fig. 9.3c), 12 kJ/kg in this example. The next step is to choose process variables that can be controlled such as screw speed, torque, or mixing time. In Fig. 9.3d processing time is chosen giving 700 and 800 s at 250 and 150 rpm, respectively. More data would be needed to extend the model to other compositions and processing temperatures.

It is also useful to cast the data reported in this thesis in the S-PRO² framework proposed by Prof. Erich Windhab (Windhab, 2009). Windhab envisaged rheology as a macroscopic manifestation of dynamic changes in structure (micro to macro level) which can be controlled by processing. The S-PRO² diagram for model cheese is presented in Fig. 9.4 which shows the functional relationship structure ($D_{4,3}$)-process (shear work)-properties (melt score). Structuring (S) of model cheese takes place during processing (PRO-1), i.e. working, that can affect finished product properties (PRO-2) e.g. pizza baking or meltability. Altering the process may create a desired structure or rheological response to the finished product to deliver the desired functional property.

Major conclusions from this study are presented below:

1. Model Mozzarella cheese demonstrated transient viscoelastic effects, wall-slip and structural failure during rheological measurements at high temperature and higher shear rate when existing techniques were used. Methods were devised to give good data for steady shear viscosity that overcome these hurdles.
2. Model cheese work thickens which adversely affects melt functionality. Excessive working (>70 kJ/kg) leads to macroscopic structural failure, loss of serum and brittle texture. The work thickening model describes the rheological changes very well. The work thickening phenomenon is attributed to major changes in the protein phase.
3. Cheese during working at 70 °C goes through a systematic viscoelastic transition from liquid to solid state. A critical stage during this transition was successfully identified using frequency sweeps which matched well with the occurrence of the peak torque values during working. A model proposing a structural transition from entangled polymer network through cross-linked protein to randomly aggregated matrix explains the observed rheological changes.
4. Laminar mixing of the fat and protein phases in the twin auger Blentech cooker through layering, stretching and folding actions leads to striated microscopic and macroscopic structures. These striated structures are the basis for the mechanical and structural anisotropy observed in fat-protein networks which is required for the right characteristics to have good pizza functionality. Further mixing (shear work input >58 kJ/kg) results in striation break up, a more uniform distribution of fat within the protein network, the disappearance of anisotropic fibre structures, much finer fat particles and poor melt.
5. Strain hardening and anisotropy were observed during tensile testing of elongated, sheared model cheeses. This phenomenon was also observed with nonfat cheese and cheese with added TSC. This indicates a major role for the protein phase in both anisotropy and strain hardening. Overworked cheese samples did not strain harden and exhibited no anisotropy.

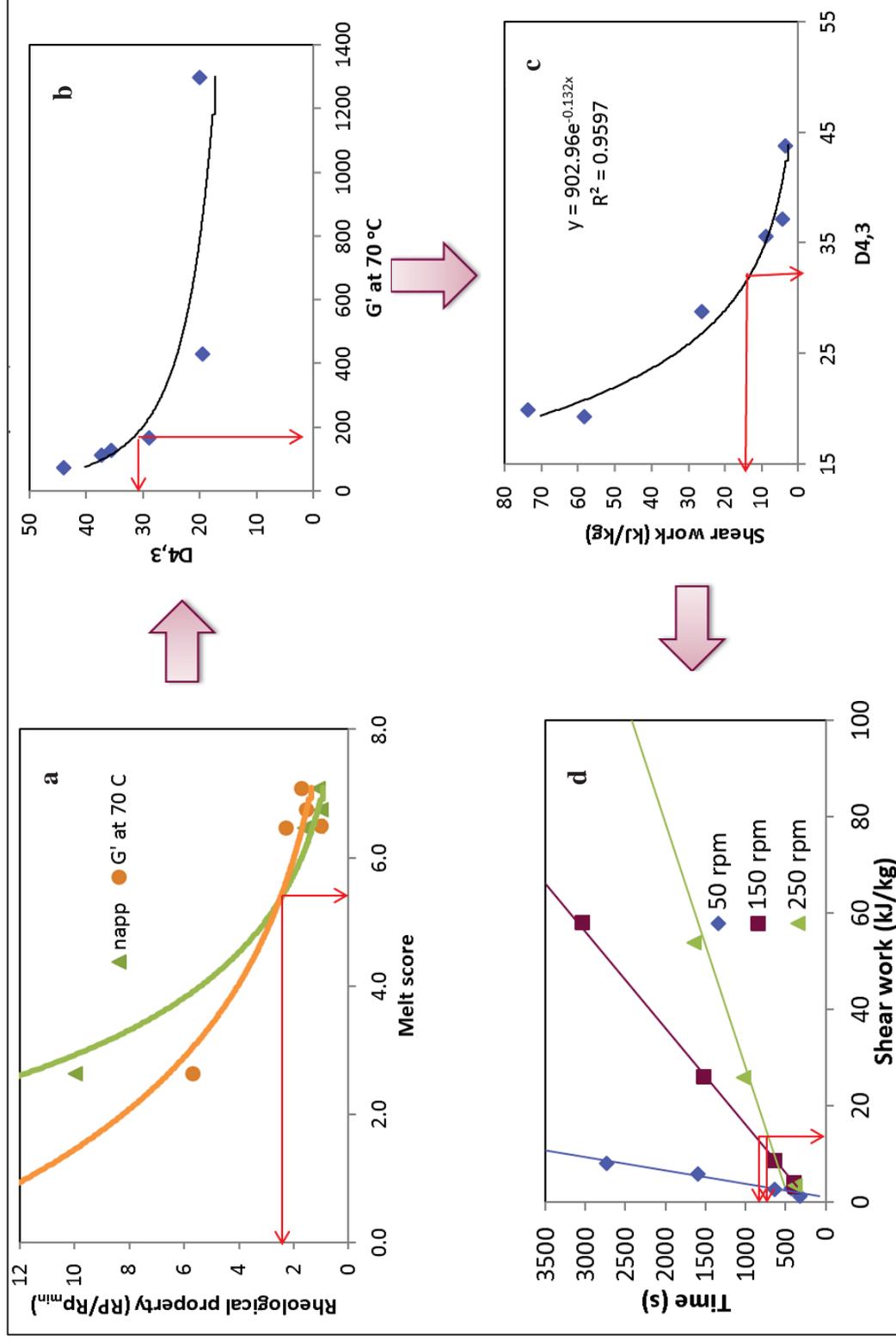


Figure 9.3 Structure-Process-Property linkages discovered for model Mozzarella cheese.

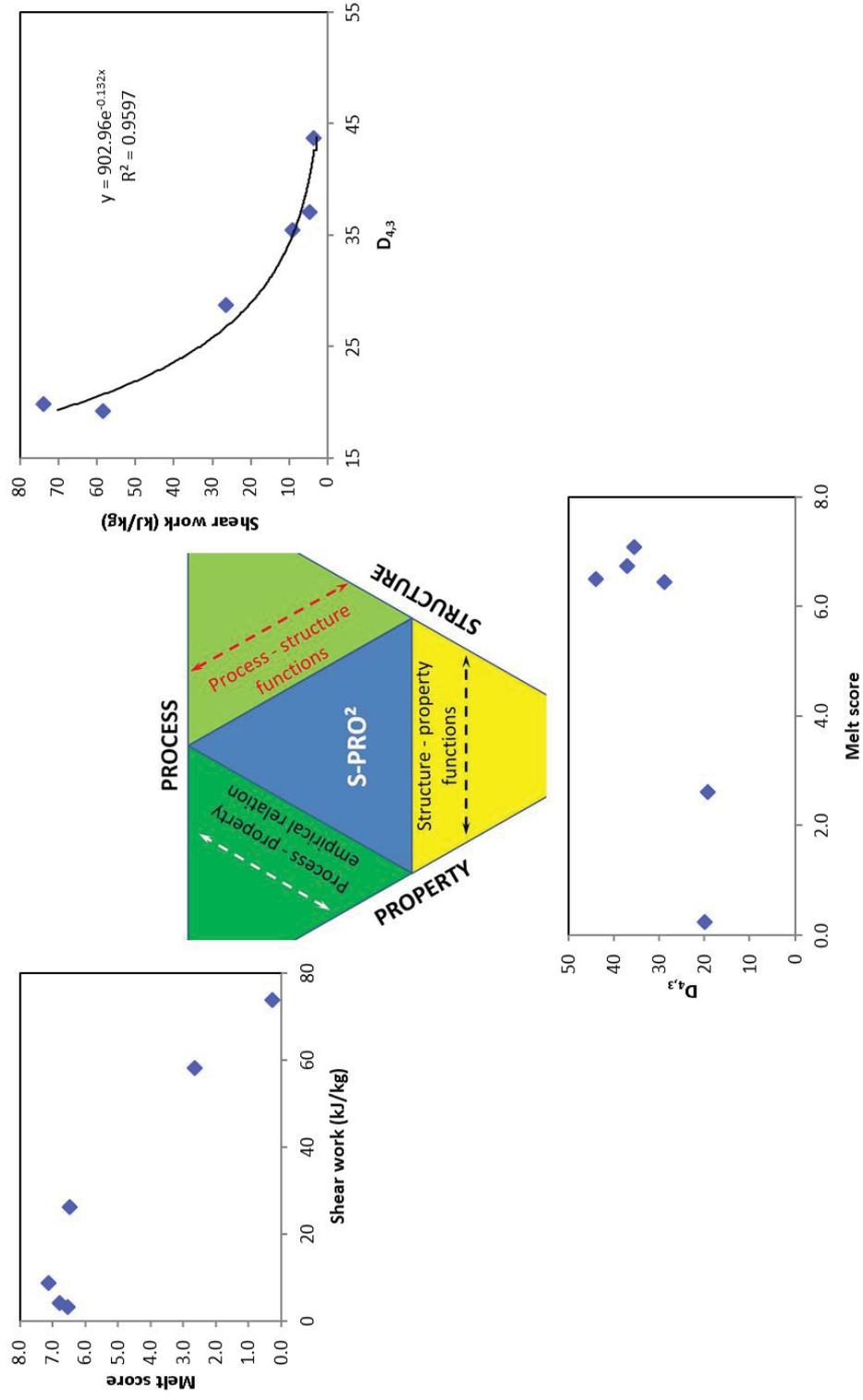


Figure 9.4 Windhab S-PRO² scheme for model Mozzarella cheese.

10.0 Recommendations for further research

The current study establishes and validates links between rheology and melt functionality and correlates this with structural changes during processing model Mozzarella cheese. We present below some of the ideas that should be considered to extend this work.

1. The anisotropy observed in nonfat cheese was intriguing. We tried to put together a model of the gel phase consisting of individual and cross linked protein elements. However, we had no experimental evidence to prove it. Therefore, elucidation of the structure at lower length scales such as the molecular and meso scales of sheared nonfat cheese using advanced techniques such as cryo-TEM/SEM, SAX and SANS would be interesting to get further insights into the working process. Network characteristics of the protein phase can also be studied by image analysis of cryo-SEM/TEM micrographs, by 3-D imaging and simulation or by using constitutive models.
2. It is proposed that increased protein-protein interactions (calcium-mediated) were the major cause of work thickening. It is also proposed a structural model consisting of entangled polymer networks with or without crosslinks. These suggestions were based on the available literature. We understand that it is difficult to comment on the exact nature and extent of interactions just from rheology. Therefore, a thorough study is proposed on the chemical (molecular) aspects of work thickening which could potentially establish the nature and extent of the various interactions taking place, e.g. protein-protein and fat-protein.
3. Developing constitutive equations using normal stress difference measurements, rigorous time-temperature superposition, and determination of relaxation spectrums at processing conditions would be useful to further understand the material behaviour during working of cheese. This information can also be useful for process modelling.
4. It is not clear during working and subsequent storage of model cheese whether protein-water interactions are modified. Therefore, it could be worthwhile to study the water binding properties of renneted casein gel and sheared cheeses using the methods developed by Prof. Kees de Kruif (de Kruif, Anema, Zhu,

Havea and Coker, 2015). This may open an alternative way of explaining the work thickening phenomenon.

5. It was observed that fat particle size decreases simultaneously with increased strength of the cheese matrix upon working. This exposes large fat surface areas to interact with the surrounding environment. If fat is considered as a filler material, its increased interactions with the surrounding matrix could reinforce the matrix, thus contributing to the observed work thickening phenomenon. However, the literature on filled composites/emulsions proposes that the role of interaction is negligible if volume fraction is less than 0.60 and further the modulus of fat at 70 °C is much less (order of magnitude less) than that for the protein phase. We, therefore, conveniently ignored that hypothesis. However, we believe that primary changes in the protein phase upon working are causing more interactions with the fat phase. We therefore, propose a study to look at changes in the interfacial properties of the protein and fat phases during prolonged working of model cheese. Studies on TSC addition to nonfat cheese would also assist.
6. Working of Mozzarella cheese involves large deformations and sometimes long time scales. Large strain rheology applying LAOS techniques would give more insights to changes in the elastic and viscous nature of molten cheese during processing. We obtained Lissajous plots for the model cheese from the preliminary LAOS experiments (Appendix 2). A more thorough study is recommended to obtain useful information from this technique, including strain hardening behaviour.
7. Studying melt fracture phenomena in model cheeses at higher temperature and high shear rates using a multipass capillary rheometer would develop further understanding on the material response during processing.
8. The effect of fat volume fraction on shear work sensitivity of model cheese would be useful to further elucidate process-structure-rheology-functionality links.
9. The shear work concept can be applied for process control on a continuous cheese production system using online rheometry tools. This could also be used to study scale up for continuous and large scale operations.
10. Investigations on fat and moisture uptake by the protein matrix at lower levels of shear work would add useful insights on the working process.

11. The proposed structure-rheology-functionality linkage can be tested on other structured products (e.g. yoghurt, processed cheese and emulsions) using the shear work hypothesis.
12. Elongation is considered a more efficient means of energy transfer than shear. Comparing the effects of working through pure elongation or pure shear on rheology, structure and functionality of model Mozzarell cheese would be an interesting study.

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Tensile testing to quantitate the anisotropy and strain hardening of mozzarella cheese



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ABSTRACT

We explored anisotropy of mozzarella cheese: its presence is debated in the literature. Tensile testing proved a good method because the location and mode of failure were clear. Mozzarella cheese cut direct from the block showed no significant anisotropy, though confocal microscopy showed good structure alignment at a microscale. Deliberately elongated mozzarella cheese showed strong anisotropy with tensile strength in the elongation or fibre direction $\sim 3.5\times$ that perpendicular to the fibres. Temperature of elongation had a marked impact on anisotropy with maximum anisotropy after elongation at 70 °C. We suggest the disagreement on anisotropy in the literature is related to the method of packing the mozzarella cheese into a block after the stretching stage of manufacture. Tensile stress/strain curves in the fibre direction showed marked strain hardening with modulus just before fracture $\sim 2.1\times$ that of the initial sample, but no strain hardening was found perpendicular to the fibre direction.

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1. Introduction

A hot-water stretching and working step forms part of the production of mozzarella cheese. In this step the proteins in the cheese curds coalesce into larger protein strands oriented in the direction of stretching, resulting in a typical fibrous microstructure based on protein networks (McMahon, Fife, & Oberg, 1999). This fibrous structure is also visible on a macroscopic level, for instance by tearing the cheese manually along its fibres. This fibrous structure suggests the likelihood of anisotropy, i.e., physical and mechanical properties dependent on the direction of examination. However, the literature on mozzarella cheese anisotropy is conflicting. Cervantes, Lund, and Olson (1983) used compression tests and reported anisotropy in some samples but not in others. Ak and Gunasekaran (1997) found anisotropy using tensile testing whereas others specifically looked for anisotropy and did not find it (Muliawan & Hatzikiriakos, 2007, using an extensional rig similar to tensile testing; Olivares, Zorrilla, & Rubiolo, 2009, using creep/recovery tests). No attempt was made in these later papers to explore or discuss the reasons for the differences with earlier work.

Rheological properties are closely related to the functional characteristics of melted mozzarella cheese such as meltability, stretchability, elasticity, oiling-off and browning. The orientation of protein fibres is expected to impact in particular the melting and stretching properties of mozzarella cheese (Kindstedt & Fox, 1993) suggesting a correlation between the extent of anisotropy and the functional characteristics of mozzarella cheese. Olivares et al. (2009) also suggest that the extent of anisotropy is related to the functionality of mozzarella cheese.

Anecdotal experience suggests that mozzarella cheese shows work thickening or strain hardening behaviour or both. For example the stretching or working step in the manufacture process makes the cheese mechanically stronger. Strain hardening is defined as the phenomenon in which the stress required to deform a material increases more than proportionally to the strain – at constant strain rate and increasing strain (Kokelaar, van Vliet, & Prins, 1996; Van Vliet, 2008). Work thickening is a broader term and we define it as an increase in mechanical strength when a material is worked. To the best of our knowledge no work thickening or strain hardening behaviour has been reported for mozzarella cheese or for any other cheese. Strain hardening has, however, been reported for fine stranded whey protein isolate gels (Lowe, Foegeding, & Daubert, 2003), for weak β -lactoglobulin gels (Pouzot, Nicolai, Benyahia, & Durand, 2006) and for gels formed by

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acidifying transglutaminase cross-linked casein (Rohm, Ullrich, Schmidt, Lobner, & Jaros, 2014). For some texturing proteins, such as gluten in wheat dough, strain hardening is well explored. In wheat dough strain hardening plays an important role in the gas holding capacity, the gas cell stability and the extension behaviour during fermentation and baking. The strain hardening behaviour of wheat dough is also related to breadmaking performance (Kokelaar et al., 1996; Van Vliet, Janssen, Bloksma, & Walstra, 1992). Mozzarella cheese has some similarities in mechanical behaviour to bread dough suggesting that it could exhibit strain hardening.

We began our study with three hypotheses that, if true, might help to explain the conflict in the literature: (1) extent of anisotropy depends on the degree of alignment of the fibrous cheese structure; (2) melting the cheese will remove alignment of protein fibres and so remove anisotropy; (3) holding the cheese at an elevated temperature (but below melting temperature) will reduce alignment and decrease anisotropy.

The studies referred to above all evaluated mechanical properties of mozzarella cheese slices cut directly from the block. In addition to this methodology we decided to use deliberate elongation of the mozzarella cheese to induce fibre formation in the direction of elongation. If hypothesis 1 is true this fibre formation should lead to anisotropy. We considered various methods of elongation and decided on manual rolling because of its simplicity and because Muliawan and Hatzikiriakos (2008) had used rolling. Tensile tests were chosen for three reasons – they are easier to interpret because fracture location is clear, there are no complicating factors such as friction or change in sample orientation under load, and analysis of tensile stress–strain curves should indicate strain hardening if it is present. Tensile fracture in the cheese fibre direction is assumed to fracture the fibres themselves. Tensile fracture perpendicular to the protein fibre direction is more likely to cause fracture between the protein fibres.

2. Materials and methods

2.1. Materials

Mozzarella cheese directly from a Fonterra cheese plant in New Zealand (named “factory cheese”) was obtained as two 10 kg blocks frozen at -20°C . The cheese had been frozen at the age where its functionality was optimal for application as pizza cheese. The 10 kg blocks were thawed for 3 d at 4°C , cut into smaller blocks of ~ 300 g, vacuum-packed into plastic bags and stored at -30°C . These blocks were tempered to 4°C for 2–7 d before use.

Perfect Italiano Semi Soft mozzarella cheese (Fonterra Brands Pty. Ltd., Melbourne, Australia; named “supermarket cheese”), string cheese (Bega Original Stringers[®], Bega Cheese Ltd., Bega, Australia) and butter (Fonterra Brands, Auckland, New Zealand) were obtained from a local supermarket and stored at 4°C . The compositions of the cheese samples ($\text{g } 100 \text{ g}^{-1}$) were determined by Fonterra Research and Development Centre: factory cheese, 48.9 moisture, 22.1 fat, 24.5 protein, 1.26 salt and 3.06 ash; supermarket cheese, 47.8 moisture, 22.6 fat, 24.8 protein, 1.57 salt and 3.44 ash; string cheese, 46.5 moisture, 21.8 fat, 27.4 protein, 1.53 salt and 3.20 ash.

2.2. Sample preparation for tensile testing

2.2.1. Elongated factory and supermarket cheese – standard procedure

First ~ 300 g cheese were heated to 60°C using a water bath and a leak proof stainless steel container. Excess liquid (always < 2 g) was then decanted quickly. The melted cheese was placed near one end of a large aluminium metal plate ($750 \text{ mm} \times 250 \text{ mm} \times 20 \text{ mm}$)

with aluminium strips ($600 \text{ mm} \times 40 \text{ mm} \times 3 \text{ mm}$) fixed on both sides as rails (to ensure minimum thickness of 3 mm) and rolled manually towards the other end with a granite rolling pin. The thickness of the elongated cheese mass was uniform and $\sim 3\text{--}4 \text{ mm}$. The metal plate, strips and rolling pin had been stored at 4°C for at least 14 h. The cheese was rolled in one direction for a total elongation time of 120 s at a frequency of $10 \text{ rolls min}^{-1}$. One roll means steady movement of the roller from one end of the cheese sheet to the other; each roll lasted for 6 s. The rationale for elongating the cheese on a cooled, highly heat conductive surface with large thermal mass was to cool the cheese quickly to lock in any structure generated by elongation. The elongated cheese was covered with plastic wrap, stored for 2 h at 4°C and cut with a scalpel into tensile samples using a template (Fig. 1) following the pattern shown in Fig. 2. This resulted in 16 longitudinal samples and 12 perpendicular samples per trial. The samples were individually wrapped in plastic and kept at 21°C for at least 1 h before tensile testing. Variations from this standard procedure were made to explore the effects of experimental variables as explained in the results section.

2.2.2. String cheese and original factory and supermarket cheese

Slices $3\text{--}4 \text{ mm}$ thick were cut longitudinally from the string cheese sticks. Samples were cut from these slices in longitudinal and perpendicular orientation to the stick axis. The string cheese sticks were only $\sim 15 \text{ mm}$ diameter but the same template was still used for longitudinal samples. The perpendicular string cheese samples were short and were cut into a dumbbell shape without a template. A 300 g factory cheese block was cut into slices $3\text{--}4 \text{ mm}$ thick. One of the slices was used to define the preferred orientation of the cheese fibres by tearing along these fibres. In this way the

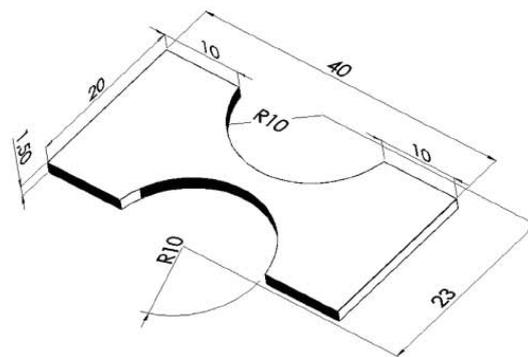


Fig. 1. Dumbbell-shaped template used for cutting cheese samples. Dimensions are in mm.

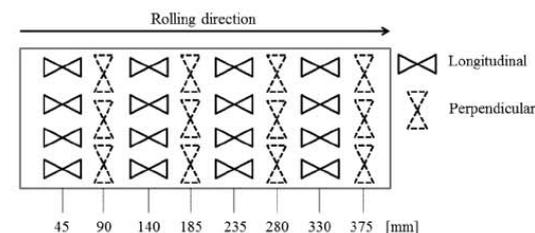


Fig. 2. Sample cutting in longitudinal and perpendicular orientations.

orientation of the template for cutting was determined. Two tensile samples were cut from each slice, one in each orientation. The procedure for obtaining supermarket cheese samples was exactly as for factory cheese. Samples were kept at 21 °C for at least 1 h before tensile testing.

2.3. Tensile testing

Tensile tests were carried out with a TA.XTplus Texture Analyser (Stable Micro Systems Ltd., Godalming, UK) in a laboratory controlled at 21 °C. Crosshead speed was 2 mm s⁻¹ and trigger force was 0.05 N. Serrated jaws were used and were tightened enough to prevent slippage. The initial dimensions of the smallest cross-section of each sample were measured with vernier callipers accurate to 0.01 mm. The initial gap between the tensile jaws was 23 mm and the final gap 63 mm (except for the string cheese samples with perpendicular orientation). As expected the cheese samples all fractured in the narrow area of the dumbbell where stress would be greatest.

2.4. Data analysis and image acquisition

Force-displacement data from tensile tests were converted into a true stress (σ)-Hencky strain (ϵ) format using the following equations:

$$\sigma = \frac{F(t)}{A(t)} \quad (1)$$

$$\epsilon = \ln \frac{L(t)}{L_0} \quad (2)$$

where $F(t)$ was force, $A(t)$ minimum cross sectional area, and $L(t)$ the length of the narrow mid part of the sample all at time t and L_0 was the initial length of the narrow mid part of the sample (20 mm). $A(t)$ was estimated as follows:

$$A(t) = \frac{V}{L(t)} = \frac{V}{L_0 + \Delta L} = \frac{L_0 W_0 T_0}{L_0 + \Delta L} \quad (3)$$

where V was the volume of the narrow mid part of the cheese sample, calculated from the initial width and thickness W_0 and T_0 of the narrowest part of the cheese samples and L_0 . ΔL is the displacement recorded. Charalambides, Williams, and Chakrabarti (1995) similarly assumed constant volume during cheddar cheese testing and stated that this assumption was fairly accurate for cheese. Rohm, Jaros, and deHaan (1997) experimentally demonstrated volume constancy during compression testing of Gouda cheese. The maximum modulus was the maximum slope before fracture in the σ - ϵ diagram. The extent of anisotropy R was calculated as $\sigma(\text{longitudinal})/\sigma(\text{perpendicular})$ and similarly for other parameters.

To check the assumptions made in calculation of $A(t)$ (Equation (3)) three different tensile tests were filmed. A camera was fixed on a tripod with the front of the lens parallel to the front side of the cheese. A mirror at a 45° angle and a small ruler were placed next to the sample. For each of the three tests images were analysed at 3 time points: beginning, mid-test and immediately before fracture. Width and thickness were estimated for all images using the freeware image analysis tool ImageJ 1.47t (<http://rsbweb.nih.gov>).

2.5. Confocal scanning laser microscopy

Confocal scanning laser microscopy (CSLM) was used to determine the microstructure of cheese samples after various treatments

including fracture. Cheese samples were cut and frozen (-20 °C) before being sectioned into 50 μm slices on a microtome. Slices were immediately stained with 0.4% Nile red and 0.2% fast green (made in citifluor to minimise photobleaching) and covered with a coverslip. The sectioned samples were then stored at 4 °C for a minimum of 24 h before imaging. Images were taken using a confocal microscope (Leica DM6000B, Heidelberg, Germany) with excitation wavelengths of 488 nm and 633 nm.

2.6. Statistical analysis

Significant differences ($P < 0.05$) in the results were analysed by SPSS 12.0 software using single factor ANOVA and the Duncan post hoc test to compare means. All experiments with elongated supermarket and factory cheese were performed at least twice and sub-sampled, resulting in $n \geq 32$ longitudinal replicates and $n \geq 24$ perpendicular replicates, unless otherwise stated. For string cheese and original factory cheese at least 6 slices were cut from the cheese stick/block resulting in $n \geq 6$ longitudinal and perpendicular samples.

3. Results

3.1. Tensile test basics

Tensile force versus displacement curves for both longitudinal and perpendicular samples decreased in slope with stretching as the area of the sample decreased. σ - ϵ curves (Fig. 3) showed a slope (tensile modulus) that slightly decreased with strain for perpendicular samples. For longitudinal samples the maximum modulus just before fracture was about 2.5 times the initial modulus. Longitudinal samples strain hardened during the tensile test.

σ values calculated using $A(t)$ from image acquisition were in reasonable agreement with the corresponding σ values calculated from Equations (1) and (3). Image acquisition plus direct visual observation of fracture indicated that longitudinal samples usually fractured at roughly a 45° angle to the stretching direction. Mohr's circle analysis for pure tension indicates shear failure. In most cases the fractured surface was rather stringy and in many cases it was shaped randomly. In contrast, perpendicular samples fractured mainly at a 90° angle to the stretching direction. Mohr's circle analysis for pure tension indicates that this means tensile failure.

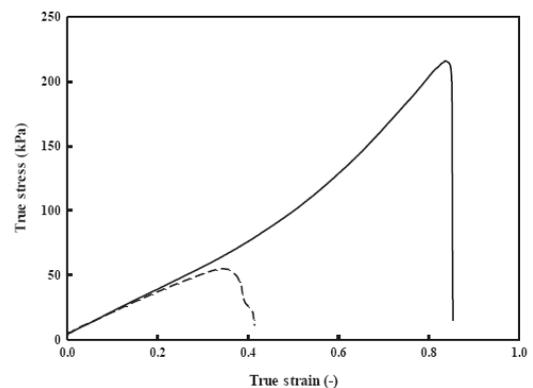


Fig. 3. True stress versus Hencky strain for tensile testing of one longitudinal (—) and one perpendicular (---) sample of factory mozzarella cheese prepared under standard conditions.

Ak and Gunasekaran (1997) similarly noted 45° fracture angles for longitudinal samples and 90° fracture angles for perpendicular samples during tensile testing of mozzarella cheese.

3.2. Reproducibility of the overall method

Four trials were carried out at standard conditions using elongated factory cheese to check reproducibility (Table 1). The standard deviations were rather high. One reason for this is variability in manual elongation, particularly variation in the first roll to produce a cheese sheet. Another reason is the inherent variability in tensile fracture, because failure is related to the random occurrence of structural weaknesses or imperfections where cracks may initiate and propagate.

Manski, van der Zalm, van der Goot, and Boom (2008) and Grabowska, van der Goot, and Boom (2012) produced fibrous materials from dense calcium caseinate-fat dispersions cross-linked by transglutaminase and similarly reported variability in their tensile measurements and attributed this to the fibrous nature of the samples with some samples breaking all at once and others in multiple stages. Fracture stress (σ_f) and fracture strain (ϵ_f) showed no significant differences between the four trials, but some differences in modulus were observed at a 5% significance level. These differences were not significant at a 10% level. In spite of the variability in the method significant differences were found between longitudinal and perpendicular samples and between sample treatments.

3.3. Comparison of cheese types

Table 2 shows tensile fracture behaviour for the five cheese types. String cheese had the highest extent of anisotropy with an R value of 6.0 for σ_f and 5.7 for ϵ_f . Original factory cheese and original supermarket cheese showed no significant anisotropy. Elongated supermarket cheese indicated significant anisotropy for ϵ_f but not for σ_f or maximum modulus. Elongated factory cheese showed pronounced anisotropic characteristics with large differences between longitudinal and perpendicular samples. All subsequent

experiments were therefore carried out with elongated factory cheese.

3.4. Effect of elongation conditions

Fig. 4 shows σ_f versus sample location along the rolled cheese sheet. σ_f for longitudinal samples showed a maximum at 140 mm. σ_f at 140 mm was significantly different from σ_f at 45 mm and 330 mm but not from σ_f at 235 mm. The higher variability towards the ends of the rolled sheets suggested less uniformity in sample preparation. For the remaining experiments only the data for 140 mm and 235 mm for longitudinal samples ($n = 8$ per trial) and 90, 185 and 280 mm for perpendicular samples ($n = 9$ per trial) were used. This removed the end locations that had higher standard deviations and gave enough replicates for good statistical significance.

Anisotropic characteristics of elongated factory cheese were investigated for 5 different elongation temperatures (Fig. 5, Table 3). Elongation temperature was the cheese equilibration temperature before placing on the 4 °C plate and elongating. For longitudinal samples σ_f increased with elongation temperature to a maximum at 70 °C. Further increase of elongation temperature to 80 °C resulted in a decrease of σ_f . There was no statistical difference between the means for σ_f at 40, 50 and 80 °C. σ_f at 60 °C was significantly higher than these but at the same time significantly lower than σ_f at 70 °C. No significant differences were found between σ_f values for perpendicular samples.

For longitudinal samples σ_f decreased significantly with longer elongation times (Table 4). However, perpendicular samples showed no significant differences for σ_f with elongation time. It appeared that the alignment of cheese fibres depended mainly on the first roll, which produced a flat cheese sheet. Further rolls generated only small changes in the length and thickness of the sheet. Consequently, the orientation of fibres and thus the degree of anisotropy are likely to be mainly influenced by the procedure when cheese is converted from a molten mass into a flat sheet. This largely took place during the first roll.

An elongation frequency of 3 min⁻¹ produced a significantly lower σ_f and modulus than 10 min⁻¹ for longitudinal samples

Table 1
Reproducibility of the elongation and tensile testing method with four independent experimental trials under standard conditions.^a

Experimental trial	Fracture stress (kPa)			Fracture strain (–)			Maximum modulus (kPa)		
	Longitudinal	Perpendicular	R	Longitudinal	Perpendicular	R	Longitudinal	Perpendicular	R
1	197 ± 64 ^a	58 ± 21 ^b	3.4	0.78 ± 0.11 ^a	0.38 ± 0.08 ^b	2.1	337 ± 114 ^a	146 ± 27 ^c	2.3
2	215 ± 31 ^a	72 ± 18 ^b	3.0	0.75 ± 0.05 ^a	0.41 ± 0.08 ^b	1.8	418 ± 72 ^b	170 ± 21 ^c	2.5
3	209 ± 39 ^a	71 ± 17 ^b	2.9	0.74 ± 0.08 ^a	0.40 ± 0.08 ^b	1.9	393 ± 80 ^{ab}	170 ± 19 ^c	2.3
4	199 ± 54 ^a	63 ± 19 ^b	3.2	0.81 ± 0.09 ^a	0.45 ± 0.11 ^b	1.8	340 ± 91 ^a	143 ± 27 ^c	2.4

^a Standard conditions were: elongation temperature, 60 °C; plate temperature, 4 °C; elongation time, 120 s; elongation frequency, 10 rolls min⁻¹. Values are means with standard deviations from $n = 16$ longitudinal samples and $n = 12$ perpendicular samples for each trial; means for the same parameter, e.g., fracture stress, with different superscript letters are significantly different ($P < 0.05$).

Table 2
Effect of cheese type and cheese treatment on tensile properties.^a

Cheese type	Fracture stress (kPa)			Fracture strain (–)			Maximum modulus (kPa)		
	Longitudinal	Perpendicular	R	Longitudinal	Perpendicular	R	Longitudinal	Perpendicular	R
String cheese	204 ± 21 ^a	34 ± 6 ^{b,c}	6.0	0.65 ± 0.04 ^a	0.11 ± 0.01 ^d	5.7	450 ± 36 ^a	387 ± 55 ^c	1.2
Original supermarket cheese	34 ± 8 ^{b,c}	24 ± 8 ^b	1.4	0.39 ± 0.04 ^b	0.34 ± 0.15 ^b	1.0	76 ± 17 ^b	71 ± 9 ^b	1.1
Elongated supermarket cheese	60 ± 12 ^{c,d}	40 ± 8 ^{b,c}	1.5	0.76 ± 0.07 ^c	0.60 ± 0.06 ^a	1.3	88 ± 21 ^b	65 ± 13 ^b	1.4
Original factory cheese	36 ± 11 ^{b,c}	33 ± 12 ^{b,c}	1.1	0.34 ± 0.12 ^b	0.33 ± 0.08 ^b	1.0	101 ± 19 ^b	97 ± 11 ^b	1.0
Elongated factory cheese	204 ± 47 ^a	68 ± 18 ^d	3.0	0.76 ± 0.09 ^c	0.41 ± 0.08 ^b	1.9	383 ± 95 ^c	162 ± 24 ^d	2.4

^a Elongation conditions were: temperature, 60 °C; plate temperature, 4 °C; elongation time, 120 s; elongation frequency, 10 rolls min⁻¹. Values are means with standard deviations from the following: $n \geq 6$ longitudinal and perpendicular samples for both string cheese and original factory cheese, $n = 3$ longitudinal and $n = 4$ perpendicular samples for original supermarket cheese; $n = 48$ longitudinal and $n = 36$ perpendicular samples for both elongated supermarket and factory cheese, from 3 trials. Means for the same parameter, e.g., fracture stress, with different superscript letters are significantly different ($P < 0.05$).

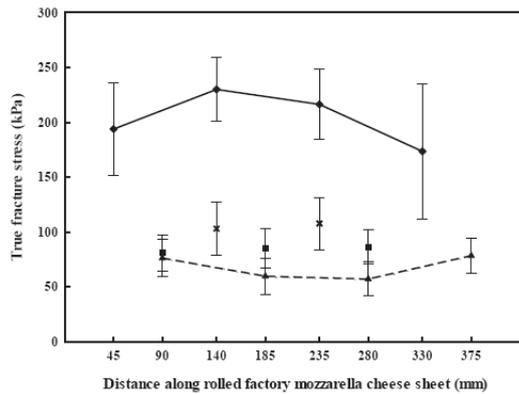


Fig. 4. True fracture stress versus distance along the rolled sheet for factory mozzarella cheese prepared under standard conditions in the longitudinal (\blacklozenge) and perpendicular (\blacktriangle) orientations, and where standard conditions were altered by holding the elongated cheese at 60 °C for 2 h before cooling to 4 °C for 2 h then cutting dumbbell samples in longitudinal (\times) and perpendicular orientations (\blacksquare). Error bars represent one standard deviation from the mean.

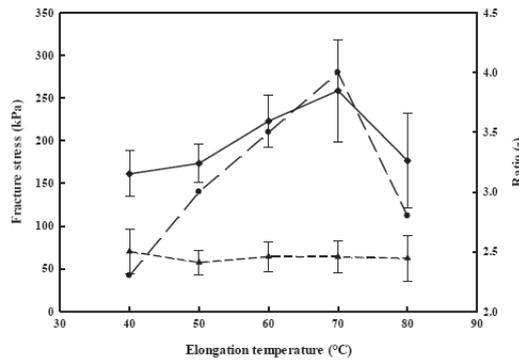


Fig. 5. True fracture stress as a function of elongation temperature in the longitudinal (\blacklozenge) and perpendicular (\blacktriangle) orientations and the corresponding ratio R (\bullet). Error bars represent one standard deviation from the mean.

(Table 5). An elongation frequency of 10 min^{-1} is close to the practical limit for manual rolling. The impact of elongation frequency might also be explained by the first roll. A faster first roll produces a significantly higher σ_f . If the molten cheese was rolled

too slowly a thick and poorly elongated cheese sheet resulted from the first roll. With further rolls the sheet did not change much as the deformability of the cheese decreased quickly with temperature reduction. Cracks would sometimes appear in the cheese sheet when trying to adjust after a slow first roll. It appears that there is an interplay between the rate of deformation and the rate of solidification. It is important to complete the first roll before the cheese has solidified too much.

3.5. Effect of plate and storage temperature

The metal plate and granite rolling pin were preconditioned to the experimental temperature for at least 14 h before rolling, and the elongated cheese sheet was then stored at the same temperature for 2 h. To minimise moisture loss during storage at 21 °C and 37 °C the elongated cheese was covered with plastic film and also placed in a sealed snaplock plastic bag. σ_f of both longitudinal and perpendicular samples decreased with increasing plate and storage temperature, although the differences between σ_f at 21 °C and 37 °C were not statistically significant (Table 6).

Hypothesis 3 suggested that anisotropy would decrease on holding at an elevated temperature. However, although σ_f reduced with storage temperature, longitudinal and perpendicular σ_f decreased by different amounts and anisotropy increased with increasing storage temperature. This experiment has not confirmed hypothesis 3.

3.6. Effect of remelting elongated cheese

The remelting experiment was designed to test hypothesis 2. The standard procedure was followed, but after storing the elongated cheese at 4 °C for 2 h the elongated sheet was wrapped in plastic film, placed in a sealed snaplock bag to reduce moisture loss and stored at 60 °C in an oven for 2 h. The remelted sheet was then stored at 4 °C for another 2 h before sample cutting and tensile testing. While remelting caused a large decrease in R values to 1.2 for σ_f and modulus and 1.1 for e_f , significant differences in means were observed between longitudinal and perpendicular samples for σ_f , e_f and modulus (Table 7). This shows that anisotropy is greatly reduced by remelting at 60 °C, but not completely removed. Longitudinal σ_f was reduced by around 50%, but perpendicular σ_f increased by around 30% (Fig. 4). An impact of moisture loss on the results is possible, but would not be directionally selective. Some condensation build up was observed inside the snaplock bag and this moisture had not completely reabsorbed after 2 h further storage at 4 °C. The moisture contents of the cheese before and after the 2 h storage at 60 °C were not significantly different. After 2 h storage at 60 °C the sheet length in the elongation direction had reduced by 15%. This is an indicator that locked in strain was being relaxed. The longitudinal samples after remelting showed very little strain hardening.

Table 3
Effect of elongation temperature on tensile properties.^a

Elongation temperature (°C)	Fracture stress (kPa)			Fracture strain (-)			Maximum modulus (kPa)		
	Longitudinal	Perpendicular	R	Longitudinal	Perpendicular	R	Longitudinal	Perpendicular	R
40	161 ± 27 ^a	70 ± 26 ^d	2.3	0.78 ± 0.06 ^{ab}	0.43 ± 0.12 ^{d,e}	1.8	322 ± 56 ^a	127 ± 19 ^d	2.5
50	174 ± 23 ^a	57 ± 14 ^d	3.0	0.79 ± 0.05 ^{ab}	0.45 ± 0.08 ^d	1.8	355 ± 47 ^a	131 ± 20 ^d	2.7
60	223 ± 31 ^b	64 ± 18 ^d	3.5	0.80 ± 0.07 ^a	0.38 ± 0.07 ^e	2.1	421 ± 69 ^b	161 ± 23 ^{d,e}	2.6
70	259 ± 60 ^c	64 ± 19 ^d	4.0	0.73 ± 0.08 ^b	0.32 ± 0.07 ^f	2.3	494 ± 114 ^c	216 ± 42 ^f	2.3
80	177 ± 56 ^a	62 ± 27 ^d	2.8	0.63 ± 0.13 ^c	0.32 ± 0.07 ^f	1.9	359 ± 93 ^{a,b}	192 ± 41 ^{e,f}	1.9

^a Elongation conditions were: plate temperature, 4 °C; elongation time, 120 s; elongation frequency, 10 rolls min^{-1} . Values are means with standard deviations from $n = 16$ longitudinal and $n = 18$ perpendicular samples for elongation temperatures of 40, 50, 70 and 80 °C, plus $n = 24$ longitudinal and $n = 27$ perpendicular samples for an elongation temperature of 60 °C. Means for the same parameter, e.g., fracture stress, with different superscript letters are significantly different ($P < 0.05$).

Table 4
Effect of elongation time on tensile properties.^a

Elongation time (s)	Fracture stress (kPa)			Fracture strain (–)			Maximum modulus (kPa)		
	Longitudinal	Perpendicular	R	Longitudinal	Perpendicular	R	Longitudinal	Perpendicular	R
18	242 ± 21 ^a	66 ± 17 ^d	3.7	0.80 ± 0.05 ^a	0.39 ± 0.08 ^b	2.0	467 ± 52 ^a	180 ± 32 ^c	2.6
120	223 ± 31 ^b	64 ± 18 ^d	3.5	0.80 ± 0.07 ^a	0.38 ± 0.07 ^b	2.1	421 ± 69 ^b	161 ± 23 ^c	2.6
180	204 ± 33 ^c	72 ± 16 ^d	2.8	0.78 ± 0.06 ^a	0.42 ± 0.07 ^b	1.8	398 ± 60 ^b	174 ± 24 ^c	2.3

^a Elongation conditions were: temperature, 60 °C; plate temperature, 4 °C; elongation frequency 10 rolls min⁻¹. Values are means with standard deviations from n = 16 longitudinal and n = 18 perpendicular samples for elongation times of 18 and 180 s, plus n = 24 longitudinal and n = 27 perpendicular samples for an elongation time of 120 s. Means for the same parameter, e.g., fracture stress, with different superscript letters are significantly different ($P < 0.05$).

Table 5
Effect of elongation frequency on tensile properties.^a

Elongation frequency (min ⁻¹)	Fracture stress (kPa)			Fracture strain (–)			Maximum modulus (kPa)		
	Longitudinal	Perpendicular	R	Longitudinal	Perpendicular	R	Longitudinal	Perpendicular	R
3	197 ± 45 ^a	65 ± 22 ^c	3.0	0.77 ± 0.07 ^a	0.39 ± 0.10 ^b	2.0	370 ± 88 ^a	166 ± 32 ^c	2.2
10	223 ± 31 ^b	64 ± 18 ^c	3.5	0.80 ± 0.07 ^a	0.38 ± 0.07 ^b	2.1	421 ± 69 ^b	161 ± 23 ^c	2.6

^a Elongation conditions were: temperature, 60 °C; plate temperature, 4 °C; elongation time, 120 s. Values are means with standard deviations from n = 16 longitudinal and n = 18 perpendicular samples for an elongation frequency of 3 min⁻¹, plus n = 24 longitudinal and n = 27 perpendicular samples for an elongation frequency of 10 min⁻¹; means for the same parameter, e.g., fracture stress, with different superscript letters are significantly different ($P < 0.05$).

Table 6
Effect of plate and storage temperature on tensile properties.^a

Plate temperature (°C)	Fracture stress (kPa)			Fracture strain (–)			Maximum modulus (kPa)		
	Longitudinal	Perpendicular	R	Longitudinal	Perpendicular	R	Longitudinal	Perpendicular	R
4	223 ± 31 ^a	64 ± 18 ^c	3.5	0.80 ± 0.07 ^a	0.38 ± 0.07 ^b	2.1	421 ± 69 ^a	161 ± 23 ^c	2.6
21	192 ± 35 ^b	46 ± 12 ^d	4.2	0.79 ± 0.07 ^a	0.40 ± 0.06 ^b	2.0	372 ± 69 ^b	120 ± 22 ^d	3.1
37	179 ± 39 ^b	41 ± 18 ^d	4.4	0.77 ± 0.09 ^a	0.38 ± 0.07 ^b	2.0	353 ± 67 ^b	112 ± 43 ^d	3.2

^a Elongation conditions were: temperature, 60 °C; plate temperature, 4 °C; elongation time, 120 s; elongation frequency 10 rolls min⁻¹. Values are means with standard deviations from n = 16 longitudinal and n = 18 perpendicular samples for plate and storage temperatures of 21 and 37 °C, plus n = 24 longitudinal and n = 27 perpendicular samples for a plate and storage temperature of 4 °C. Means for the same parameter, e.g., fracture stress, with different superscript letters are significantly different ($P < 0.05$).

Table 7
Effect of remelting MC after elongation on tensile properties.^a

Treatment	Fracture stress (kPa)			Fracture strain (–)			Maximum modulus (kPa)		
	Longitudinal	Perpendicular	R	Longitudinal	Perpendicular	R	Longitudinal	Perpendicular	R
Elongated MC	223 ± 31 ^a	64 ± 18 ^c	3.5	0.80 ± 0.07 ^a	0.38 ± 0.07 ^c	2.1	421 ± 69 ^a	161 ± 23 ^c	2.6
Remelted MC	105 ± 23 ^b	84 ± 16 ^d	1.2	0.52 ± 0.06 ^b	0.47 ± 0.07 ^d	1.1	232 ± 48 ^b	199 ± 36 ^d	1.2

^a Elongation conditions were: temperature, 60 °C; plate temperature, 4 °C; elongation time, 120 s; elongation frequency 10 rolls min⁻¹ after elongation to remelt the cheese was held at 60 °C for 2 h. Values are means with standard deviations from n = 16 longitudinal and n = 18 perpendicular samples for remelted elongated MC, plus n = 24 longitudinal and n = 27 perpendicular samples for MC elongated under standard experimental conditions. Means for the same parameter, e.g., fracture stress, with different superscript letters are significantly different ($P < 0.05$).

3.7. Confocal scanning laser microscopy

Confocal micrographs of the original factory cheese showed clear structural anisotropy at this scale with longitudinal samples showing aligned fat phase between the protein strands (Fig. 6a) and perpendicular samples showing little alignment as we are looking end on at the aligned structure (Fig. 6b). The melted and elongated cheese similarly showed alignment for the longitudinal sample (Fig. 6c) but no alignment for the perpendicular sample (Fig. 6d). The fat phase in the elongated cheese showed more coalescence and less individual fat globules than the original factory cheese. The melted, elongated and remelted cheese showed no alignment for either the longitudinal (Fig. 6e) or perpendicular (Fig. 6f) samples. Confocal micrographs that show the fracture surface after tensile testing (Fig. 6g and h) indicate a high concentration of fat near or at the fracture surface suggesting that weak fat planes in the cheese may be the location of fracture initiation.

3.8. Tensile testing of butter

Tensile testing of butter was attempted to determine whether the milk fat component of mozzarella cheese contributes any tensile strength. The same method of sample preparation was used as for original factory cheese but without choosing direction of cutting. Sample cutting and tensile testing proved to be impossible at 21 °C because the butter was too soft. The butter was therefore stored at 4 °C before cutting. The dumbbell samples were also stored at 4 °C for at least 2 h before tensile testing. They were then tested as quickly as possible in the 21 °C laboratory. Of the 8 samples prepared, 5 samples broke before testing or slipped out of the tensile grips. The remaining 3 samples had σ_f of 5.73 ± 1.49 kPa, ϵ_f of 0.082 ± 0.025 and modulus of 49.6 ± 15.1 kPa. The samples were not isothermal during tensile testing with measured temperatures of 14 °C–20 °C. Nevertheless these results showed that the tensile strength of milk fat at 21 °C is very small.

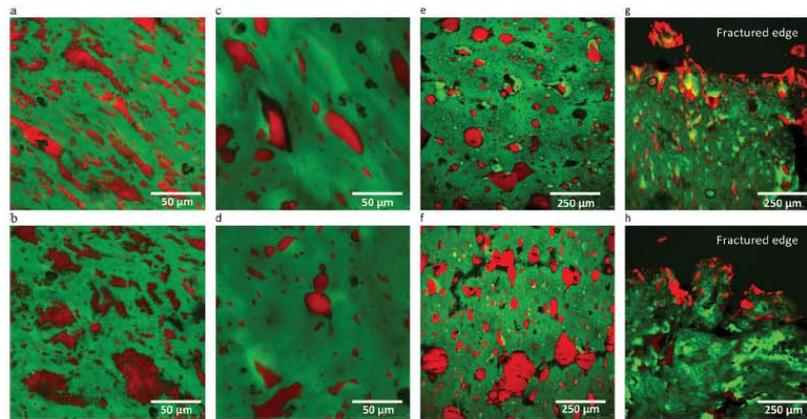


Fig. 6. CSLM images of factory mozzarella cheese samples: red, fat; green, protein. Original cheese in the longitudinal (a) and perpendicular (b) direction; elongated cheese in the longitudinal (c) and perpendicular (d) direction; elongated and remelted cheese in the longitudinal (e) and perpendicular (f) direction; and fractured edges of elongated cheese in the longitudinal (g) and perpendicular (h) direction. Longitudinal samples were always viewed across the fibre direction whereas perpendicular samples were viewed end on to the fibre direction. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

As milk fat has very low tensile strength at 21 °C, we conclude that the tensile properties of mozzarella cheese are largely due to the strength of the protein network. Tensile testing is therefore a good method to determine protein network strength and the effect of any processing changes. String cheese exhibited the highest degree of anisotropy with an R value for σ_f of 6.0. String cheese is produced by extrusion into the shape of sticks (Chen, Wolle, & Sommer, 2009) and shows a very fibrous character at a macroscopic level. The highly anisotropic character is therefore not surprising. Manski et al. (2008) reported R values for σ_f up to 14.2 for their fibrous calcium caseinate materials formed by simultaneous shear and transglutaminase action showing that casein molecules are capable of producing highly anisotropic structures. The elongated supermarket cheese showed statistically significant anisotropy only for ϵ_f whereas elongated factory cheese was strongly anisotropic for all fracture properties. This might be because the supermarket cheese was described as semi-soft, indicating a high level of proteolysis (Chen et al., 2009). The effect of proteolysis on mozzarella functionality is well reported (e.g., Kindstedt & Fox, 1993). It is likely that the extent of anisotropy depends on the degree of proteolysis and therefore is affected by the ripening time of mozzarella cheese.

The variation of σ_f with distance along the plate (Fig. 4) suggests that anisotropy increased with the force applied and the amount of flow induced while elongating. During the first roll at lower distances the amount of cheese in front of the roller was higher, requiring higher forces and more flow induction. At higher distances the thickness of the cheese sheet decreased continuously so that the required force and amount of flow decreased. Perhaps the higher rolling force and higher flow near the beginning of the plate resulted in better alignment of the samples at these distances.

Elongation temperature had a bigger impact on the degree of anisotropy than any other parameter. It is clear that elongation builds up or strengthens a network structure in the cheese. Presumably this strengthening is caused by increasing protein–protein interactions. Bryant and McClements (1998) note that hydrophobic protein–protein interactions increase in strength as temperature rises up to a maximum at about 60–70 °C, above which

hydrophobic interactions begin to reduce again as the temperature is further increased. Hence, the increase in σ_f from 40 to 70 °C and the decrease in σ_f to 80 °C are probably linked to changes in hydrophobic protein–protein interactions. An alternative explanation for the temperature effect is changes to the milk mineral system at high temperatures and various possibilities are discussed by Udyarajan, Horne, and Lucey (2007). Calcium has higher affinity for α_{S1} -casein as the temperature increases. In addition calcium and phosphate in the serum phase of the cheese may form new insoluble calcium phosphate at higher temperature that could interact with the caseins. Commercially mozzarella cheese is usually stretched in hot water circulating at a temperature of approximately 72 °C (Chen et al., 2009), near the temperature where we found the greatest effect of elongation on σ_f .

There are several indications of both strain hardening and work thickening of mozzarella cheese. The shapes of the longitudinal $\sigma-\epsilon$ curves (Fig. 3) show a more than doubling of tensile modulus between the start of the test and fracture, i.e., significant strain hardening. Perpendicular $\sigma-\epsilon$ curves did not show strain hardening. Similar behaviour was reported by Manski, van der Goot, and Boom (2007) for their tensile testing of fibrous materials produced by shearing fat-free calcium caseinate dispersions. Strain hardening was found in the fibre direction but not perpendicular to the fibre direction. Three trials under standard conditions were analysed to quantify strain hardening. The ratio of maximum tensile modulus to initial modulus was 2.12 ± 0.38 ($n = 24$). Curve fitting showed two distinct regions on the $\sigma-\epsilon$ curves. A linear model accurately fitted $\sigma-\epsilon$ data up to a strain of about 0.4. From 0.4 to a point near fracture, $\sigma-\epsilon$ data was best modelled by an exponential curve. Clearly all the strain hardening is in the exponential part of the curve. Van Vliet (2008) notes that for bread dough there is often an exponential relationship between σ and ϵ , though for bread dough this fits the whole $\sigma-\epsilon$ curve rather than just the portion at high strain.

The reason for two distinct regions on the longitudinal $\sigma-\epsilon$ curve is not clear. One possible explanation is that in the linear region, curves or bends in the fibre network are merely straightened. The concept of the straightening of curved strands was used by Lakemond and van Vliet (2008) to explain the fracture behaviour of acid skim milk gels. At higher strains, where all the strands are

now straight, the protein fibres must either stretch or move past one another, increasing the interactions between fibres. This may cause an increase in the strength of the protein network. The protein fibres in the longitudinal orientation also become progressively closer together because of the reducing cross-sectional area thus increasing interactions. In perpendicular samples, fibres would be pulled further apart, resulting in no strain hardening. Many biopolymers have been shown to strain harden both as single molecules, e.g., collagen, and also as network structures resulting in a significant body of biophysics literature on the topic, e.g., pectin (Vincent, Mansel, Kramer, Kroy, & Williams, 2013), rubber (Horgan & Saccomandi, 2006). At the molecular level the strain hardening arises from limits to chain extensibility and to the “stickiness” of adjacent polymer chains. There are many reports of strain hardening of flour dough (e.g., Kokelaar et al., 1996; Van Vliet, 2008; Van Vliet et al., 1992).

Work thickening is also evident. The original factory cheese had σ_f of 36 kPa parallel to the fibres and 33 kPa perpendicular to the fibres (Table 2). After elongation the longitudinal σ_f had increased by 5.7 times to 204 kPa and the perpendicular σ_f had increased by 2.1 times to 68 kPa. Presumably the elongation operation at 60 °C has increased the strength of the casein network, i.e., increased protein–protein interactions. This stronger protein structure is aligned because of the elongation. σ_f of the remelted perpendicular samples is 84 kPa, about 30% higher than σ_f before remelting (Table 7), whereas σ_f of the remelted longitudinal samples is 105 kPa, about 50% lower than the 223 kPa σ_f before remelting (Table 7). One possible explanation is that the increased protein–protein interactions formed during elongation at 60 °C are not destroyed by remelting, but the alignment is relaxed. Some of the increased protein–protein interactions thus increase σ_f in the remelted perpendicular samples. During mechanical flour dough development there are similarly significant increases in mechanical strength, or work thickening (e.g., Zheng, Morgenstern, Campanella, & Larsen, 2000).

Do these results help to explain the disagreement in the literature about anisotropy of mozzarella cheese? No significant anisotropy was found for original factory cheese when slices were cut from the block, but when this cheese was elongated strong anisotropy was observed (Table 2). Today, the final stages of continuous mozzarella cheese manufacture generally involve a stretching machine followed by a moulding step to form a block (Chen et al., 2009). We expect that the original cheese from stretching would be highly aligned and therefore anisotropic as it is transferred through a pipe to moulding. However, during moulding into blocks it is expected that this aligned structure will pack randomly with different orientations in different parts of the block. An aligned microstructure is retained in the confocal micrographs (Fig. 6a) but when a slice is cut and a large enough sample taken for tensile testing isotropic behaviour is observed. The length scale of anisotropy in the formed cheese block will vary between packing operations and might be expected to depend on the diameter of the cheese pipe feeding the block, any motion of the pipe around the space of the block, the presence of vibration to aid packing (eliminate air) and the packing rate.

All previous studies that tested anisotropy of mozzarella cheese used samples cut from original cheese blocks. In the recent papers no anisotropy was observed as for our results with original factory cheese (Muliawan & Hatzikiriakos, 2007; Olivares et al., 2009). However, in older papers anisotropy was clearly demonstrated, e.g., Ak and Gunasekaran (1997). One possibility is that their cheese samples were from smaller scale batch operations where large masses of cheese were placed into the moulds at once leading to anisotropy on a macroscale. Part of our thinking was that if the cheese was packed hot then relaxation of the structure could lead

to the disappearance of anisotropy. However, the confocal micrographs show clear alignment at the microscale, so that is not the correct explanation.

5. Conclusions

Original factory mozzarella cheese showed no anisotropy on tensile testing but confocal micrographs indicated clear alignment in the structure at the microscale. The structure produced by melting and elongating the factory cheese was shown to be highly anisotropic both by tensile testing and by confocal microscopy. Elongation temperature had a significant impact on the extent of anisotropy. Remelting the mozzarella cheese after elongation gave a non-aligned structure that showed very little anisotropy. We suggest the disagreement on anisotropy in the literature is related to the method of packing the cheese into a block after the stretching stage of manufacture. During tensile testing elongated factory cheese showed strain hardening behaviour in the longitudinal direction but not in the perpendicular direction. Tensile testing was a good method to demonstrate and quantitate anisotropy and strain hardening in mozzarella cheese.

Acknowledgements

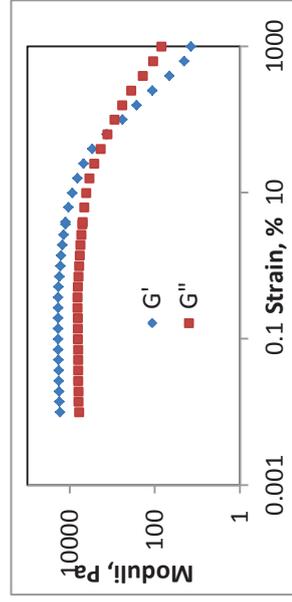
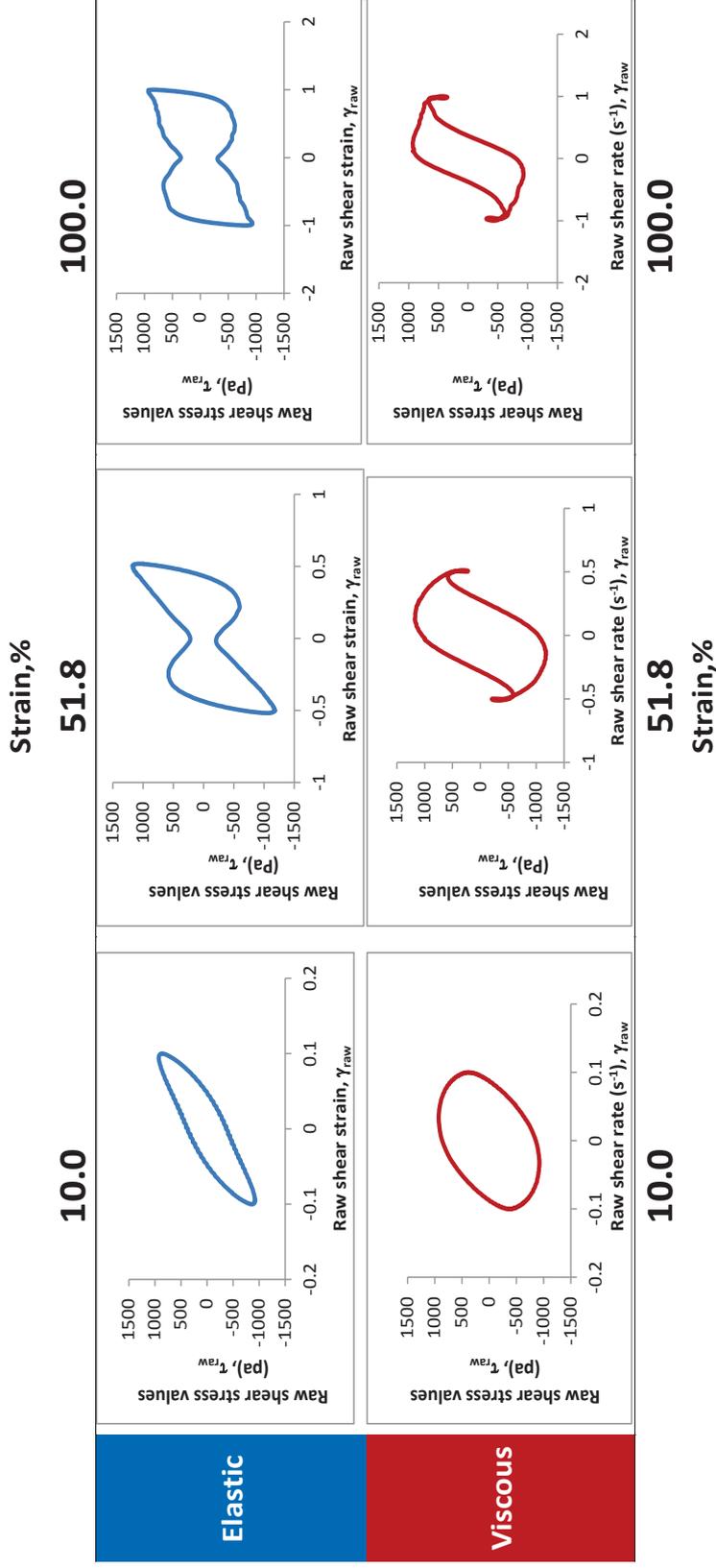
We are grateful to Fonterra Co-operative Group and the Ministry of Primary Industries for funding this work via the Dairy Primary Growth Partnership Programme in Food Structure Design. We thank Janiene Gilliland, Byron McKillop, Warwick Johnson, Garry Radford and Steve Glasgow for expert technical assistance. Discussions with Ton van Vliet, Thom Huppertz and Martin Williams were very useful in helping to understand the strain hardening data.

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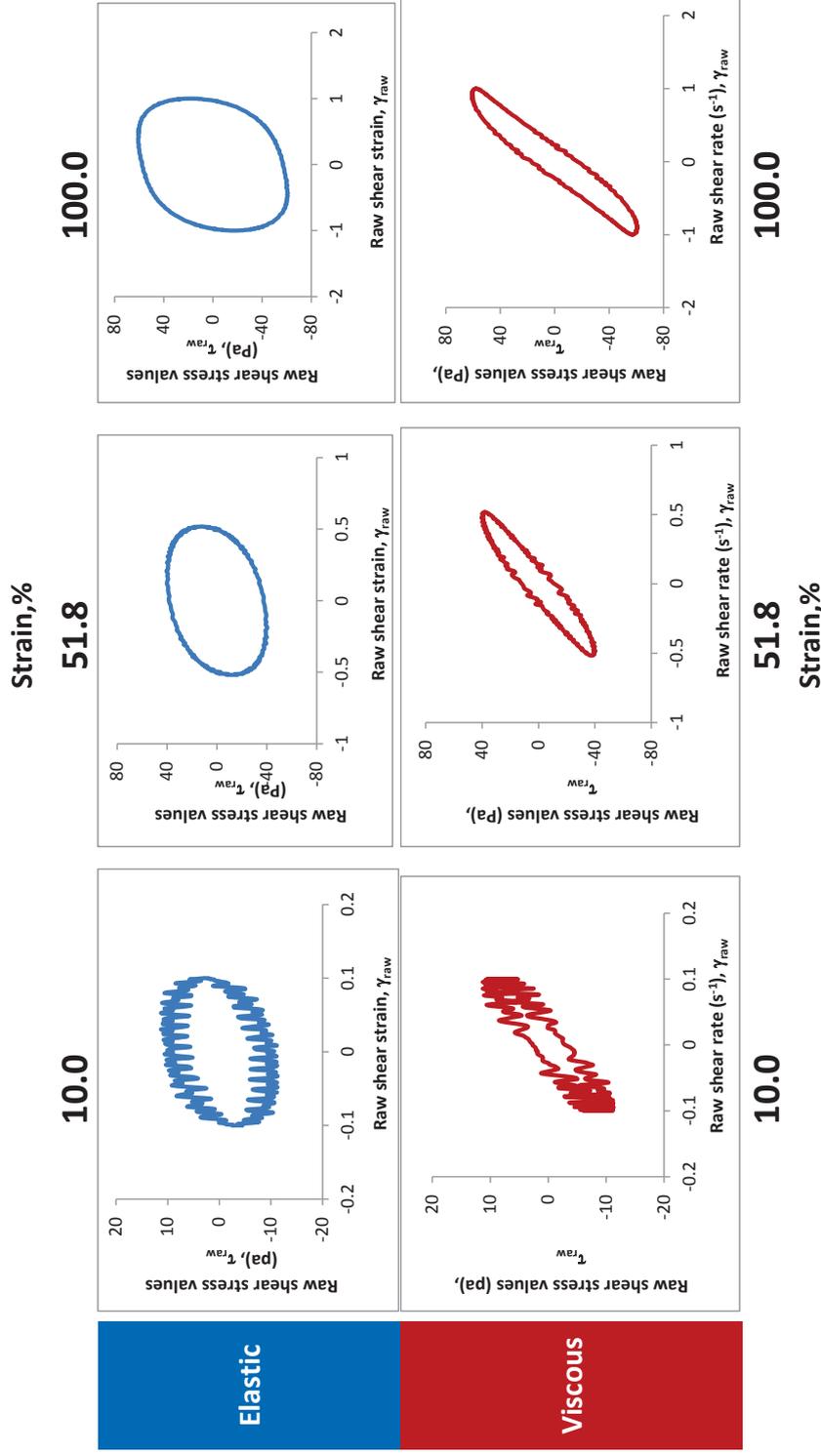
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Lissajous plots –model Mozzarella cheese at 20 °C (8.8 kJ/kg)



Lissajous plots –model Mozzarella cheese at 70 °C (8.8 kJ/kg)





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Effect of Shear Work on Viscoelastic Properties, Structure, and Melt Functionality of a Mozzarella-Type Cheese

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Abstract

We studied the effect of shear work on viscoelastic properties and melt functionality of Mozzarella type cheese and the relationship between the two. Cheese samples with varied amounts of shear work were prepared by working mollen cheese mass at 70°C in a twin screw cooking-stretching vessel, Blentech™. Screw speed (50, 150 and 250 rpm) and residence time (0 to 60 min) in the cooking vessel were used as processing variables. Values of dynamic moduli (G' and G'') were higher for cheese having larger amounts of shear work. LT_{max} decreased with increasing shear work input [Fig. 2]. An inverse relationship was found between melt score and the cross over temperature [Fig. 6]. However LT_{max} correlated (positively) well with melt score and could be regarded as a structure sensitive parameter during working of Mozzarella like cheeses.

Introduction

Working of mollen cheese curd by stretching and kneading is an important step in the manufacture of pasta-filata type cheeses¹. The mechanical energy imparted to the cheese facilitates calcium-mediated protein-protein interactions and so builds up the typical fibrous texture². The energy imparted affects the viscoelastic properties of the cheese. The viscoelastic properties of a Mozzarella-type cheese and the relationship with structure and melting characteristics.

Research hypothesis

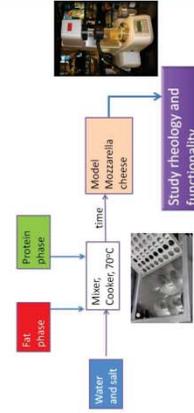
Energy supplied to the mass in the form of shear work results in strong attractive protein-protein interactions. Unmelt is caused by stretching of the cheese mass and excessive flow is caused by underworking of the cheese mass. Some measures of cheese rheology should correlate with level of working

Materials and Methods

Cheese samples with varied amounts of shear work were prepared by working mollen cheese mass at 70°C in a twin screw cooking-stretching vessel, Blentech™. Screw speed (50, 150 and 250 rpm) and residence time (0 to 60 min) in the cooking vessel were used as processing variables.



Oscillatory rheology techniques such as temperature and frequency sweeps were employed to study viscoelastic behaviour. Dynamic light scattering (DLS) and confocal laser scanning microscopy were used to study structural changes upon shearing. Melting characteristics were evaluated using a modified Schreiber melt test³.



Samples from experiments were placed in plastic containers and cooled rapidly in salt-ice-water slurry. Subsequently samples were frozen and stored at -20°C till testing. Shear work input to the mollen cheese mass was obtained by calculating area under the torque-time curve [4].

Results and Discussion

The Stretch appearance of excessively worked model Mozzarella cheese, changed dramatically [Fig. 1]. Progressive working led to loss of stretch, exudation of serum liquid.



Fig. 1. Stretch appearance of model Mozzarella cheese produced in Blentech cooker at 70°C using 150 rpm. a. Optimum melt functionality after shear work input of 6.6 kJ/kg; b. Excessive shear work input of 73.7 kJ/kg.

Values of dynamic moduli (G' and G'') were higher for cheese having larger amounts of shear work. LT_{max} decreased with increasing shear work input [Fig. 2]. An inverse relationship was found between melt score and the cross over temperature [Fig. 6]. However LT_{max} correlated (positively) well with melt score and could be regarded as a structure sensitive parameter during working of Mozzarella like cheeses.

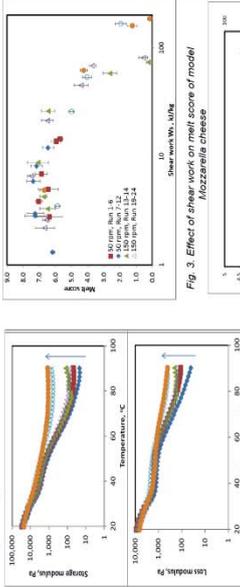


Fig. 2. Temperature sweeps on model Mozzarella cheese. Storage Modulus Pa, Loss Modulus Pa, Temperature, °C.

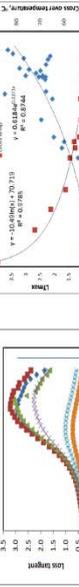


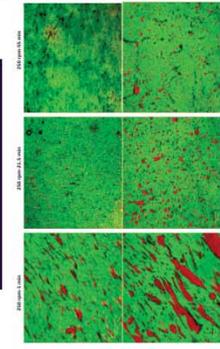
Fig. 3. Effect of shear work on melt score of model Mozzarella cheese.



Fig. 4. Relationship between LTmax, cross over temperature and melt score.

Excessively worked samples (>70 kJ/kg) had the lowest value of LT_{max} (0.7) and didn't exhibit a cross over temperature. The samples were solid upon working. Melt score decreased with shear work input [Fig. 3]. Cheese samples with >70 kJ/kg work input had a poor melt score (< 0.7) and some barely melted at all. Confocal images and particle sizing show that the fat particle size decreased upon prolonged working (Fig. 4 & 5).

Results and Discussion





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Techniques to measure steady shear viscosity of Mozzarella cheese at higher shear rates

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Introduction

Steady shear viscosity measurements on rheologically complex materials such as mozzarella cheese are difficult because of transient viscoelastic effects, wall slip and structural changes taking place in the sample during shearing^[1]. Because of these difficulties a limited amount of work has been conducted on steady shear rheology of mozzarella cheese^[2]. Therefore, the aim of this study was to generate reliable and consistent data on steady shear viscosity at higher shear rates and higher temperatures.

Materials and Methods

Samples of commercial mozzarella cheese, a model mozzarella cheese and a renneted casein gel were obtained from Fonterra Co-operative Group. Disc shaped samples were prepared for rotational shear measurements. Cheese cylinders of appropriate diameter were drawn from a cheese block using a cork borer. Discs ~2-3 mm thick were cut from the cheese cylinder using a wire cutter. The cheese was then placed between the parallel plates of the rheometer. Preliminary studies were conducted on a stress controlled AR-G2 rheometer (TA Instruments). Subsequent studies were conducted on an MCR 301 rheometer (Anton Paar, Austria) using a Peltier temperature hood (H-PTD 200). The measurement gap was set by closing the gap till normal force was 5N. All rheological measurements were conducted at 70°C.

Results and Discussion

Initial experiments conducted on model Mozzarella cheese using smooth plates suggested the presence of wall slip, observed as a sudden drop in viscosity at shear rates ~5 s⁻¹ [Fig. 1]. Maybe the presence of molten fat near the surfaces causes loss of grip resulting in early wall slip. The grip between the plates and cheese was improved by gluing sand paper onto the smooth surfaces, or by using sandblasted plates or serrated plates. Best results were obtained with serrated plates, followed by sand paper and then sandblasted plates [Fig. 2].

However, surface modification just changed the location of slip from the walls to within the material. This slip within the sample is referred to as shear banding or melt fracture in the polymer literature^[3].

Continuous shearing of sample at higher shear rates resulted in expulsion of sample from the measurement gap in the form of a thick strand with aligned protein fibers [Fig. 3].

A smooth flow curve up to 250 s⁻¹ was obtained for Mozzarella cheese by allowing longer measurement durations at low shear rates to avoid viscoelastic transient effects and selecting definite shear steps in order to limit accumulated shear strain [Fig. 4].

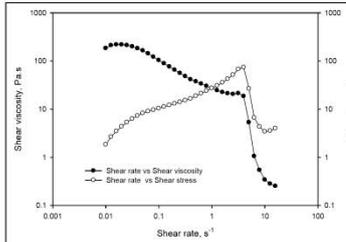


Fig. 1. Steady shear viscosity measurements on model Mozz cheese at 70°C using 20 mm Dia smooth plate

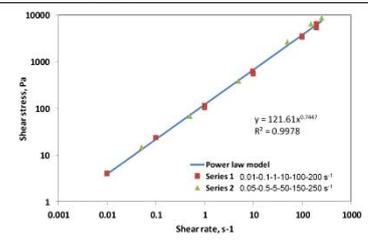


Fig. 4. Optimized flow curve for mozzarella cheese at 70°C.

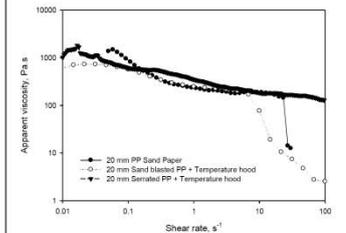


Fig. 2 Steady shear viscosity measurement on model Mozz cheese at 70°C using surface modification of parallel plates

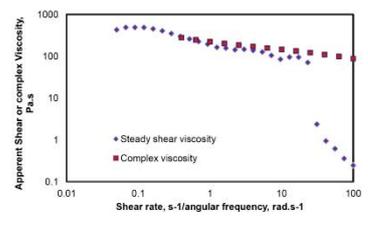


Fig. 5. Applicability of Cox-Merz rule for renneted casein gel. (Oscillation amplitude=0.5%)

These structural changes posed significant challenges in viscosity measurement. Time-dependent viscoelastic behavior of Mozzarella cheese was observed at low shear rates (<1 s⁻¹) and that indicated a longer time was needed to reach steady state shear conditions.

Applicability of the Cox-Merz rule was assessed for renneted casein gel by comparing shear viscosity and complex viscosity [Fig. 5]. A good agreement between the two was observed, suggesting the use of oscillatory data to estimate shear viscosity at higher shear rates.

Conclusions

The best conditions to obtain reliable and consistent data up to 250 s⁻¹ on steady shear viscosity of Mozzarella cheese were: 1. Use of 20 mm serrated plates with Peltier temperature hood; 2. Longer measurement duration for low shear rates and; 3. Using fewer shear steps in the flow curve. The Cox-Merz rule was valid for renneted casein gel at 70°C.

Acknowledgement

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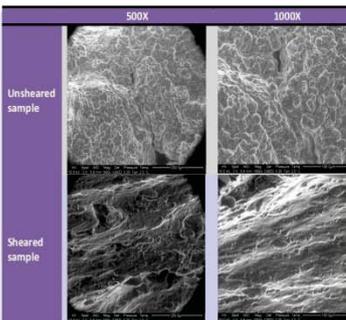


Fig. 3 SEM micrographs of Model Mozzarella cheese samples sheared in the rheometer with sand paper attached to the parallel plates



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Bast R, Sharma P, Easton HKB, Dessev TT, Lad M & Munro PA (2015) Tensile testing to quantitate the anisotropy and strain hardening of mozzarella cheese. *International Dairy Journal*, 44, 6-14.

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