Interpretive Summary

Strain hardening and anisotropy in tensile fracture properties of sheared model Mozzarella cheeses. By Sharma et al.

Mozzarella cheese has a fibrous appearance that is created during the working process involving kneading and stretching action. Energy imparted to the cheese during working determines its characteristics. The fibrous character of the cheese suggests the possibility of direction dependent (anisotropic) properties. This work investigates the effect of shear work input on strain hardening and anisotropy in the tensile properties. It also proposes schematic models to explain the observed anisotropy and strain hardening in sheared cheeses.

Strain hardening and anisotropy in tensile fracture properties of sheared model Mozzarella cheeses

Prateek Sharma[*‡1] **, Peter A. Munro**[*]**, Tzvetelin T. Dessev**[*]**, Peter G. Wiles**[†]**, E. Allen Foegeding**[§]

[*]*Riddet Institute, Massey University, Private Bag 11222, Palmerston North, New Zealand*

[†]*Fonterra Research and Development Centre, Private Bag 11029, Palmerston North 4442, New Zealand*

[‡]*Dairy Technology Division, National Dairy Research Institute, Karnal-132001, Haryana, India*

[§]*Department of Food, Bioprocessing and Nutrition Sciences, North Carolina State University, Box 7624, Raleigh, NC, 27695-7624, USA*

[1] *Corresponding Author: Tel: +91 7082261083*
Email address: P.Sharma@massey.ac.nz; prateek.sharma@icar.gov.in; *prateeksharma2000@yahoo.com*

ABSTRACT

We studied tensile fracture properties of model Mozzarella cheeses with varying amounts of shear work input (3.3-73.7 kJ/kg). After manufacture cheeses were elongated by manual rolling at 65 ºC followed by tensile testing at 21 ºC 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. Although the distortion of the fat drops in the cheese structure associated with the elongation could account for some of the anisotropy observed, the presence of anisotropy in the elongated nonfat samples reflected that shear work and rolling also aligned the protein structure.

Key words: Tensile testing, Strain hardening, Anisotropy, Mozzarella cheese

INTRODUCTION

Hot water stretching and kneading form 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 et al., 1999). The fibrous structure is visible on a macroscopic level (Oberg et al., 1993; Sharma et al., 2016a). Sharma et al. (2016a, b; 2017) 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 ºC. Shear work inputs were extended well beyond normal manufacturing limits to exaggerate any changes in the cheese caused by working. Mechanical properties were characterized using a range of rheological methods including steady shear viscosity, strain sweeps, frequency sweeps, temperature sweeps, and creep behavior. With increase in shear work input cheeses showed increases in steady shear viscosity and storage modulus. Frequency sweeps at 70 ºC demonstrated a shift 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 major macroscopic structural changes to the cheese network with disappearance of the fibrous structure, loss of stretch and melt, and serum syneresis. 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 (Sharma et al., 2017). The observed phenomena were attributed mainly to an increase in the strength of protein-protein interactions with prolonged working.

Bast et al. (2015) developed a tensile testing method to quantitate the anisotropy and strain hardening of commercial Mozzarella cheese. The method involved deliberate elongation of cheese at 60 ºC by manual rolling on a cooled metal surface to ensure that the structure was systematically aligned. Mozzarella cheeses showed strong anisotropy for both fracture stress and strain after elongation and also showed significant strain hardening in the longitudinal or fiber 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 et al., 2003), weak β-lactoglobulin gels (Pouzot et al., 2006) and gels formed by acidifying transglutaminase cross-linked casein (Rohm et al., 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 and Fox, 1993; Olivares et al., 2009).

Strain hardening behavior expresses the underlying arrangement of structural units, is therefore useful for understanding functional properties of food materials. 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 et al., 2006; Peressini et al, 2008; Kokelaar et al., 1996; Van Vliet et al., 1992; Van Vliet, 2008). The effect of mechanical work 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. Structural analogy of anisotropic nature of gluten network and Mozzarella cheese indicates possibilities of adapting testing procedures from dough rheology for better understanding of strain hardening in Mozzarella type cheeses.

The objectives of this paper 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 we have used; 2. To explore whether our 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.

MATERIALS AND METHODS

Materials

Frozen blocks (-20 ºC) of renneted, acidified protein gel prepared from skim milk were obtained from Fonterra Research and Development Centre (FRDC) pilot plant (Palmerston North, NZ). The protein gel was typically about 50% moisture and 46% protein. The frozen blocks were thawed for 1 d at 11 ºC and ground to 6 mm grind size. Cream was obtained from FRDC as a fresh lot on each trial day. Cheese salt and tri-sodium citrate (TSC) were procured from Dominion Salt (Mount Maunganui, New Zealand) and Jungbunzlauer (Basel, Switzerland), respectively.

Manufacture of model Mozzarella cheeses

Model Mozzarella cheese was manufactured by mixing, cooking and working protein gel, cream, water and salt together using 150 rpm at 70 ºC in a counter rotating twin-screw cooker (Blentech, model CC-0045, Blentech Corporation, Rohnert Park, CA, USA) (Sharma, et al., 2016a). Three model cheeses were prepared – full fat, nonfat and full fat with 0.5 % tri-sodium citrate (TSC) as a chelating agent. The target composition of full fat cheese was 23% fat, 21 % protein, 53% moisture and 1.4 % salt. The same protein to salt and protein to moisture ratios as in full fat cheese were used in nonfat cheese. Further details of processing methods, sampling times, sample storage conditions and product compositions were given by Sharma et al. (2016a). Each experimental run was repeated twice on a different day at least one month interval in order to ensure that no variation arising from raw materials with similar composition but obtained from different lots.

All cheeses used in this study were frozen after manufacture. Shear work input was estimated by numerical integration of power-time curves (Sharma et al., 2016a). Shear work inputs ranged from 3.3 to 73.7 kJ/kg.

Sample preparation for tensile testing

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 closed container at 65 °C water bath for about 2 h. Melted cheese was manually rolled on a cooled (4 °C) aluminum plate using a granite rolling pin (4 °C) to form a sheet. Aluminum guide strips were attached to the plate sides to achieve a sheet thickness of 3-4 mm. The term elongation is used throughout the paper for this process. Elongation 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. Samples were kept at 21 °C for at least 1 h before tensile testing. Each rolling treatment was performed twice.

Tensile testing and data analysis

Tensile testing on elongated cheese samples was performed on a TA.XT2plus Texture Analyzer (Stable Micro Systems Ltd., Godalming, UK) using tensile grips 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 calipers. Dumbbell-shaped samples were placed carefully on both jaws avoiding any fracture during sample transfer.

Force-displacement data were converted into true stress (σ, Pa) 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.

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.

$$\sigma \ = \mathrm{K}_{SH}\ \epsilon_H^{n_{SH}} \qquad (1)$$

where K_{SH} is the strength coefficient (Pa) and η_{SH} is strain hardening index (SHI). Values of n_{SH} >1 indicate strain hardening behavior. Equation (1) was fitted (R^2~0.98-0.99) to stress-strain data over the strain range 0.4 to "0.05 before fracture".

Strain hardening is observed directly 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)

$$Strain\ hardening\ ratio\ (\mathrm{SHR}) = \frac{Maximum\ Modulus\ near\ fracture}{Initial\ modulus} \qquad (2)$$

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 localized thinning allowing much larger extensions to occur, and allowing the material to resist further thinning by locally increasing the resistance to further deformation

(Dobraszczyk and Vincent, 1999). According to the Considère criterion for necking stability in uniaxial extension

$$\frac{d\sigma}{d\varepsilon} = \sigma \quad (3)$$

$$\text{Apparent strain hardening (ASH)} = \frac{d \ln \sigma}{d\varepsilon} \quad (4)$$

ASH values > 1 indicate strain hardening (van Vliet et al., 1992, van Vliet, 2008; Peighambardoust et al., 2006).

Microscopy

Confocal scanning laser microscopy (CSLM) was done with a Zeiss LSM 510 META confocal microscope (Carl Zeiss AG, Oberkochen, Germany) according to the method of Sharma et al. (2017). 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).

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 of Sharma et al. (2015) for steady shear rheology and of Sharma et al. (2016b) 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 ºC 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 ºC using the method developed by Sharma et al. (2015) and a power law model fitted to the data to obtain consistency coefficient K and flow behavior 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 ºC. Rheological measurements were conducted in triplicate.

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 analyzed using single factor ANOVA and the Duncan post hoc test to compare means.

RESULTS

Tensile fracture properties of sheared model Mozzarella cheese

Both longitudinal and perpendicular samples exhibited non-linear stress/strain behavior (Fig. 1). At low strains ($\varepsilon < 0.25$) both longitudinal and perpendicular samples behaved in a linear manner with similar values of initial modulus. At small deformations, Hookean behavior is expected in food materials. However, at higher strain levels ($\varepsilon > 0.25$) nonlinear behavior was observed. Longitudinal samples demonstrated strain hardening with a significant increase in tensile modulus. Further measures to quantify strain hardening are explored in section 3.2. Perpendicular samples exhibited slight strain softening. Perpendicular samples fractured at much lower strain.

For full fat cheese, all longitudinal samples at shear work levels ≤26.3 kJ/kg produced similar stress-strain curves (Fig. 2a). At small strains ($\varepsilon < 0.25$), initial modulus of longitudinal samples was about 129 kPa at shear work levels ≤26.3 kJ/kg but much higher (216 kPa) at 58.2 kJ/kg, indicating the creation of a stiffer structure upon working. Perpendicular samples (Fig. 2b) showed much more variation in stress-strain curves with reduction in fracture strain with increasing shear work input. Longitudinal and perpendicular samples indicated strain hardening and strain weakening behavior respectively. When a shear work of 58.2 kJ/kg was used to make the cheese, both longitudinal and perpendicular samples showed strain weakening behavior and had a low fracture strain. When comparing longitudinal samples of the 3 model cheeses (Fig. 2c), the order of both initial stiffness and extent of non-linear behavior was nonfat >full fat >TSC added cheese.

Longitudinal samples of full fat cheese indicated no significant difference in fracture stress, fracture strain or curve shape with shear work input up to 26.3 kJ/kg (Table 1), indicating similar structure and strength. However, there was a dramatic decrease in both fracture stress and strain at 58.2 kJ/kg. The decrease ($P<0.05$) in fracture stress with increase in shear work suggested that the cheese matrix had 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 (Table 2). Fracture stress did not change significantly with shear work input for perpendicular samples (Table 1), whereas fracture strain decreased significantly as shear work increased. Fracture strain is usually regarded as an indicator of structural arrangement, so decrease in fracture strain with increasing shear work indicates significant differences in structure, e.g. more inherent weaknesses in the structure causing crack initiation, propagation and fracture (Table 1, Fig. 2b). The large percentage variations in fracture strain for perpendicular samples (Table 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 (σ_f = 115-128 kPa) and fracture strain (ε_f = 0.74-0.77) than the perpendicular samples (σ_f = 27-40 kPa; ε_f = 0.29-0.37). Anisotropy index was higher for fracture stress (3.0-4.5) than for fracture strain (2.0-2.6). 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 1).

Fracture stress for nonfat cheese was ~33 % higher (P<0.05) for longitudinal samples and ~59 % higher (P<0.05) for perpendicular samples than that for full fat cheese with similar shear work input (Table 1). Nonfat cheese had a much higher protein content than full fat cheese, so more structural protein elements were present per unit cross-sectional area in the nonfat cheese giving rise to higher values of fracture stress. Adding TSC to full fat cheese resulted in ~32% lower (P<0.05) fracture stress than full fat cheese for both longitudinal and perpendicular samples. This may be attributed to chelation of calcium by added TSC, resulting in loose binding of cheese matrix (Sharma et al., 2016a).

The different cheeses behaved very differently during the elongation process (Fig. 3) and this had an impact on their tensile fracture behavior. At low and moderate shear work inputs, the cheese elongated well giving a smooth, homogeneous cheese layer (Fig. 3a). At 58.2 kJ/kg shear work, the cheese did not flow well giving a heterogeneous cheese layer with a number of weak spots (Fig. 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 sheet was highly heterogeneous (Fig. 3c). Representative dumbbell tensile samples could not be cut from the cheese sheet.

Strain hardening of model Mozzarella cheeses

Strain hardening parameters for samples cut in longitudinal orientation are presented in Table 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. In contrast, ASH, K_H and SHI were estimated from fits to the whole non-linear portion of the fracture curve and were 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 3 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), so strain hardening is significant. In contrast, 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. Decreased ($P<0.05$) strain hardening with an increase in shear work input from 26.3 kJ/kg to 58.2 kJ/kg suggested 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 2). The 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 (2.57 and 1.32) with similar shear work input (Table 2). Table 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 (3.55 and 1.42) than at constant crosshead speed (2.84 and 1.35).

Structural anisotropy in model cheeses

Cheeses before elongation exhibited microstructural anisotropy with orientation of fat in one direction (Fig. 4a1, a2). Tensile testing of these unrolled cheeses during preliminary studies, however, showed no significant anisotropy for either fracture stress or fracture strain. After melting and elongation the same cheeses still revealed signs of microstructural anisotropy with the fat channels enlarged (Fig. 4b1, b2, b3). The structure showed globular fat in some regions (Fig. 4b3) and coalesced, elongated fat particles in other regions (Fig. 4b2). This microstructural alignment of fat particles was presumably 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. 4c). A simple photograph of nonfat cheese macrostructure also suggested orientation in the direction of rolling (Fig. 3d). Similar structural orientation has been reported previously at various length scales for nonfat cheeses (Mizuno and Lucey, 2005) supporting our observations.

Small strain oscillatory shear rheology of model cheeses

Mechanical spectra at 20 ºC of full fat, nonfat and TSC added cheeses are shown in Fig. 5. All three cheeses exhibited viscoelastic solid behavior (G' > G" across all frequencies tested) with low and similar frequency dependence (slope, n_f ~0.16 - 0.18), suggesting the presence of a physically stable network. The n_f values are consistent with those reported for casein gels and fat-filled casein gels (Zhou and 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 ºC (Zhou and Mulvaney, 1998). The storage modulus of milkfat (G_f' = 292 kPa) was higher than that of the cheese matrix (G_m' = 164 kPa) at 20 ºC (Yang et al., 2011), so fat would be expected to reinforce the matrix.

Steady shear rheology of elongated model cheeses

Bast et al. (2015) noted that the manual rolling process caused considerable work thickening in as little as 18 s, whereas shear in the Blentech caused almost no work thickening after 4000 s at 50 rpm (Sharma et al., 2016a). It is interesting to know whether the work thickening from elongation was only evident in tensile fracture properties or whether it also caused changes in steady shear viscosity. Consistency coefficient and apparent viscosity at 0.01 s^{-1} increased by 1.43 and 1.52 times respectively ($P<0.05$) after elongation (Table 3). This indicates significant work thickening upon elongation. Elongation also increased tensile fracture stress by 5.7 times parallel to the fibers and by 2.1 times perpendicular to the fibers (Bast et al., 2015). The type of deformation is quite different in measurement of the two properties though. Tensile fracture measures the strength of a material while pulling in one direction whereas steady shear rheology measures resistance to shear flow.

DISCUSSION

The anisotropy index range for fracture stress of 3.01-4.49 (Table 1) for our 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 el 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 were 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 the fibrous character in both the examples quoted.

To help explain anisotropy in full fat, elongated model Mozzarella cheese, we propose a structural model (Fig. 6a). The proposed model is based upon assumption that fracture process uses viscolastic mode of energy release in isothermal conditions. A continuous protein-gel contains a dispersed phase of fat particles elongated in the direction of rolling as observed by CSLM (Fig. 4b). 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. 1). As strain increased perpendicular to the long axis of the fat particles, fracture was initiated at low strains because of the large amount of structurally weak fat and fat-protein interface in this orientation. This resulted in lower values of fracture stress and strain for the perpendicular orientation (Table 1). As strain increased parallel to the long axis of the fat particles, fracture occurred in the gel phase as there was much less fat-protein interface. Fracture was reached at higher strains and therefore higher fracture stress. The anisotropy of cooked meat has been explained in a similar way with the muscle fibers having higher strength than the connective tissue between the fibers (Purslow, 1985).

Nonfat cheese was also highly anisotropic (Table 1) and showed evidence of structural alignment at a macroscopic/visual scale (Fig. 3d) and a microscopic scale (Fig. 4c). Structural alignment at a microscopic scale was also observed by Mizuno and Lucey (2005). Nonfat mozzarella cheese has been shown to contain serum pockets (Paulson et al., 1998; Pastorino et al., 2002; McMahon et al., 2005). These pockets would be aligned in the direction of rolling forming weak interfaces between the protein fibers as depicted in Fig. 6a. An alternative explanation is that shearing in the Blentech or elongation by rolling may be causing localized 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). Strain exerted in the perpendicular orientation may cause early fracture because of weak bonding between fibers (Taneya et al, 1992; Ak and Gunasekaran, 1997), while strain in the longitudinal orientation requires fracture of the fibers 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 changes and distortions of the fat phase were probably an indicator of related microstructural changes occurring in the 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 ours 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. 6b). Crosslinked elements are assumed to be stiffer. After strain hardening, three major changes are depicted – both elements are more aligned, the initial cross-links are more tightly bound and additional cross-linking has occurred. Zhang et al., (2007) proposed calcium induced junction zones as a possible reason for strain hardening behavior in alginate gels. The crosslinks depicted in Fig. 6b would have a similar role to these junction zones in causing strain hardening and calcium is again likely to be involved.

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 et al., 1998; McMahon et al., 1999; Mizuno and Lucey, 2005; McMahon et al., 2005). Sharma et al. (2017) 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 et al., 1983; Ak and Gunasekaran, 1997; Manski et al., 2008; Bast et al., 2015; Sharma et al., 2015). 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 (Sharma et al., 2016a, 2017). This cheese showed no structural or mechanical anisotropy. Parallel behavior was reported for prolonged working of flour dough in a z-blade mixer (Peighambardoust 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 was 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 was expected to result in reduced but still flexible interactions between proteins in the structure, leading to a weaker but still cohesive structure (Sharma et al., 2016a). 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 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 (Sharma et al., 2016a) but did not reduce strain hardening very much (Table 2). Cheese with TSC added still strain hardens as observed by all three measures used. The role of calcium in the strain hardening bonding mechanism appears to be less important as compare with the work thickening bonding mechanism. It would be interesting to look at the effect of extent of chelation of calcium in detail on rheological and fracture properties of cheese.

Steady shear viscosity increased 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 (Sharma et al., 2016a). The one dimensional elongational flow caused by rolling with simultaneous cooling into the more elastic region was 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 volume. On the other hand, remelting of elongated cheese leads to relaxation of structure and decrease in anisotropy with some loss of strength (Bast et al., 2015).

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. Based on the previous discussion by Bast et al. (2015), we suggest one possible mechanism. In the initial or linear region on the stress-strain curve, strain is merely straightening curves or bends in the protein network, which may be the same in the two orientations. In the exponential or strain hardening region, where straightening has reached its limit, the fibers in the longitudinal direction need to be stretched or move past one another, leading to strain hardening. In the perpendicular orientation the fibers are pulled further apart, the interfaces are weak and maybe there are also more microcracks for initiation of fracture. This plausible mechanism will require further experimental evidence for validation.

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 behavior. 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. We attribute this to the presence of macroscopic protein fibers 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.

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Table 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	121.02 ± 26.97[a]	40.24 ± 14.51[be]	3.01	0.75 ± 0.07[ac]	0.37 ± 0.09[d]	2.04
	4.3	115.72 ± 22.14[a]	32.25 ± 12.07[bf]	3.59	0.75 ± 0.09[a]	0.34 ± 0.10[de]	2.23
	8.8	125.45 ± 16.40[a]	27.94 ± 8.18[bf]	4.49	0.74 ± 0.06[ac]	0.29 ± 0.09[e]	2.54
	26.3	128.61 ± 21.50[a]	30.60 ± 12.20[bf]	4.20	0.77 ± 0.07[a]	0.30 ± 0.09[e]	2.58
	58.2	44.36 ± 14.55[be]	41.33 ± 11.20[be]	1.07	0.21 ± 0.05[b]	0.22 ± 0.06[b]	0.94
Nonfat cheese	6.8	159.24 ± 33.53[c]	47.74 ± 17.65[e]	3.34	0.68 ± 0.14[c]	0.34 ± 0.07[de]	2.03
Nonfat cheese – constant strain rate	6.8	140.77 ± 42.76[c]	40.08 ± 8.06[e]	3.5	0.58 ± 0.10[c]	0.31 ± 0.05[de]	1.87
TSC added full fat cheese	4.4	78.50 ± 19.24[d]	21.92 ± 3.88[f]	3.58	0.70 ± 0.09[ac]	0.29 ± 0.04[e]	2.45

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 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, $d\ln\sigma/d\varepsilon_H$	Strength coefficient, K_H (Pa)	Strain hardening index, n_H
Full fat cheese	3.3	129.31 ± 10.05[a]	235.48 ± 42.25[a]	1.81 ± 0.24[a]	2.60 ± 0.15[a]	184.91 ± 20.89[ab]	1.33 ± 0.05[a]
	4.3	128.70 ± 17.74[a]	219.73 ± 34.26[a]	1.79 ± 0.26[a]	2.57 ± 0.16[a]	171.78 ± 29.36[ab]	1.32 ± 0.05[a]
	8.8	141.89 ± 14.77[a]	231.46 ± 28.29[a]	1.65 ± 0.27[a]	2.52 ± 0.13[a]	189.53 ± 13.16[b]	1.30 ± 0.05[a]
	26.3	142.13 ± 21.05[a]	238.74 ± 40.92[a]	1.72 ± 0.28[a]	2.51 ±0.10[a]	181.20 ± 22.30[ab]	1.31 ± 0.05[a]
	58.2	248.44 ± 59.74[b]	188.87 ± 48.07[b]	0.76 ± 0.07[b]	-	3.63 ± 1.31[c]	0.49 ± 0.03[b]
Nonfat cheese	6.8	174.01 ± 27.91[c]	313.96 ± 58.86 [c]	1.76 ± 0.37[a]	2.84 ± 0.09[b]	273.52 ± 38.73[d]	1.35 ± 0.08[a]
Nonfat cheese - constant strain rate	6.8	198.14 ± 13.39[e]	334.16 ± 9.25[c]	2.03 ± 0.59[a]	3.55 ± 0.20[d]	366. 97 ± 6.74[f]	1.42 ± 0.01[d]
TSC added full fat cheese	4.4	104.93 ± 5.97[d]	148.30 ± 27.01[d]	1.41 ± 0.23[c]	2.39 ± 0.09[c]	125.11 ± 14.47[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 ($P<0.05$).

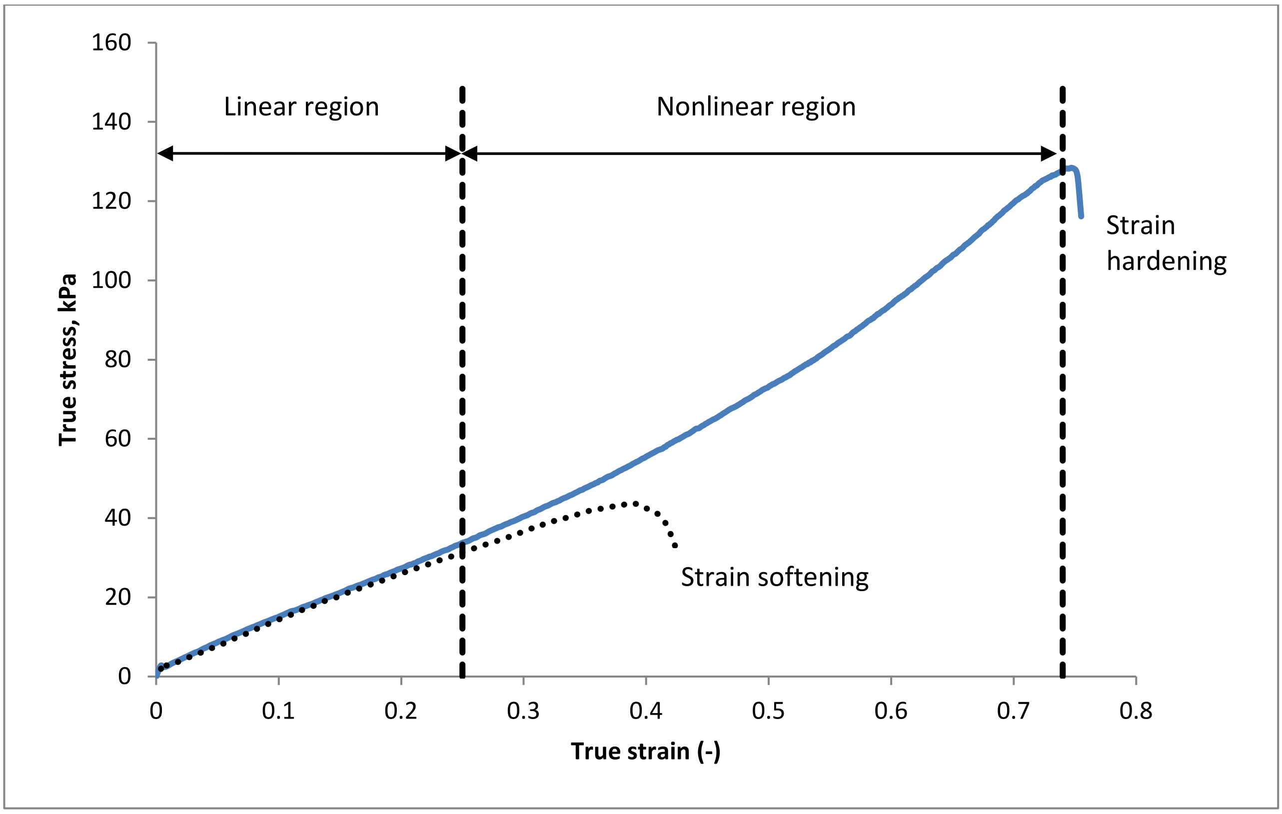
Table 3. Effect of rolling on steady shear rheology of model Mozzarella cheese* at 70°C

	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 ($P<0.05$)

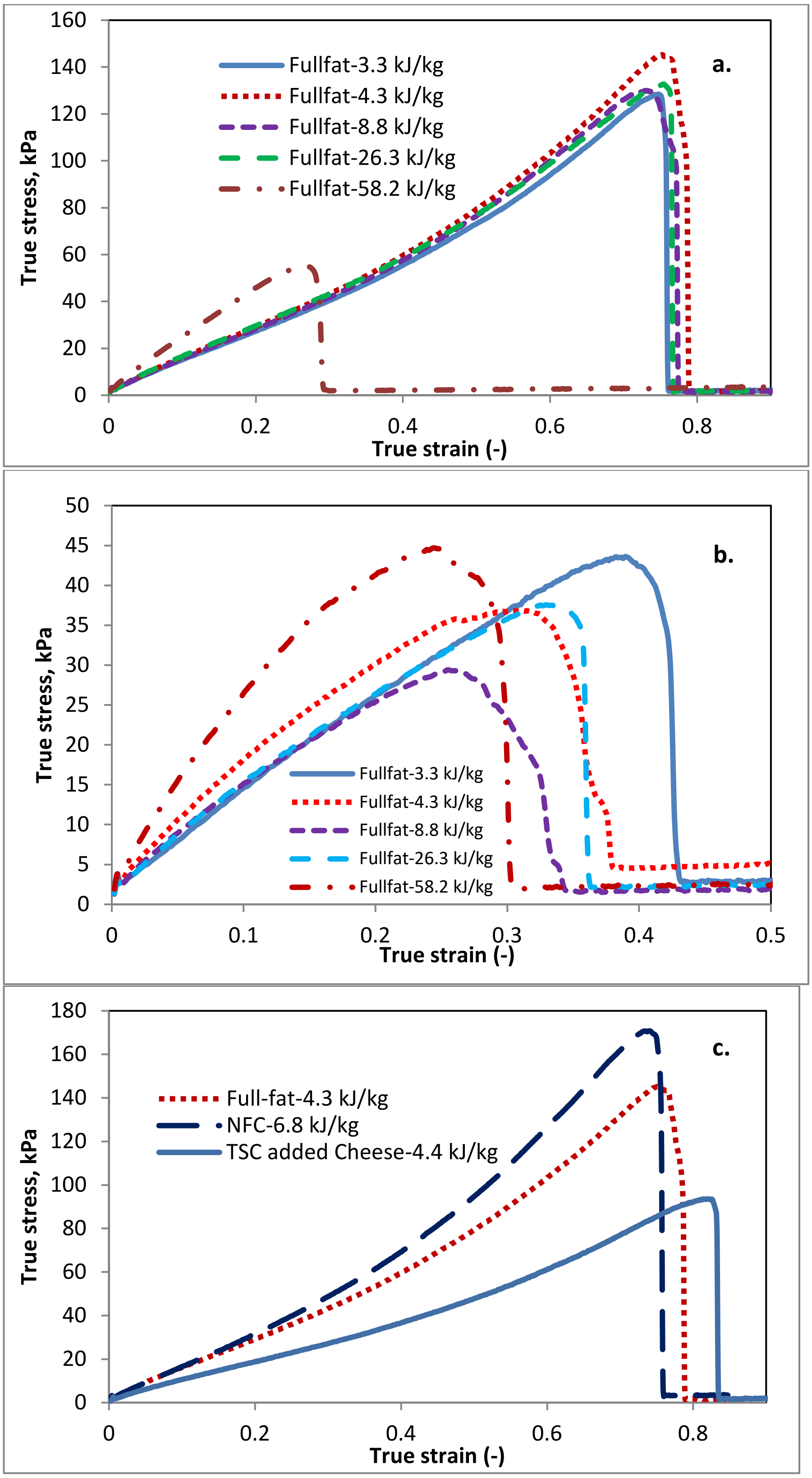
Sharma Fig 1.



160
140
120
100
80
60
40
20
0
True stress, kPa
Linear region
Nonlinear region
Strain hardening
Strain softening
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
True strain (-)

Fig. 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 ºC using 150 rpm screw speed.

Sharma Fig. 2



a.
True stress, kPa
True strain (-)
Fullfat-3.3 kJ/kg
Fullfat-4.3 kJ/kg
Fullfat-8.8 kJ/kg
Fullfat-26.3 kJ/kg
Fullfat-58.2 kJ/kg
b.
True stress, kPa
True strain (-)
Fullfat-3.3 kJ/kg
Fullfat-4.3 kJ/kg
Fullfat-8.8 kJ/kg
Fullfat-26.3 kJ/kg
Fullfat-58.2 kJ/kg
c.
True stress, kPa
True strain (-)
Full-fat-4.3 kJ/kg
NFC-6.8 kJ/kg
TSC added Cheese-4.4 kJ/kg

Fig. 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 ºC using 150 rpm screw speed. Typical curves close to the mean behaviour were chosen from n=16 longitudinal samples and n=18 perpendicular samples.

Sharma Fig. 3

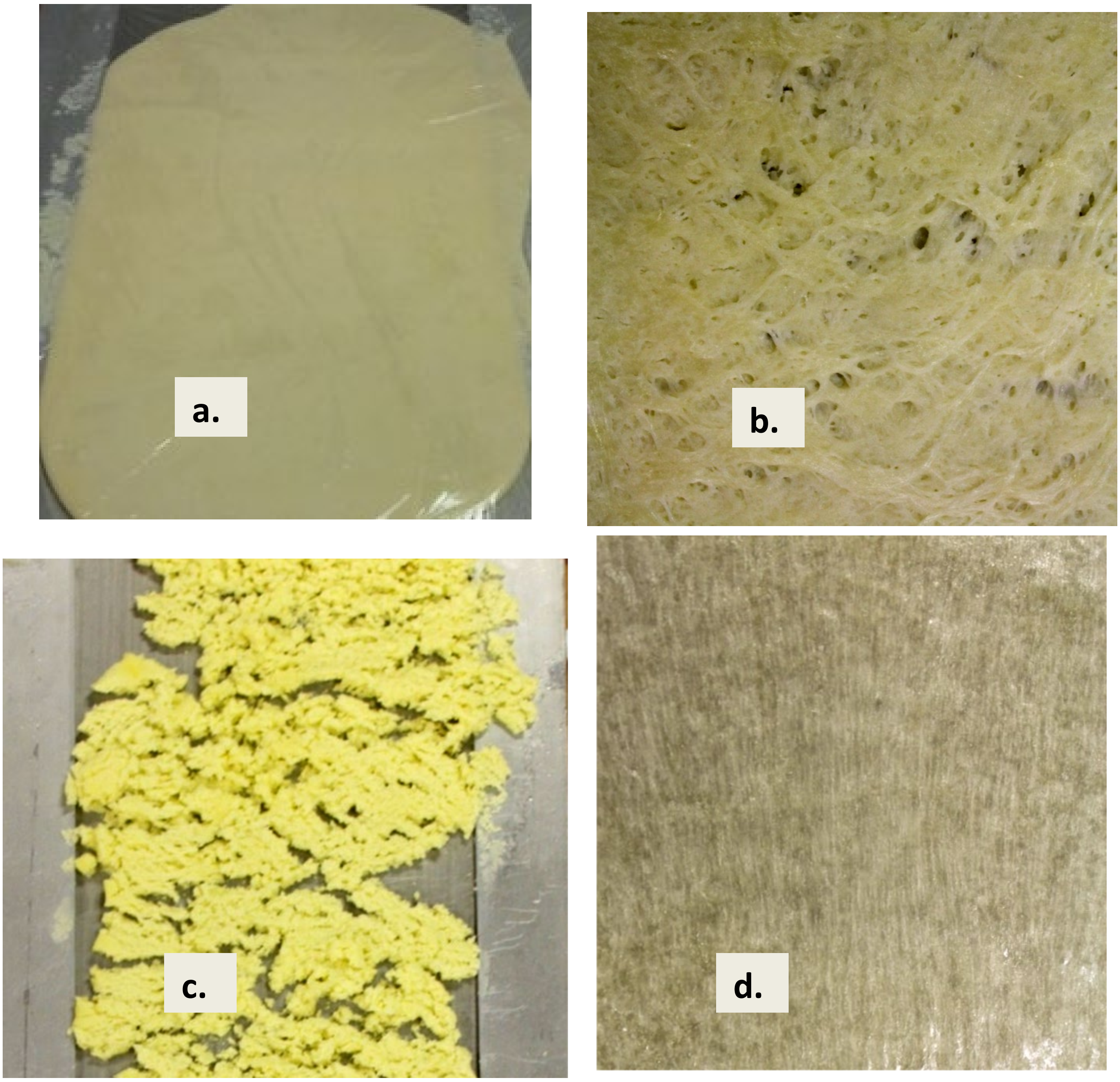


Fig. 3. Visual appearance of elongated model Mozzarella cheeses. Cheese samples were prepared in the Blentech cooker with varied amounts of shear work input at 70 ºC and 150 rpm; full fat cheese a. 8.8, b. 58.2, c. 73.7 kJ/kg, nonfat cheese d. 6.8 kJ/kg

Sharma Fig. 4

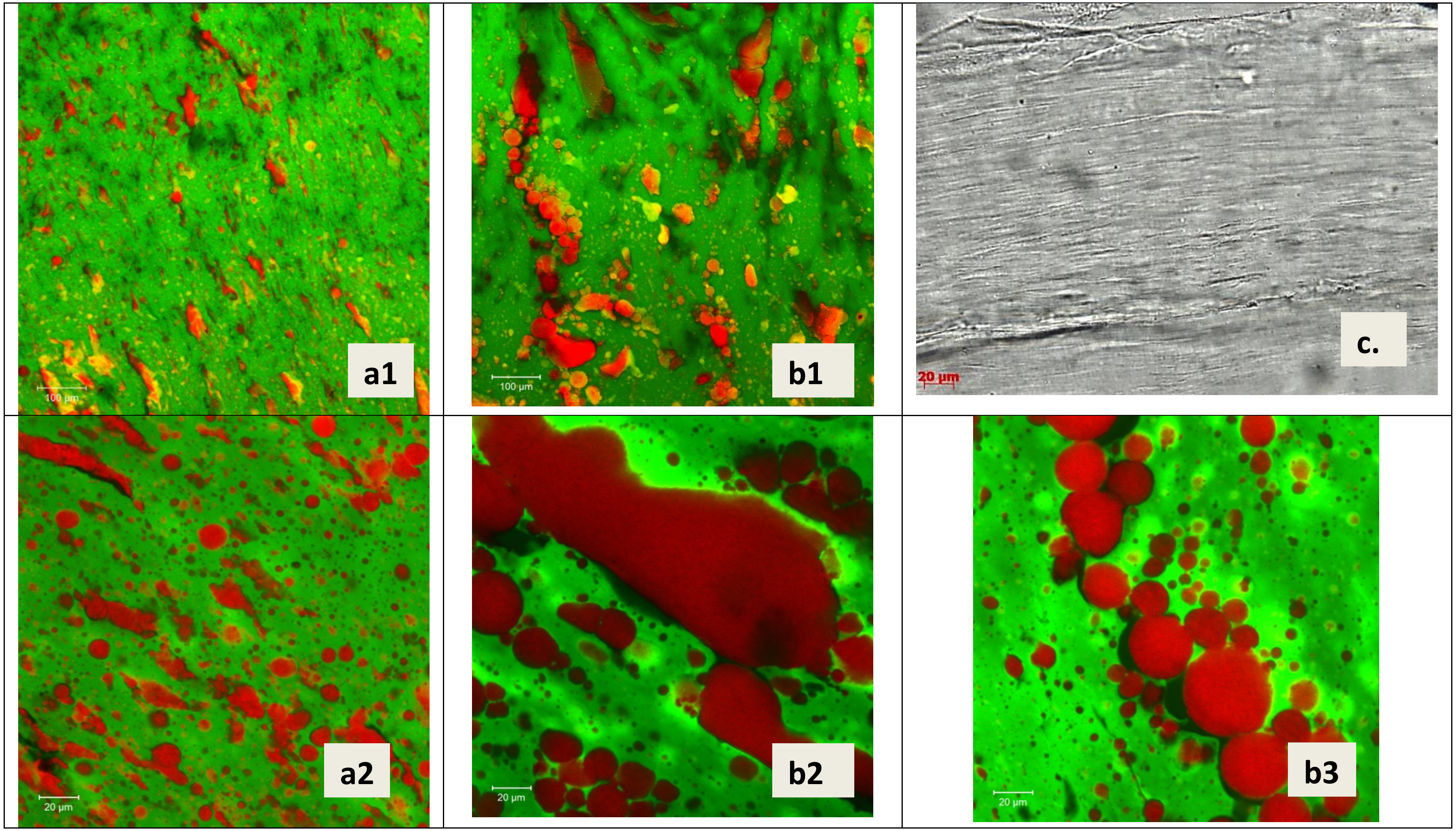
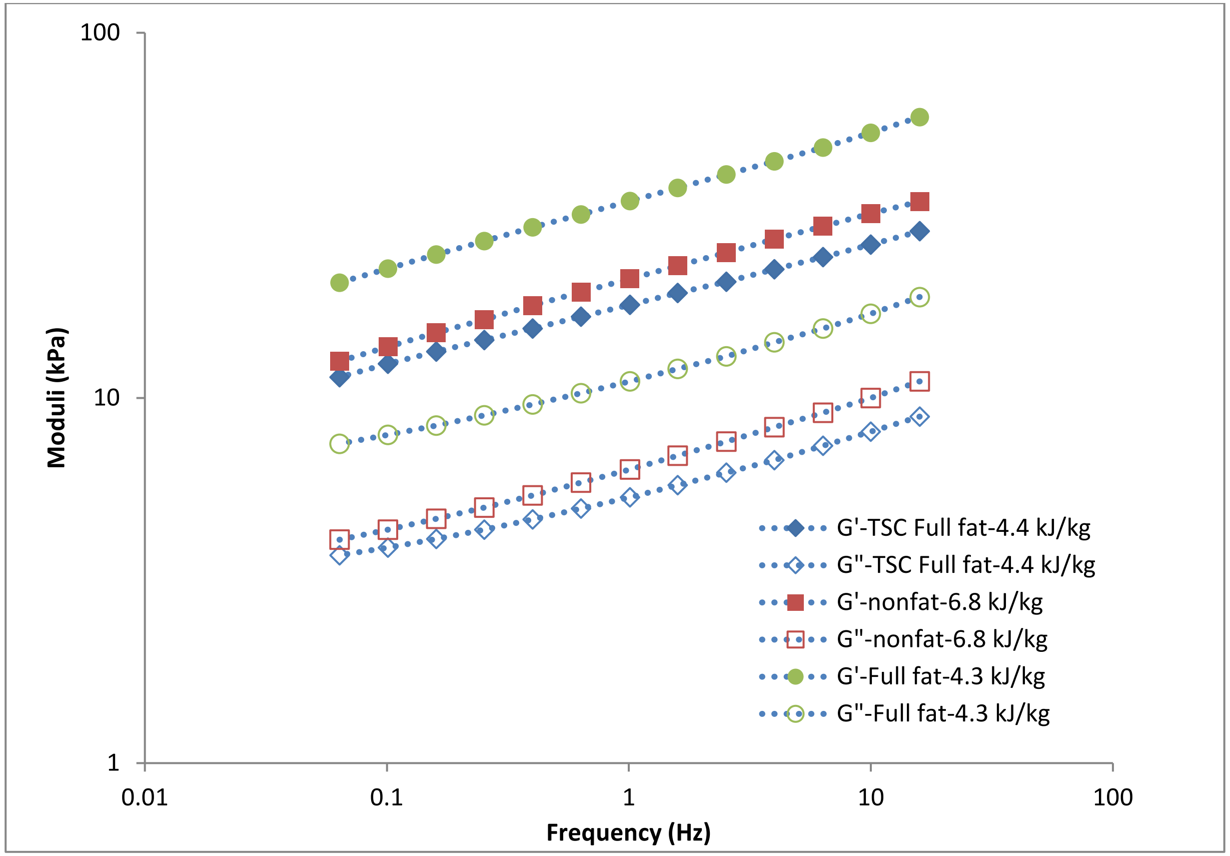


Fig. 4. Microstructures of model Mozzarella cheeses; CSLM images of normal (a1,a2) and elongated (b1,b2,b3) full fat Mozzarella cheeses after 26.3 kJ/kg of shear work input; Light microscopy (LM) image of nonfat Mozzarella cheese (c) after 6.8 kJ/kg of shear work input. Cheese samples were prepared in the Blentech cooker at 70 ºC and 150 rpm. Red - fat, green - protein and black - air/water.

Sharma Fig. 5



100
10
1
Moduli (kPa)
0.01
0.1
1
10
100
Frequency (Hz)
G'-TSC Full fat-4.4 kJ/kg
G"-TSC Full fat-4.4 kJ/kg
G'-nonfat-6.8 kJ/kg
G"-nonfat-6.8 kJ/kg
G'-Full fat-4.3 kJ/kg
G"-Full fat-4.3 kJ/kg

Fig. 5. Storage moduli (closed symbols) and loss moduli (open symbols) of model Mozzarella cheeses obtained from frequency sweeps at 20 ºC 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 ºC and 150 rpm.

Sharma Fig. 6

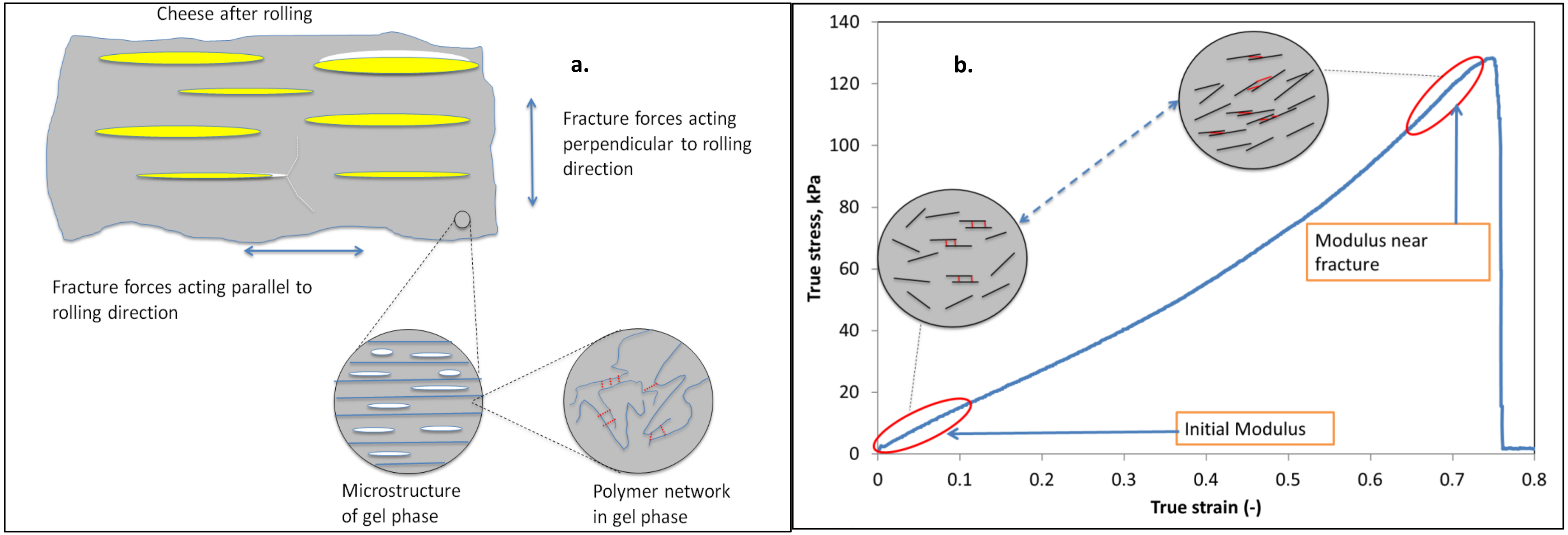

Cheese after rolling
a.
Fracture forces acting perpendicular to rolling direction
Fracture forces acting parallel to rolling direction
Microstructure of gel phase
Polymer network in gel phase
b.
140
120
100
80
60
40
20
0
True stress, kPa
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
True strain (-)
Modulus near fracture
Initial Modulus

Fig. 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. White pockets in gel phase indicate serum/water portion. Further structural elements within the gel phase are presented in magnified grey circles.