Microstructural transformations in anisotropy and melt-stretch properties of low moisture part skim mozzarella cheese

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Abstract

Mozzarella cheese is primarily consumed in its melted form due to its desirable melt and stretch characteristics when heated. Understanding the relationship between the anisotropic structure and melt-stretch properties is critical for controlling functionality. A novel *ex situ* sample extraction system was developed to produce melted and stretched mozzarella. New structure analytics were established to reveal transient changes during deformation of mozzarella under dynamic heat-shear conditions. Transformations in anisotropy were examined using various microscopy techniques coupled with image analysis. Coalescence of milk fat aggregates into large droplets upon heating caused loss of anisotropy. However, fat droplets broke down into channels on stretching, comprising agglomerates of smaller droplets and anisotropy was regained. When stretched further adhesion between the agglomerated fat droplets was broken by the forces exerted from the contracted protein fibres. Large fat droplets are necessary as they act as shock absorbers that enable protein fibres to become pliable; allowing greater stretch.

1.0 Introduction

Mozzarella cheese is a widely used ingredient in both home cooking and food services sector. Mozzarella production is growing, in 2015 in the US alone it represented (at 1.8 x 10^6 kg) the largest volume cheese sold to restaurants, accounting for 29% of total cheese usage (largely driven by demand for pizza). (Dequaine, 2016) Consumers' perception of mozzarella is dominated by its desirable melt, stretch and stringy characteristics that emerge upon heating. These signature textural attributes are classified as functional properties (Gunasekaran & Ak, 2003) and are frequently attributed to the *pasta-filata* structure (McMahon & Oberg, 1999).

The *pasta-filata* microstructure in the final mozzarella product originates from the critical stretcher-cooker process during manufacture (Kindstedt & Guo, 1997; McMahon, Oberg & McManus, 1993;). The structure is heterogeneous and anisotropic combination of networks of long parallel aligned *para*-casein fibres, with elongated channels of fat oriented in the direction of stretch (Everett & Auty, 2008; Kindstedt, 2004; Kinstedt & Guo, 1997; McMahon & Oberg, 1999). Cheese manufacturers aim to produce mozzarella with structures that deliver the physical functional properties specified by the end-user (Kindstedt & Guo, 1997; McMahon *et al*., 1993). By quantifying the link between structure and property, manufacturers can design and fabricate products using a more methodical and controlled basis (McClements, 2007).

Meltability and stretchability are the key functional properties that emerge on baking of mozzarella and are perceived as the signature attractive characteristic of this cheese (Chen, Wolle & Sommer, 2009; Guinee, Feeney, Auty & Fox, 2002; Rowney, Roupas, Hickey & Everett, 1999). Whilst these properties are frequently attributed to the anisotropic microstructure little attention has been paid to the transformation of that structure during melting.

Meltability is defined as the ability of the cheese structure to flow and form a uniform continuous melt upon heating, and it is the first point of major alteration in the structure during cooking (Kindstedt, Carlc & Milanovic, 2004; Rowney, Roupas, Hickey & Everett, 1998). As the mozzarella is heated, aggregates of milk fat globules between the protein fibres liquefy at 40 °C (Joshi, Muthukumarappan & Dave, 2004; Lopez, Camier & Gassi , 2007). The liquid fats coalesce and enhance the meltability by lubricating the surfaces of adjacent para-casein network to facilitate displacement of adjoining layers of the protein matrix and effectively increase cheese flow (Kindstedt, 1993; Lucey, Johnson & Horne, 2003). However, there is currently a lack of structural information on *melted* mozzarella.

Stretchability is known to be one of the most significant functional properties of mozzarella, yet it is the most difficult aspect to measure (Fife, McMahon & Oberg, 2002). Stretchability is defined as the capacity of melted cheese to form fibrous strands that extend without breaking under tension (Rowney *et al.*, 1999). Lucey *et al.* (2003) have provided a more precise definition; stretch is the ability of the casein network to maintain its integrity when a continuous stress is applied to the cheese.

Many authors (Ak, Bogenrief, Gunaskaran & Olsen, 1993; Ak & Gunasekaran, 1995; Apostopoulos, 1993; Cavella, Chemin & Masi, 1992; Fife *et al.*, 2002; Ma, James, Zhang & Emanuelsson-Patterson, 2012; Wang, Muthukumarappan, Ak & Gunasekaran., 1998) have implemented objective stretch tests that provides quantitative rheological measurements of the cheese stretched under controlled environmental conditions. However, even with the possibility of quantifying stretch, the *mechanism* of stretch still remains unclear as to how the microstructure deforms.

One difficulty in directly visualising the effect of heat on the microstructure of mozzarella arises from the difficulty in handling soft mozzarella because of its viscoelastic behaviour. As strained materials are sampled stress relaxation immediately alters the structure. Simply put, cutting a sample from stretched cheese alters the microstructure before analysis.

Thus, designing a method to extract the strained structure with the loading orientation and strained microstructure intact is critical to producing samples for microstructural analysis. The purpose of the current study was to reveal changes in microstructure on melting and stretching in order to quantify their role in mechanism of melt flow and stretchability. Melted and stretched mozzarella samples were produced using a sampling system capable of extracting samples with the loading orientation and the strained microstructure intact. These samples were then analysed using advanced complementary microscopy techniques combined with image analysis to quantify key structural parameters in the mozzarella microstructure under controlled heat-shear conditions.

2.0 Materials and methods

Low moisture, part-skim mozzarella samples (Galaxy Mozzarella Cheese, Fonterra Brands Ltd., Auckland New Zealand) were purchased as 200 g blocks from a local supermarket. The composition of the mozzarella was: 19.9 g fat per 100 g cheese, 26.7 g protein per100 g cheese, 50.9 g water per100 g cheese and 0.5 g salt per100 g cheese. The mozzarella was reported to be packed at the age of approximately 4 to 8 wk, vacuum sealed and stored at 2 °C.

2.1 Sample extraction system

A sample extraction system was designed and fabricated based on modifications of Ma *et al.*'s (2012) stretching apparatus to produce melted and stretched mozzarella to capture in-built strain in the structure, and to arrest the deformation of fat during melting and stretching for microstructure characterisation.

A 10 g sample of mozzarella was placed into a stainless steel tube and covered with aluminium foil to prevent dehydration. The tube and cheese was then heated in a hot water bath at 75 °C for 15 min to achieve a melt temperature of 70 °C. After heating a Teflon sleeve (which was also heated in the same water bath) was slid onto the tube to insulate and reduce the change in temperature of the sample. A 3-prong hook attached to the Instron crosshead was lowered into the pool of mozzarella, 5mm above the rig base, as shown in Fig. 1a.

The hook was subsequently rotated clockwise by 90° and locked into position. The mozzarella was pulled vertically at 1000 $mm.min^{-1}$. The strained structure was immediately frozen in place by pouring liquid N_2 into the rig as illustrated in Fig. 1b. Removing the sample from the rig prior to freezing could not be accomplished without further deformation of the sample. As such quenching the stretched mozzarella using liquid nitrogen was found to be the most effective method of preserving the strained microstructure & arresting the transient deformations. Instantaneous solidification prevented further deformation from gravity and minimised artefacts during handling of samples that were in a viscous or malleable state.

The mozzarella sample was taken (Fig. 1c) from the centre of the stretched sample. Extracted samples were placed inside an airtight container and immediately stored at -20 °C in a freezer. Samples were taken at stretched lengths of 50 mm, 100 mm and 150 mm, giving strains of 280 %, 580 % and 900 % respectively at a strain rate of about 10 s^{-1}. Unstretched samples were also taken by melting the cheese using the steps above, freezing then unsealing the tube.

2.2 Microstructure characterisation

Confocal laser scanning microscopy (CLSM), environmental scanning electron microscopy (ESEM) and cryo-SEM were used to observe the microstructure of the unmelted, melted and strained mozzarella. This set of microscopy techniques were selected as they accommodate the inspection of fully hydrated samples, use mild or no sample preparation and less inclined to introduce artefacts (James, 2009).

2.2.1 Confocal laser scanning microscopy

CLSM was used to observe the change in fat size and shape of fat globules and channels within the protein matrix for unmelted, melted, and melted and stretched mozzarella. Samples, previously stored at -20 °C as described above, were cut to 10 mm×10 mm×10 mm. Specimens were cryo-sectioned to thickness of 50 µm along the longitudinal axis at -20 °C using a cryostat (Leica CM1850 Cryomicrotome, Buffalo Grove, IL, USA).

The sectioned specimens were placed onto flat glass microscope slides. Two drops of nile red/FCF fast green stain (Sigma-Aldrich, St. Louis, MO, USA) were applied to the coverslip using a pipette and placed onto the specimen. The stain was composed of 0.5 % nile red and 0.2 % fast green FCF dyes diluted in AF1 Citifluor (a glycerol-PBS solution containing an amine antifadent) (Sigma-Aldrich, St. Louis, MO, USA). The prepared slides were placed in a light-proof container to prevent photo bleaching and left to stain overnight in a refrigerator at 4 °C.

Confocal micrographs were taken using a 40× magnification oil immersion lens (Leica TCS SP2, Buffalo Grove, IL. USA: objective lens specifications HCX Plan Apo 40X/1.25 oil with numerical aperture 1.25). An He/Ne laser with a wavelength of 633 nm was used to excite the nile red dye and emissions were collected at 575-600 nm for the fat phase. Kr/Ar laser with a wavelength of 488 nm was used to excite the fast green and emissions were collected

above 645 nm for the protein phase. Images consisting of 512 × 512 pixels were acquired at approximately 20 to 25 µm below the sample surface using a line average of 8.

2.2.2 Environmental scanning electron microscopy

ESEM was conducted to investigate the microstructure of mozzarella in its native state at a high resolution. The internal structure of the unmelted, melted and stretched mozzarella samples were examined. The internal microstructure was revealed by thawing the samples at room temperature for 1 min and peeling along the fibre direction. The peeled section was cut into a rectangular section to a dimension of 4 mm by 4 mm (length, width) by 1 mm thickness. The peeled surface (not the cut edges) was viewed.

Scanning electron microscopy was conducted in an environmental scanning electron microscope equipped with a peltier cooling stage (Quanta 200F SEM, FEI, Eindhoven, The Netherlands). Water vapour was used as the imaging gas and the sample chamber was pumped through five cycles (minimum pressure of 430 Nm^{-2} and maximum of pressure 930 Nm^{-2} Torr) to stabilise the water vapour pressure. An accelerating voltage of 10 kV and working distance of approximately 5 mm-6 mm was used. Pressure of 430 Nm^{-2} Torr and temperature of 2.0 °C was used to establish a relative humidity of 60 % during imaging.

2.2.3 Cryogenic scanning electron microscopy

Cryo-SEM was also used to observe the internal structure of the cheese. Samples were cut into sections with a height of 4 mm, length of 3 mm and width of 1 to 2 mm and mounted onto a copper sample holder. The copper holder, with mounted specimen, was plunged into liquid N_2 slush at -210 °C. The frozen specimens were transferred under vacuum conditions to the preparation chamber (Alto 2500 cryo-trans system, Gatan, Pleasanton, CA, USA)

where they were cryo-fractured with a scalpel blade at -187 °C. The specimens were subsequently allowed to warm to -90 °C for 30 min to sublime water, cooled again and sputter coated with gold. The specimen was transferred onto a liquid nitrogen cooled cold-stage module, maintained at -187 °C and observed using a field emission scanning electron microscope (XL30 S-FEG, FEI/Philips, Eindhoven, The Netherlands). Images were captured with an accelerating voltage of 5 kV using a secondary electron detector. Cryo-SEM was not carried out for 100 mm and 150 mm stretched mozzarella due to the restriction of the sample geometry as these samples were too thin to handle easily for freeze-fracture, as the sample needs to be held in the sample holder, frozen then fractured in the sample preparation chamber of the cryo-transfer system.

2.2.4 Quantification of fat channels

CLSM images only containing the fat channels (obtained from 488 nm excitation) were selected for quantification using LensEye software (Engineering & Cyber Solutions, Gainesville, FL, U.S.A.). The fat channels were segmented by manually applying a binary threshold to the image. For the fat analysis, objects in contact with the edges of the image were removed. Eight images were analysed in terms of area, aspect ratio and spatial distribution for each unmelted, melted, and melted and stretched sample. Statistical treatment of the measurements was conducted by extracting a mean and 95 % confidence interval based on the number of “objects” identified. Across 8 images there were between 900 and 1300 objects included for these measurements.

Area (of fat channels) was measured by calculating the area of the segmented fat channels in pixels. The area in pixels was converted into micrometre squared. The aspect ratio was used to describe and quantify the shape of fat channels, more specifically magnitude of elongation corresponding to its unmelted, melted and strained conditions. Aspect ratio was

calculated by dividing the longest length by the shortest length of the fat channel or globule. An aspect ratio of one indicates a perfectly circular fat particle and increases as the fat elongates.

The spatial distribution of fat was quantified by measuring the minimum neighbouring distance between each individual fat channel. This function was employed to estimate the dispersion of fat in the protein matrix and the degree of fat emulsification in mozzarella. The minimum distance between the fat was measured by searching for the nearest fat from each particle count and measuring the distance (shown with an arrow, Fig. 2) between the centres of gravity (shown with a circular dot, Fig. 2, and defined as the the average x- and y-positions of a binary object) of each fat channel.

2.2.5 Quantification of fat droplets

ESEM images revealed that the fat "channels" observed in CLSM were comprised of aligned aggregates of spherical milk fat globules. Segmentation of SEM images is difficult due to the large variation of grey tones in the micrographs. In addition, artefacts can be introduced from using various filters from conventional image processing software. In this case, higher precision was obtained from manual segmentation of objects, rather than automated procedures. Therefore, manual segmentation was employed for fat droplet quantification by drawing super-imposed lines in Image Pro Premier (Mediacybernetics, Rockville, MD, USA) along the major axis of each individual fat droplet in the ESEM images. Statistical treatment of the measurements was conducted by extracting a mean and 95% confidence interval based on the number of objects (ie fat droplets) identified. At the higher magnification of ESEM a lower number of individual "objects" can be identified and between 100 and 500 objects across, up to 50 images were included for these measurements.

220

221 3.0 Results

222 Changes were observed and measured in the strained samples indicating heat and strain
223 induced changes in anisotropy and microstructure of the fat channels and the fat droplets
224 within those channels. Fat channels and fat droplets were assessed separately.

225

226 3.1 Microstructure observation and evaluation

227 3.1.2 Fat channels

228 Fig. 3 shows CLSM images showing the longitudinal fibre axis of unmelted, melted and
229 melted and stretched mozzarella. As expected unmelted mozzarella showed elongated fat
230 channels in the protein matrix, forming a highly anisotropic structure (Everett & Auty 2008).

231 Heating to 70 °C induced complete loss of detectable structural anisotropy as the elongated
232 fat channels coalesced to form large spherical fat droplets with relaxation of the protein
233 matrix. These spherical fat droplets existed in a wide range of sizes, from less than 10 µm to
234 up to as large as 200 µm in diameter. These large droplets elongated in the direction of
235 strain when the mozzarella was stretched, and began to break up when the strain increased.

236

237 3.2.2 Fat droplets

238 The fractured longitudinal sections of the unmelted, melted and melted and stretched
239 mozzarella were examined using the ESEM to show the morphology of the fat droplets
240 within the channels, shown in Fig. 4. The fat channels in unmelted mozzarella were
241 comprised of aggregates of milk fat globules with diameters ranging from 1 to 15 µm,
242 consistent with previous studies (Kuo & Gunasekaran, 2009). Similar to the observations

made in the confocal micrographs, heating resulted in a loss of anisotropy, with coalescence of milk fat globules forming large fat droplets up to 200 µm in diameter.

When a stretch yielding a strain of 280% was applied, the large droplets broke down into bundles of smaller agglomerated droplets and, with additional strain, anisotropy was regained as protein "fibres" reappeared as fat droplets broke up and channels of fat droplets became aligned in the direction of strain. The fat droplets showed distinct striations on the droplet surface indicating that the protein fibres were exerting stress perpendicular to the stretch direction during strain. Furthermore, the protein strand striation marks also indicated that protein phase possesses a relatively higher stiffness than the fat phase. This is in line with other studies that have suggested values of Young's modulus for dairy protein fibrils in the order of a few GNm^{-2}(Pan & Zhong, 2015), whereas fat networks have values in the order of a few MNm^{-2} (Maleky, Campos & Marangoni, 2007).

The degree of heterogeneity and anisotropy increased in the structure as the agglomerated droplets broke down to smaller diameters. Cryo-SEM showed similar morphology to ESEM analysis (Fig. 5).

3.2 Image analysis

3.2.1 Quantification of fat channels

The size of the fat channels in the samples was estimated by measuring the mean area of each channel, Fig. 6. The mean size of fat channels in the melted state (419±267 μm^2) was considerably larger than that in the unmelted state (179±74 μm^2). This suggests that melting allowed the coalescence of droplets from more than one visible channel, since the channels have a 3-dimensional structure this is likely a result of a single channel, with out of plane branches, collapsing to a single droplet. The fat channel area started to decrease as strain

267 was applied, levelling out after a stretch by 580 % strain. The aspect ratio of the fat
268 channels also indicates this coalescence into larger, more spherical drops as shown in Fig. 7.

269 Fig. 8 shows that minimum neighbouring distance was the lowest (13.0±0.4 μm) in the
270 unmelted mozzarella. The stretcher cooker results in a uniform distribution of narrow fat
271 channels, which, on melting and reforming under strain form fewer, fat channels more widely
272 spaced.

273

274 3.2.2 Quantification of fat droplets

275 The mean fat droplet diameter is plotted in Fig. 9. The fat channels observed in CLSM (Fig.
276 3) were comprised of these fat droplets apparent in ESEM (Fig. 4). The milk fat globules
277 within the aggregates were the smallest in the unmelted state (4.2±0.2 μm) at 4 °C and the
278 fat globule sizes were representative of the range of sizes found in raw milk and cheese
279 (Walstra, Wouters & Geurts, 2006). Upon heating the mozzarella, the aggregated milk fat
280 globules coalesced into large fat droplets (120±33 μm), which was up to 27 times larger in
281 size. When strain was applied, the large spherical fat globules broke down into agglomerates
282 of smaller spherical globules, the diameter decreasing with increasing strain.

283

284 4.0 Discussion

285 4.1 Heat/strain induced transformations in anisotropy

286 The sample extraction system successfully arrested the deformation of fat during heating
287 and stretching whilst keeping intact the loading orientation and the strained microstructure.
288 Transformations in anisotropy were observed upon heating and stretching. Fig. 10 depicts
289 the overall change in structure after melting and distribution of forces in the protein matrix
290 when subjected to stretch.

Unmelted mozzarella possessed an anisotropic structure, with parallel aligned channels of partially solid milk fat globule aggregates between protein strands. This anisotropic structure disappears on melting at 70 °C, where the mozzarella completely lost its characteristic *pasta-filata* structure, and resembled a homogeneous curd structure, with isolated large fat droplets (Fig. 5b). During heating, the protein matrix relaxed and the partially solid fat became fully liquefied (Gunasekaran & Ak, 2003). Once sufficiently liquefied, the interfacial tension dominates and causes aggregated milk fat globules to form fewer larger droplets.

The origin of the large fat globules is the coalescence of fat droplets from within channels that branch outside the plane of view of the CLSM technique. Formation of large fat droplets in melted mozzarella is crucial in order to facilitate a longer stretch. These large fat droplets act as “shock absorbers” when tensile stress is applied to the melted pool of mozzarella; enabling it to stretch. Fat droplets do not adhere to the protein matrix but are contained in slightly larger domains, predominately made of water (Kinstedt & Guo, 1997; McMahon & Oberg, 1999). These fat filled water domains deform with the stretching protein matrix. In the short stretching time little viscous dissipation is expected and the local strain rate resembles the bulk strain rate. Nonetheless, the stress transmitted to the fat droplet via the elongating water domain will not deform the fat droplet. The elongational stress transmitted across the water phase (η ~ 1 $mNm^{-2}.s$) is the product of the viscosity and the local strain rate and of the order of 10^{-2} Nm^{-2}. This stress is insignificant compared to the smallest plausible Laplace pressure of 10^{3} Nm^{-2} that assumes r ~ 100 μm and a conservatively low interfacial tension of 10 mN/m. Thus the deformation arises from the compression stress generated perpendicular to the stretch direction and is carried by the protein matrix, as illustrated schematically in Fig. 10. These compressive stresses facilitate deformation of fat while the elongated serum channel restricts its shape. After sufficient droplet deformation the Laplace pressure favours the formation of multiple smaller droplets.

Smaller droplets deform less under the same load, and thus droplet break up will stop once droplets reach a critical size where deformation resulting in break up is no longer achievable.

The sample extraction system developed for this study has successfully arrested transient deformations in fat structure in viscoelastic cheese under heat-strain conditions.. This has provided understanding of the mechanism of fat deformation and redistribution during stretching.

4.2 Evaluation of mozzarella structure using complementary microscopy techniques

The microstructure of mozzarella was explored using CLSM, ESEM and cryo-SEM. It is useful to consider the complementary information offered by each technique. Optical sectioning in CLSM permits image capture across a plane at different depths in the sample. Thus, most of the fat and protein are captured across the image plane and a representative sample for image analysis can be attained (Fig. 11a).

In ESEM, the topography of the observed surface is captured (Aguilera & Stanley, 1999). Therefore any fat buried underneath the protein matrix (indicated by the red circle in Fig. 11b) cannot be directly observed, until the sample is physically altered outside of the microscope. Nevertheless, ESEM offered the benefit of higher resolution when examining the milk fat globule microstructure of mozzarella. In the unmelted mozzarella, ESEM was able to reveal the morphology of individual milk fat globules in aggregates in unmelted mozzarella and clearly defined morphological outlines of the individual fat droplets within the fat channels in the stretched mozzarella (Fig. 4).

Cryo-SEM was also able to reveal the individual milk fat globules, the advantage of this technique is that fracture plane can bisect fat globules potentially revealing more about their interactions than ESEM. Cryo-SEM was able to reveal aggregates of the *para*-casein network that lie within the protein matrix (Fig. 5c). Sublimation of water in the fractured

sample surface exposed porosity within the protein matrix. The pores are highly likely to be the entrapped water surrounded by casein aggregates.

5.0 Conclusion

This significance of this study lies in the technique developed to sample melted and stretched cheese and analyse the structural components of relevance. In particular the *ex situ* sample extraction system for arresting coalescence and deformation of fat droplets as revealed that anisotropy was lost when mozzarella was fully melted and regained in the direction of applied strain when stretched.

Fat structuring is critical for developing the necessary functional properties in mozzarella. The interconnected fat channels allow the formation of large fat droplet fillers when heated which subsequently deform and facilitate stretch.

The advantage of using complementary microscopy techniques for microstructure characterisation was demonstrated by evaluating the mozzarella structure using CLSM, ESEM and cryo-SEM. CLSM, was ideal for quantifying fat channel dimensions whereas the EM techniques were well suited to examining individual fat globules and structuring in the protein matrix. Application of the sampling and structural analytics presented in this paper could lead to improved understanding of dynamic changes in cheese processing technologies. Such understanding can be used in manufacture and provide a guide for tailoring the cheese structure to produce desirable functional properties.

365 Acknowledgements

366 This work was funded by the Primary Growth Partnership (PGP) program, “Transforming the
367 Dairy Value Chain”, funded by Fonterra Co-operative group and the NZ Ministry for Primary
368 Industries (MPI). The authors would like to thank the Research Centre for Surface and
369 Materials Science (RCSMS) and the Biomedical Imaging Research Unit (BIRU) at the
370 University of Auckland, for access to equipment.

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441 # Figures

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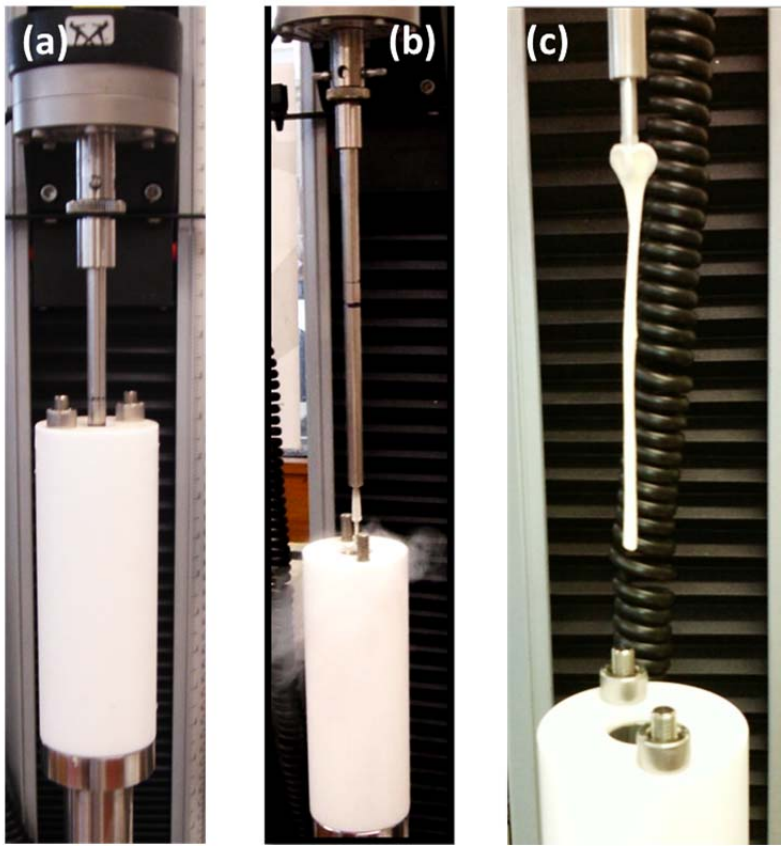

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444 Figure 1: Extraction of sample stretched to 100mm and quenched.

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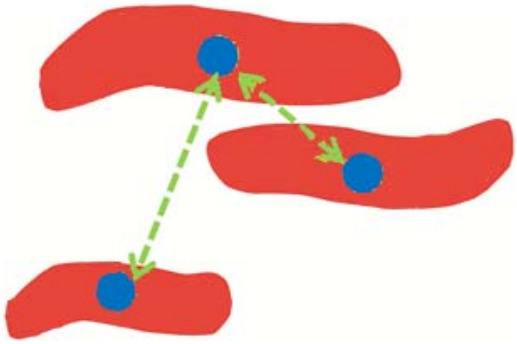
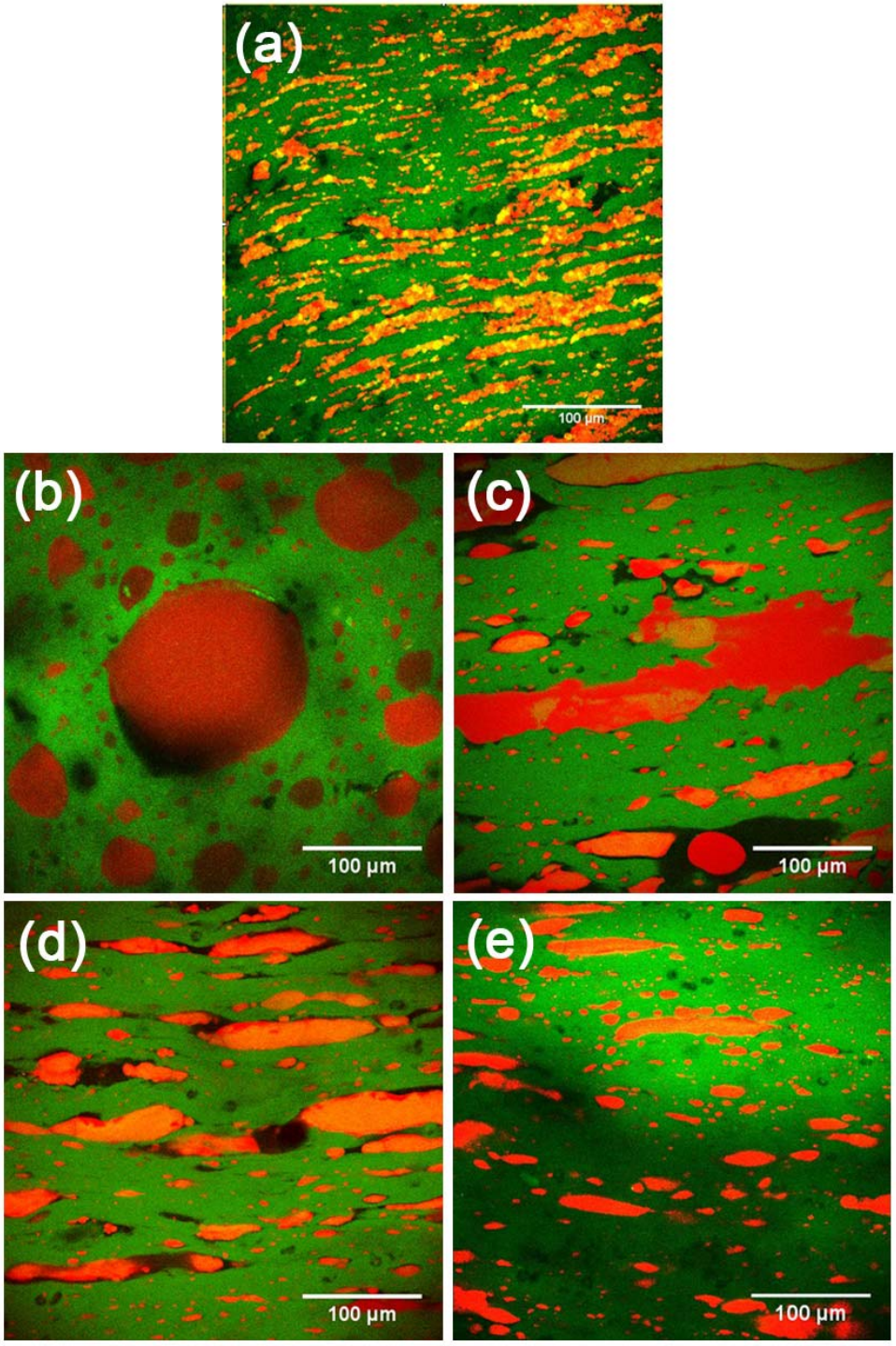
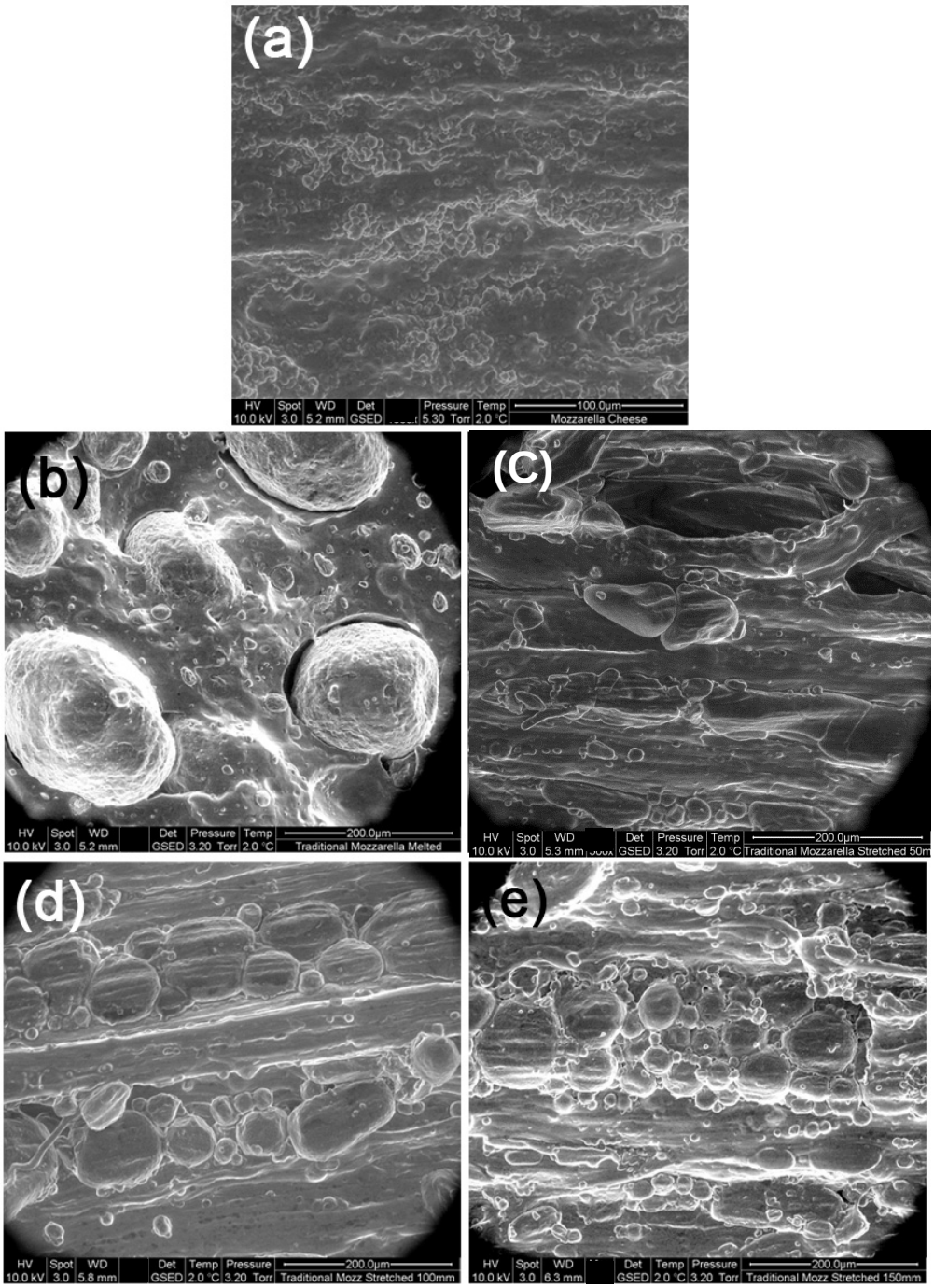


Figure 2: Schematic illustration demonstrating measurement of minimum distance to neighbouring fat channels. Distance (arrows) between the centres of gravity (circular dot) of each fat channel.



454

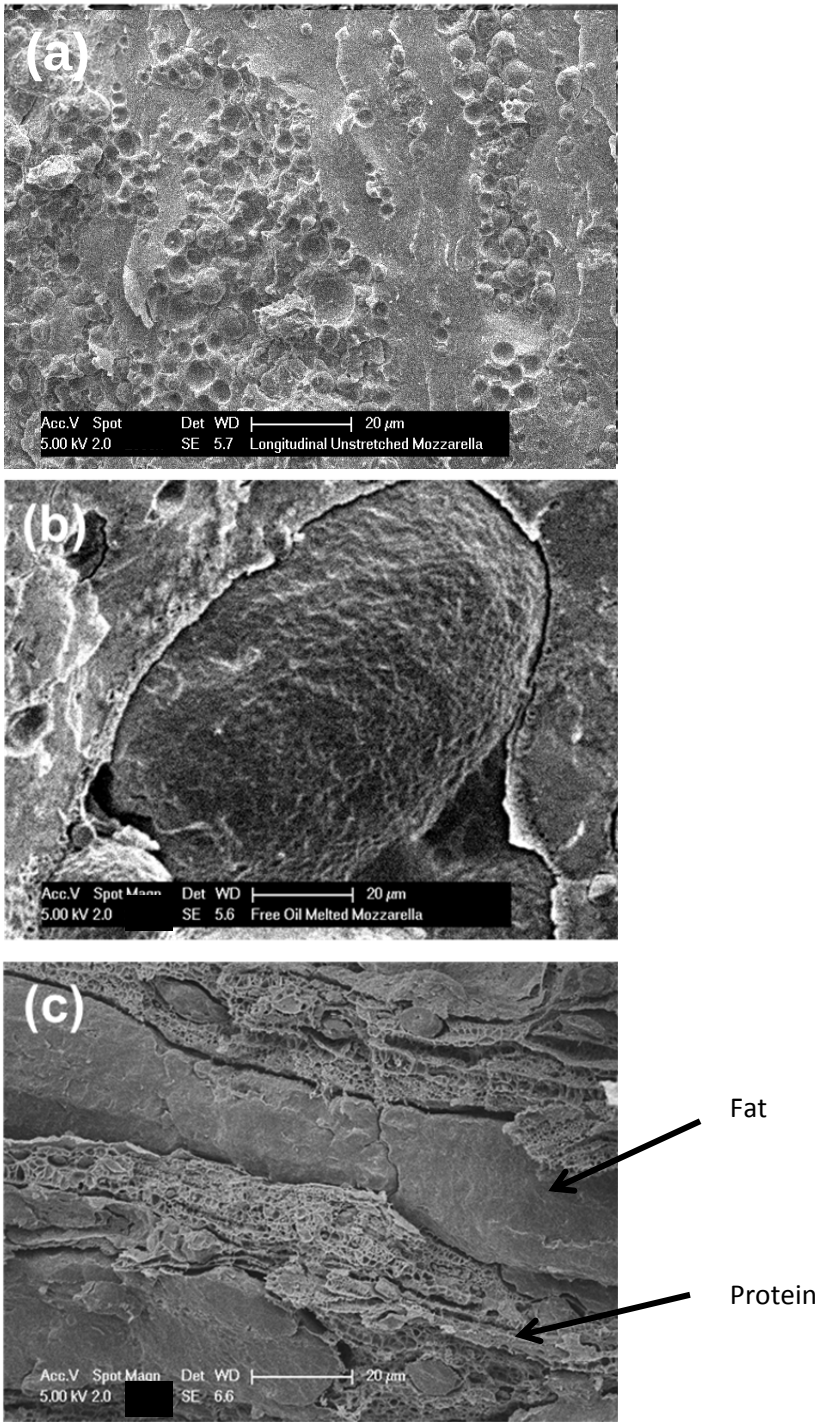
455 Figure 3: CLSM images of unmelted, melted and stretched mozzarella in the longitudinal
456 axis. (a) Unmelted mozzarella at 4°C; (b) Melted mozzarella at 70°C; (c) mozzarella
457 stretched by 280% strain ; (d) mozzarella stretched by 580% strain; (e) mozzarella stretched
458 by 900% strain. Red = Fat, Green = protein



459

460 Figure 4: Environmental scanning electron micrographs showing internal microstructure of
461 mozzarella (longitudinal axis) in different states: (a) unmelted mozzarella at 4°C; (b) Melted
462 mozzarella at 70°C; (c) mozzarella stretched by 280% strain; (d) mozzarella stretched by
463 580% strain; (e) mozzarella stretched by 900% strain.

464



465

466 Figure 5: Cryo scanning electron micrographs showing internal microstructure of mozzarella
467 (longitudinal axis) in different states: (a) unmelted mozzarella at 4°C; (b) Melted mozzarella
468 at 70°C; (c) mozzarella stretched by 280% strain.

469

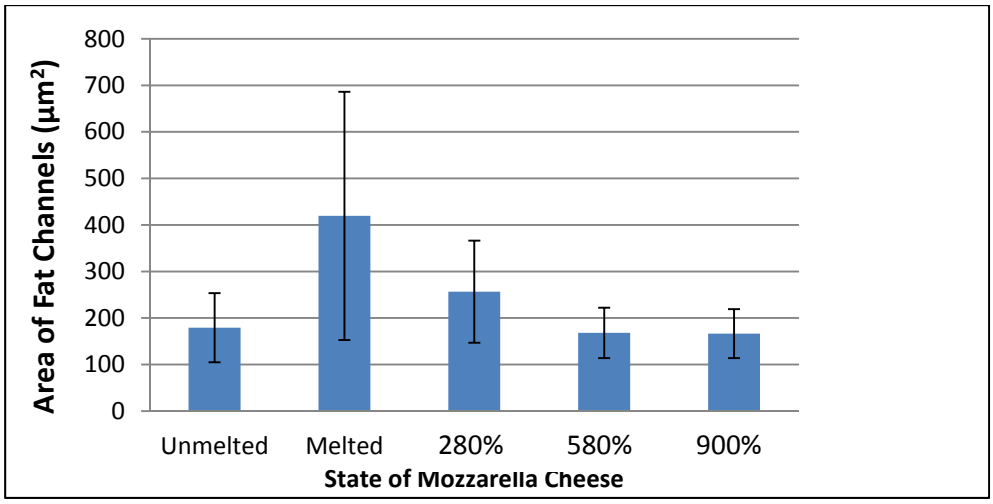
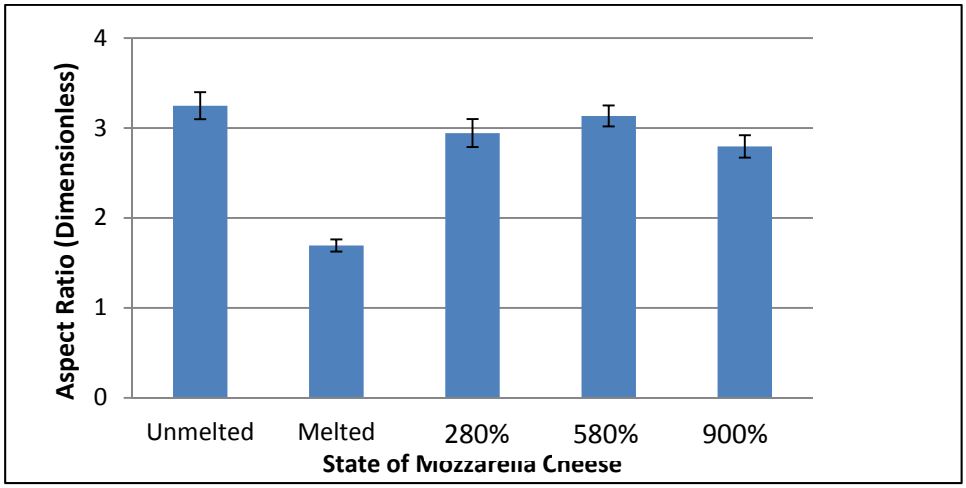


Figure 6: Change in individual size of fat channels in unmelted, melted and stretched mozzarella. Error bars are 95% confidence intervals.



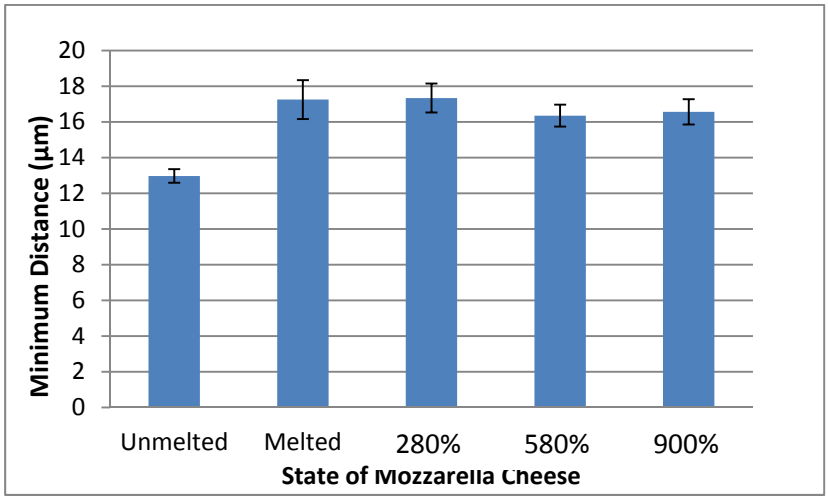
476

477 Figure 7: Change in aspect ratio of fat channels in unmelted, melted and stretched

478 mozzarella. Error bars are 95% confidence intervals.

479

480



481

482 Figure 8: Change in minimum distance between neighbouring fat channels in unmelted,

483 melted and stretched mozzarella. Error bars are 95% confidence intervals.

484

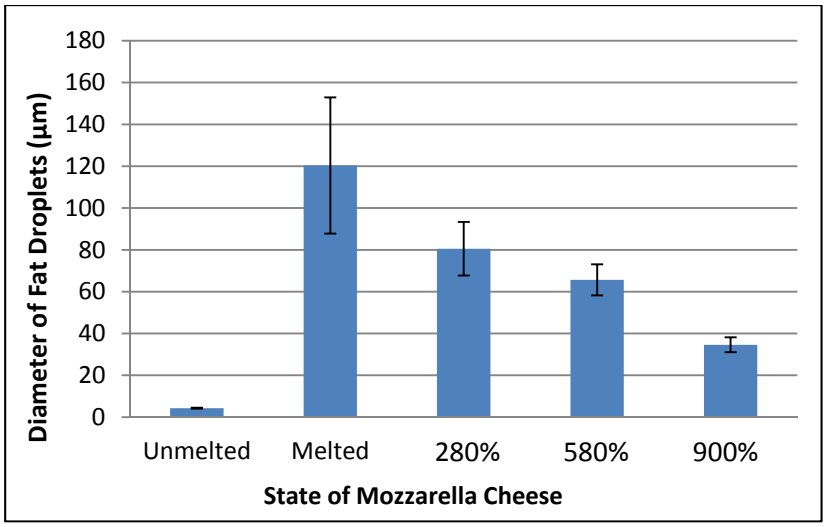


Figure 9: Change in diameter of individual fat globules and droplets within fat channels in unmelted, melted and stretched mozzarella. Error bars are 95% confidence intervals.

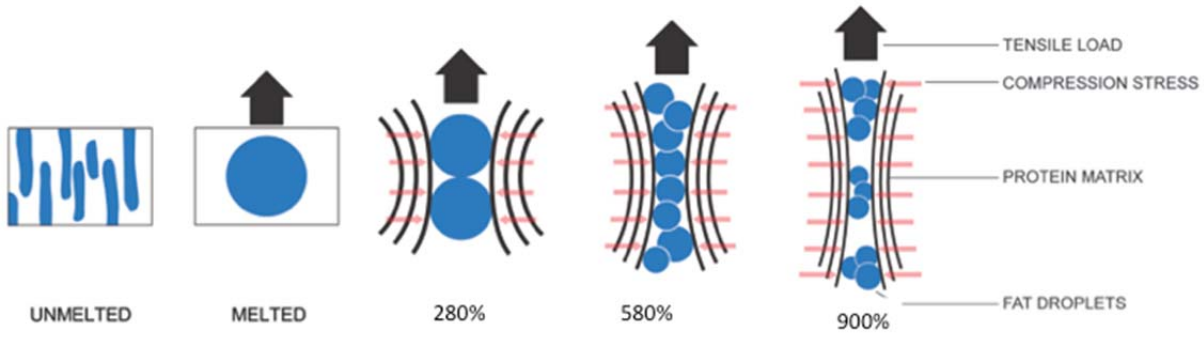


Figure 10: Diagram illustrating change in fat structure and distribution of forces in the unmelted, melted and mozzarella stretched by a strain of 280%, 580% and 900%.

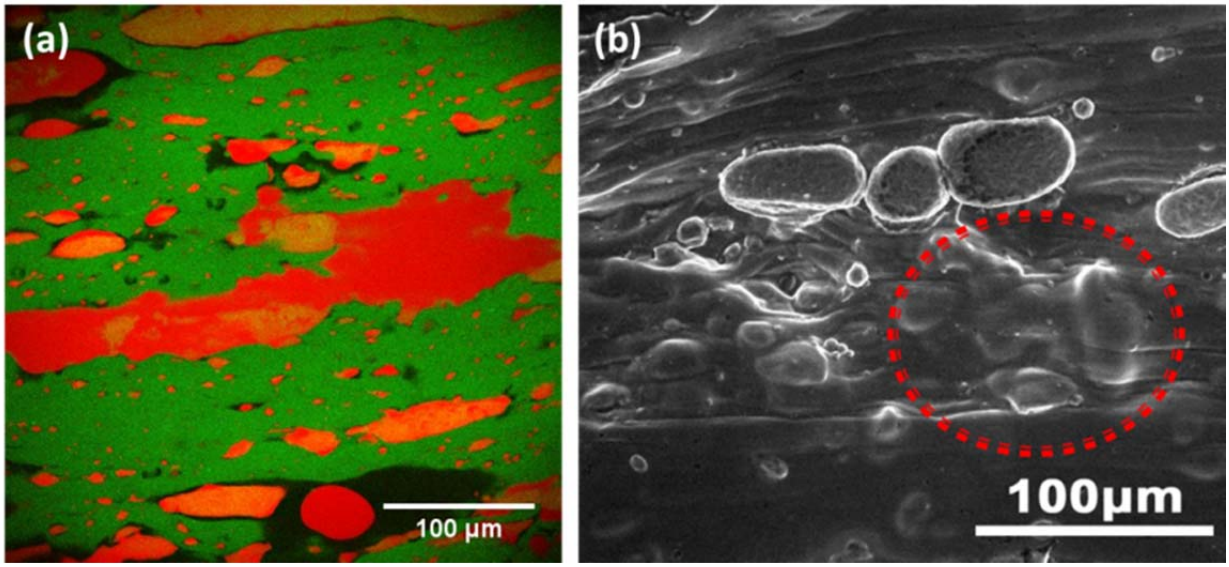


Figure 11: Comparison of image taken across a plane in CLSM and image taken from the surface in ESEM in mozzarella stretched by 280% strain.