In Situ Tensile Deformation of Plasticized zein films

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ABSTRACT

Deformation of a material is a dynamic process. Visualisation of this deformation can help to understand the local deformation and fracture behaviour. This paper describes the deformation of Zein (the prolamin protein from maize) films with different amount of plastcizers at micron scale under tension in real time by a confocal laser scanning microscope (CSLM).

INTRODUCTION

Biomaterials are widely used in food, pharmaceutical and medical industries. The strength of biomaterials is generally assessed by mechanical deformation test to predict their performance, especially in highly stressed conditions. Deformation of biomaterials like gels, biopolymer films and food materials, is a dynamic mechanical process. During deformation, load (or stress) is transferred through the material and eventually lead to fracture, failure or energy dissipation.

Microrheology is a new approach to look into the local deformation of biomaterials. It uses rheological techniques in combination with microscopy to study deformation and flow of materials in real time at micrometer length scale. For example Plucknett *et al.*[1] used CLSM with a tensile cell to explain the 'pseudo-yiedling' behaviour of gelatine-maltodextrin gel system as a result of debonding of the particle/matrix interface. Thus, real time measurement techniques can provide information on local deformation and microstructural changes during the deformation process.

This paper describes the local deformation of plasticized zein films in real time with a CLSM and a microtensile stage. Zein is the prolamin protein of maize. Zein films and coatings have received considerable attention as biomaterial coatings and films because of its unique properties[2]. These coatings and films can be both edible and biodegradable. They can be an attractive environment friendly alternative to plastic packaging made from non-renewable resources. Zein films are brittle. Plastcizers are added to produce extensible films. However, only a few edible coatings and films are used in commercial application because of their imitations in performance compared to synthetic packaging. Therefore visualisation of deformation process in real time with CLSM of plasticized zein films can provide the possibility to elucidate deformation mechanism and help to design films with better properties for end use.

EXPERIMENTAL

Materials

Zein protein was purchased from Sigma-Aldrich Chemie (Diesenhofen, Germany) and defatted sequentially with hexane[3]. Glycerol (Analar®, BDH lab supplies Poole, England) and polyethylene glycol 400 (PEG) (Merck, Honenbrunn, Germany) were the plasticizing agents. Fluospheres®

(Molecular probes, Leiden, Netherlends) were the particle tracking in the experiments. The fluospheres® are sulphate microspheres, 2 μm in diameter and yellow green fluorescent at 505/515 nm.

Methods

Preaparation of zein films

Zein films were prepared by casting method in petridishes as explained by Emmambux et al.[3] with the following modifications. After dissolution of the film forming ingredients, 2 μl of the fluosphere® was pipetted into 10 g of film forming solution. Then, aliquots (2 g) were poured into plastic petridishes (9 cm diameter), gently swirled, placed in a ventilated oven at 50 °C for 4 h to evaporate the solvent. After drying, the films were conditioned for at least 48 h at 22 °C and 50±5 % RH.

Real time measurement of tensile deformation with CLSM

Film strips of 2 mm wide were cut with a sharp scalpel, measured for thickness with a micrometer and placed in between the grips of a microtest material testing modules (Deben, Suffolk, UK) with a 2 N load cell and an initial grip separation of 10 mm. The microtest material testing module was then placed under an air objective of CLSM Leica TCS SP2 (leica Ltd, Heidelberg, Germany). The tensile deformation of the plasticized films was performed at a crosshead speed of 0.1 mm/min while observing with the CLSM. The light sources of the CLSM were Ar/ArKr laser using λ_{ex} = 488 nm to detect the fluospheres® from emitted signal at 500-540 nm. The HeNe light source at 594 nm was used in the reflection mode. An air objective with a magnification of 10x (Numerical aperture = 0.3) and a digital zoom x 2 were used. The scanning was bidirectional with a speed of 400 Hz and 2 scans were averaged out to give an image of 512 x 256 pixels every 1.015 sec as a real time series. The distance between some fluospheres® were calculated by image analysis software Image J 1.32j (Wayne Rasband, National Institutes of Health , USA, http://rsb.info.nih.gov/ij/)

RESULTS AND DISCUSSION

During tensile deformation, the stress of zein films without the addition of plastcicizer mix increased with an increase in strain (Figure 1). The average stress at break (σ_b), strain at break (ε_b) and Young's Modulus of elasticity (*E*, calculated from the linear region of the stress-strain graph) were respectively 19.4 MPa, 0.97 % and 2.1 GPa. The stress-strain curve of zein films has an elastic region, without showing a yield point and a transition to ductile or plastic region. This clearly demonstrates that zein films without glycerol and polyethylene glycol (PEG) were brittle. The stress-strain behaviour of zein films with a mixture of glycerol and PEG mix shows an elastic region, a yield point and ductile region (Figure 1). The yield stress (σ_y), σ_b, ε_b, *E* were respectively 14.9 MPa, 16 MPa, 2.5 % and 1.4 GPa for zein films with glycerol and PEG mix at 6.25 %. An increased in level of glycerol and PEG mix to 25 % decreased σ_y to 3.6 MPa, σ_b to 4.2 MPa, and *E* to 0.12 GPa, but increased the ε_b to 16.5 %. The stress-strain curve of zein films with glycerol and PEG mix showed a typical plasticization behaviour because there was an increase in strain and a decrease in stress and Young's modulus(ref).

Free standing zein films without addition of plasticizers are brittle, but a mixture of glycerol and PEG as plasticizers increased the strain and decrease the stress. Similar results were reported for zein films [4, 5], and kafirin films[6], a similar prolamin protein to zein[7]. Many theories have been proposed to explain the plasticizer action[8]. However the exact mechanism is not clear. Hydroxyl groups of plasticizers like glycerol and PEG can form numerous hydrogen bonds with the zein polypeptide chain. The plasticizer can interpose between polypeptide chains and thus can probably decrease polymer-polymer interaction to decrease film brittleness.

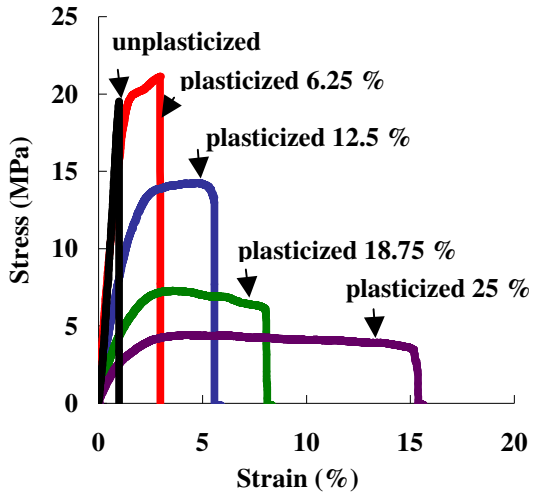

Figure 1. Effect glycerol and polyethylene glycol as plasticizer mix on the tensile properties of zein films.

Figure 2 shows the CLSM micrographs of zein films without and with the plastcicizers at different levels. The zein films without plasticizer have some imperfections in the films indicated by some black spots in the micrographs. These black spots could be some undissolved protein and/or micro-pores, which reflected the laser light differently in comparison to the zein protein matrix. Micro-pores as observed by scanning electron microscopy were also found in cast zein film[9]. Micro-pores or other imperfections in films can be sites for micro-crack formation and propagation that lead to fracture. The reflection mode of the laser HeNe at 594 nm was used instead of the emission mode because in preliminary experiments of this work, the imperfections as black spots were more visible in the reflection mode.

Zein films without plasticizer were brittle (Figure. 1). The CLSM micrographs (Figure 2) of these films from unstrained to 0.8 % strain did not show any evident microstructural changes at the scanned position. During tensile deformation, zein films with added plastcicizers at 6.25 and 12.5 % plasticizers formed micro-cracks.

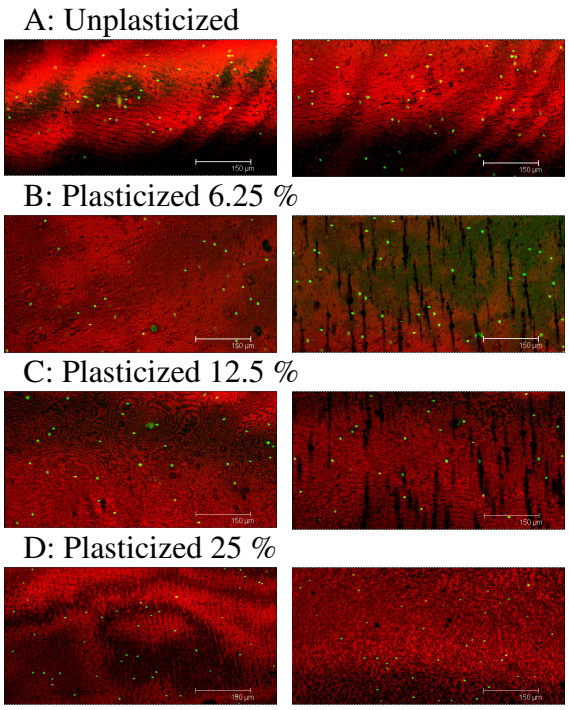

Figure 2. CLSM micrographs of unplasticised and plasticized zein films at different strain

The microstructural changes during tensile deformation in terms of micro-cracks formation and propagation were different for zein films added with different amount of plasticizers. At 3 % strain of zein films with 6.25 % plasticizers, the micro-cracks were thin and long normal to the tensile axis (Figure 2 B). At 4 % strain of deformed zein films plasticized at 12.5 %, the observed micro-cracks were thick and short normal to the tensile axis in comparison to the micro-cracks of zein films plasticized at 6.25 %. This shows that increasing plasticization can delay the formation and propagation of microcracks during tensile deformation. The effects of plasticization on the microstructural changes during tensile deformation of zein films can be further observed Figure 2 (D and E). The CLSM micrographs of zein films plasticized at 18.75 and 25 % show few cracks or no

cracks at different strain level during tensile deformation. Only some micro-pores probably formed from the black spots can be interpreted from the CLSM micrographs.

Figure 3 shows the relationship between the overall tensile strain (calculated by grip to grip separation) and local tensile strain (calculated by image analysis of fluospheres®). Statistical analysis showed a significant (P<0.001) linear response between the overall and local strain of all the treatments. However large standard deviation of local strain within a specific overall tensile strain suggests that tensile deformation of the film is not homogeneous.

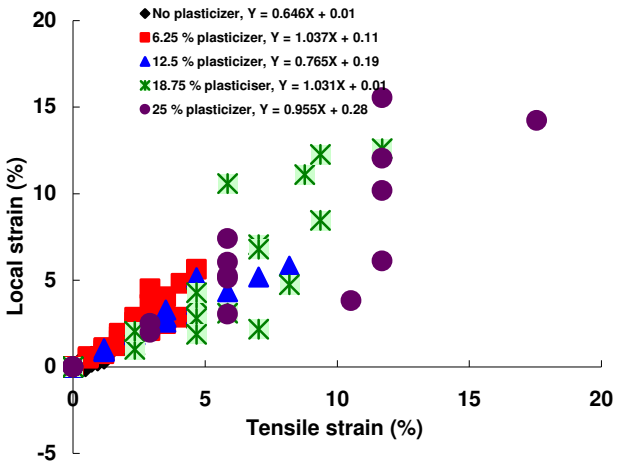

Figure3. Relationship between overall tensile (grip to grip separation) and local strain (fluospeheres® distances)

CONCLUSIONS

In situ visualisation by CLSM is a good technique to understand deformation mechanism. Visualisation of Tensile deformation shows that plasticization increases strain of zein films by decreasing micro-crack formation and propagation at micron scale.

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