ANNUAL TRANSACTIONS OF THE NORDIC RHEOLOGY SOCIETY, VOL. 19, 2011

# Rheology and microstructure of cereal protein melts

GÓMEZ- MARTÍNEZ, DIANA[1, 2, 3] AND STADING, MATS[1, 2]

[1]SIK- The Swedish Institute for Food and Biotechnology, Structure and Material Design, Göteborg, Sweden
[2]Chalmers University of Technology, Department of Materials and Manufacturing technology, Göteborg, Sweden
[3]Departamento de Ingeniería Química. Facultad de Ciencias Experimentales, Universidad de Huelva, Spain.

## ABSTRACT

Biomaterials from agricultural sources are becoming an interesting alternative, not only as biodegradable films, suitable for food packaging, but also as plastic materials which require improved mechanical properties.

The aim of this work was to compare rheological and microstructural properties of biomaterial melts of the cereal proteins pennisetin and zein.

## INTRODUCTION

Development of new materials to substitute synthetic polymers has become an important challenge. Cereal proteins are interesting as raw materials since are abundant and relatively inexpensive, and have the advantages of forming bioplastics which are hydrophobic, thermoplastic, and biodegradable.

Maize is globally the largest crop produced of which ~40% is produced in USA[1]. The indigenous African cereal pearl millet accounts for a small fraction of the world grain production, nevertheless, millets are extremely important for the sub-arid and sub-humid zones as staple crops.[2]

Cereal protein materials are fragile and a plasticizer is required. Plasticizers are low molecular weight molecules which modify the three-dimensional structure of proteins increasing their flexibility. The most commonly studied of plasticizers are water, glycerol, sorbitol, mannitol, diglycerol, triethylene glycol, polyvinyl alcohol, and polyethylene glycol and lipids.[3-8]

These plasticizers all plasticize the cereal proteins even if there are no known perfect plasticizers for zein,[8] and even less is known about pennisetin plasticization.

Molecular weight and its distribution (MWD) for biopolymer melts has been discussed sparsely in literature. Little is known about the structure of protein melts and the combination of MWD data from viscoelastic melt measurements and molecular information of the proteins can give new insight to the melt structure. In this work the model by Cocchini and Nobile [9,10] has been used to obtain molecular information for zein and pennisetin melts. The Cocchini and Nobile model predicts the Molecular Weight Distribution (MWD) from the mechanical spectrum ($G^*(\omega)$) of the melt.

The aim of this study was to determine the melt properties of pennisetin as compared to zein, to obtain molecular data of the proteins and to correlate it to melt microstructure and physical properties. These relationships can be used to predict melt behaviour and thermoforming. Thermoplastic cereal proteins have a wide range of applications, from injection

moulding of disposables and extruded foams to wheat-free bread.

## EXPERIMENTAL

Zein was obtained from Sigma-Aldrich (Schnelldorf, Germany). Pennisetin was provided by Dr Dlmani CSIR, South Africa. Prior to melt preparation, the proteins (zein and pennisetin) were defatted in n-hexane as decribed by Oom et al. [11] The protein content for zein and pennisetin were 95% and 99% (w/w) respectively. Polyethyleneglycol 400 (PEG 400), citric acid, glycerol and other chemicals used were of analytical reagent grade and obtained from Sigma-Aldrich.

Zein and pennisetin were hand-mixed with aqueous ethanol (70% v/v ethanol) at a ratio of solvent/protein=0.3 to form a melt. The plasticizer was then added under further mixing at room temperature to form a homogeneous melt. Two types of plasticizers, citric acid plus glycerol (2:1), and PEG 400 were used for plasticization at a ratio of plasticizer/protein=0.1.

Dynamic rheological properties of the melts were determined on a controlled strain rheometer ARES-G2 (TA Instruments, New Castle, USA), using parallel-plate geometry (20 mm diameter and 2 mm gap). The blend was placed between plates immediately after mixing, and the test was started after the melt had rested for 10 min. The exposed edges were greased with paraffin oil to reduce water loss from the sample. Strain sweep tests, at 10 rad/s, were carried out before hand on separate samples in order to identify the linear viscoelastic region of the melts. Measurements of each experimental point was performed at least in triplicate. Temperature scan tests, from 10 to 80 °C, were performed at constant frequency (10 rad/s) and strain (0.1) at a heating rate of 2 °C/min. Mechanical spectra were recorded from 100 to 0.1 rad/s in oscillatory shear, at a constant strain of 0.01, at constant temperatures of 10º, 20º, 30º, 40º, 50º and 60 ºC.

Characterization of the molecular properties of zein and pennisetin was performed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS PAGE) under reducing conditions as described by Da Silva and Taylor [24], but using a 4-12% acrylamide gradient gel prepared as described by Byaryhanga et al.[12].

The microstructure of different blends was analyzed with a transmission electron microscope (TEM). Small pieces of blends were chemically fixated in 2% glutaraldehyde in salt solutions as used preparation and postfixated with 1% $OsO_4$ in the same salt solution. Samples were dehydrated in grade ethanol series, followed by an infiltration in resins, LR White, and polymerized. Thin sections of ~70 nm were cut on a ultratome Reichert-Jung Ultracut E (Reichert-Jung, Germany) using a diamond knife and stained with uranyl acetate and lead citrate. The sections were examined in a transmission electron microscope, TEM, LEO 906 E, (LEO Electron Microscopy Ltd., Cambridge, England) at an acceleration voltage of 80 kV.

## RESULTS AND DISCUSSION

### *Microstructure of pennisetin and zein-based melts*

The microstructure of pennisetin and zein based melts was determined using transmission electron microscopy (TEM) as shown in Figure 1. The micrographs show that the plasticizer and protein phase separate in all samples into one phase rich in protein (dark) and one phase rich in plasticizer/solvent (bright). This effect was more pronounced in the pennisetin melts than in the zein based melts.

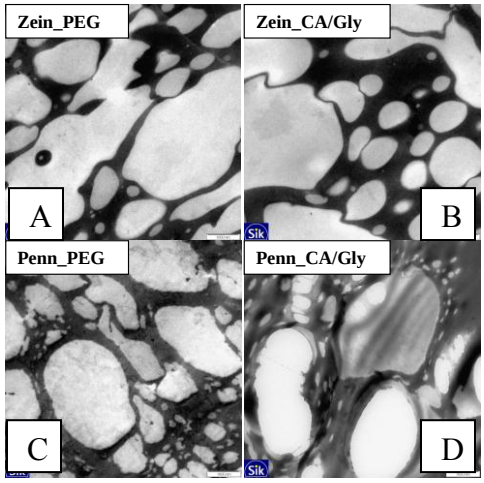


Figure 1. TEM micrographs of protein based melts (Scale bar is 1000): Zein melt with (A), glycerol/citric acid (B), Pennisetin melt with PEG (C) and glycerol/citric acid (D).

Thus, there were many spherical droplets of several sizes of plasticizers for zein based melts. Also, the small droplets of plasticizer were likely to be able to migrate in the melt and merge together creating the larger droplets. On the other hand, there were more large droplets of plasticizers of irregular shape for pennisetin based melts, which indicate a higher heterogeneity. In both zein melts the larger droplets are forming chain-like structure. It may originate from tip streaming caused by extensional flow during mixing.[13]

The pennisetin melt with glycerol/citric acid was the most phase separated sample of these four protein melts and has the largest droplets of plasticizer with the protein in thin lamellas between the plasticizer containing droplets. The high plasticizer/protein ratio is the main cause for the phase separation. The mixing could also influence the phase separation, since it involved only low mechanical energy thus involving less exposure of the protein to the plasticizer.

*Thermo-mechanical behaviour of pennisetin and zein-based melts*

Fig. 2 shows the change in G' (storage modulus) with temperature, at constant frequency, for the protein melts plasticized with PEG and citric acid/glycerol.

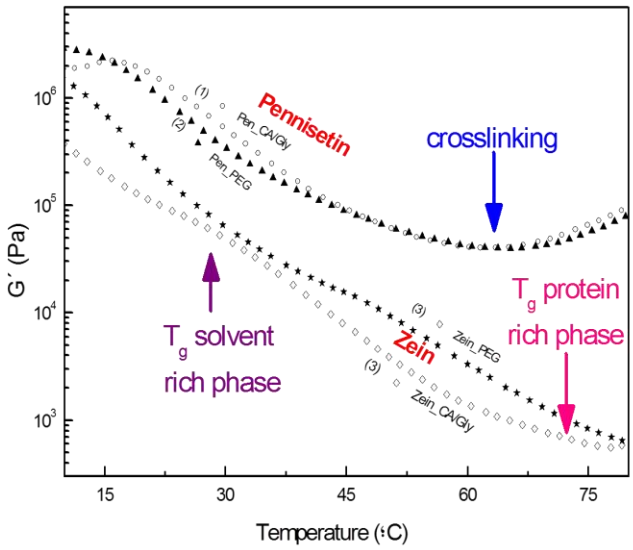


Figure 2. Temperature ramp test in oscillatory shear for zein and pennisetin based melts plasticized with PEG and citric acid/glycerol: Storage modulus (G')

Zein exhibited the expected, uniform decrease in G' with temperature typical of a melt, whereas pennisetin showed distinctly different regions as temperature was raised. Between 10° and 60°C, G' decreased, down to a plateau region, resulting from increased protein-protein interactions. Between 60° and 80°C, G' underwent an unexpected increase, which could be attributed to protein cross-linking reactions or to solvent evaporation. Crosslinking reactions in the pennisetin melts above 60°C is therefore the likely explanation of the increase in G'.

As observed in Fig. 2, the pennisetin melts obtained with both plasticizers displayed more structured systems with higher values of G'. Nevertheless, the temperature dependence of the storage

modulus was always similar for zein and pennisetin melts with both plasticizers.

SDS PAGE under reducing condition showed (Fig. 3) that the zein used in the melts contained a group of monomers at 19 and 22 kDa identified as $\alpha_1$ and $\alpha_2$, which are less rich in histidine, arginine, proline and methionine.[14]

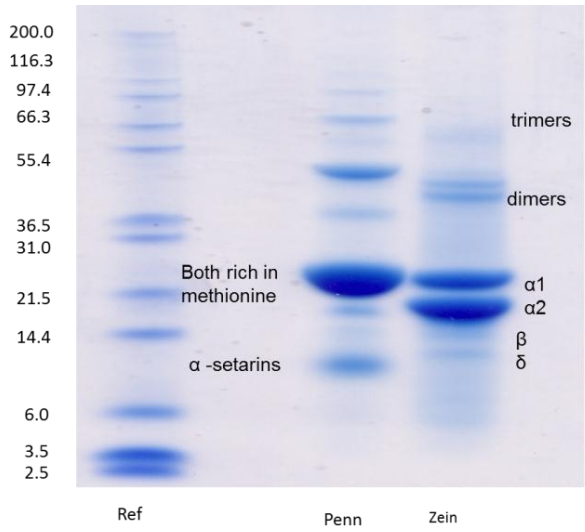


Figure 3. SDS-PAGE for pennisetin and zein pure, under reducing conditions. Lane 1: marker, lane 2: pennisetin, lane 3: zein (A), Zein based melts under reducing and non-reducing conditions.

Also, zein showed bands at 10 and 18 kDa, identified as β and δ, which are less abundant but rich in methionine.[15] In addition, three oligomers were found at 38, 49 and 60 kDa. Pennisetin exhibited two major bands at 19 and 22 kDa, which are rich in methionine and cysteine[16], the sulphur-containing amino acids. Also, pennisetin contained a band at 9 KDa, called α-setarins, which is relatively rich in methionine[17,18]. In this sense, the higher values of G' found for pennisetin melts due to protein cross-linking reactions (Fig. 2) could be associated to disulfide bridge breaking as these were not present to the same extent in zein.

*Rheological behaviour of pennisetin and zein -based melts*

Time-temperature superposition (tTS) was used to expand the time or frequency regime of the mechanical spectrum. Fig. 4 shows the resulting master curves after tTS, for for zein melts plasticized with PEG and citric acid/glycerol for mechanical spectra between 10º and 40 ºC, presented at a reference temperature of 10 ºC, for G' and G". As can be observed, the loss modulus in both samples is always slightly higher than storage modulus, in this temperature range, at high and intermediate frequency, but at low frequency the difference between G" and G' increases as expected for an amorphous melt.

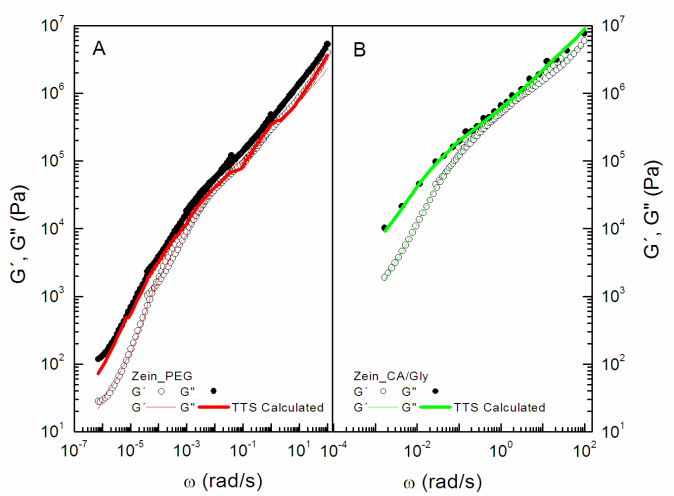


Figure 4. Master curve of the frequency dependence of the linear viscoelasticity functions for zein based melts plasticized with PEG and citric acid/glycerol

For the zein melt plasticized with citric acid/glycerol (Fig. 4B) there is a hook at low frequency which comes from the mechanical spectra obtained at 40, 50 and 60ºC. This mechanical spectrum was not included in the calculation of the master curve.

*Molecular Weight Distribution and microstructure*

The numerical prediction of Molecular Weight Distribution data using the model proposed by Cocchini and Nobile[10] for zein based melts is showed in Fig. 5. The plateau modulus was calculated from the relaxation time spectrum for each sample and the model was calibrated using the molecular weight determined by SDS-PAGE (Fig.3) in the absence of other calibration data. The same calibration constant *k* was used in both cases. This means that the model does not give absolute molecular weight data, but the effect of the two plasticizer systems can be compared.

A distinct influence of the plasticizer system can be observed on the distribution. The melts plasticized with PEG yield a broader distribution with a shoulder at ten times the main peak.

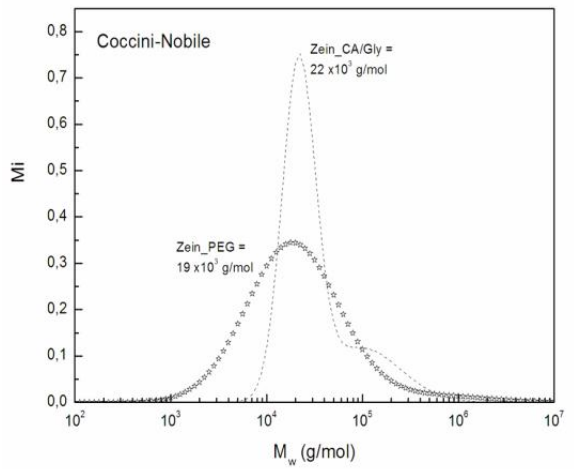


Figure 5. The numerical prediction of Molecular Weight Distribution for zein based melts plasticized with PEG and citric acid/glycerol.

The melts plasticized with glycerol/citric acid has a narrower distribution with a distinct peak at ten times the main peak. This indicates that glycerol/citric acid has a more pronounced effect on the protein conformation. We can speculate that the reducing effect of citric acid is the main factor and that this is also the effect of the different melt structure observed in the microstructure (Fig. 1).

## CONCLUSION

The cereals proteins zein and pennisetin in presence of plasticizers can form viscoelastic melts. Both protein melts form a phase separated microstructure at the present ratio of plasticizer solvent/protein of 0.5. The different phases have clearly different glass transition temperature indicating phases rich in protein and plasticizer respectively. The pennisetin melts formed crosslinkes at temperatures above 60ºC, which could be related to the high content of cysteine and methionine, as compared to zein. A general conclusion is that since protein based bioplastics need plasticization in all practical applications, the choice of plasticizer will significantly affect the behaviour of the melt. Not only may it cause a phase separated structure, it may also induce polymer-polymer interactions.

## ACKNOWLEDGMENTS

The Ministerio de Educación y Ciencia, Spain, The Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (FORMAS), the Swedish Board for Agriculture and The Swedish Innovation Agency (VINNOVA) are gratefully acknowledged for financial support. The skilled assistance by Daniel Johansson is gratefully acknowledged as well as fruitful discussions with prof. Franco Cocchini.

## REFERENCES

1. Wirsenius, S.; Azar; Berndes, G.; Global bioenergy potentials: A new approach using a model-based assessment of biomass flows and land demand in the food and agricultural sector 2030. 2nd World Biomass Conference: Biomass for Energy, Industry and Climate Protection. Eds. Van Swaaij, W.P.M., Fjällström, P.,

Helm, P. and Grassi, A., Florence, ETA-Florence. 1, 2004; pp. 471-474.
2. Belton, P. S.; Taylor, J.R.N.; Pseudocereals and Less Common Cereals. Grain Properties and Utilization Potential., Eds. Springer, Berlin; 2002.
3. Ghanbarzadeh, B.; Oromiehi, A.R. J. Food Eng.2002, 90: 517-524
4. Suyatma, N.; Tighzert, L.; Copinet, A. J. Agric. Food Chem. 2005, 53: 3950-3957.
5. Gao, C.; Stading, M.; Wellner, N.; Parker, M.L.; Noel, T. R.; Mills, E.N.C.; Belton, P.S. J. Agric. Food Chem. 2006, 54: 4611-4616.
6. Gillgren, T.; Barker, S.A.; Belton, P.S.; Georget, D.M.R.; Stading, M. Biomacromol. 2009, 10: 1135-1139.
7. (Shukla, R.; Cheryan, M. Ind. Crops Products 2001, 13: 171-192.
8. Lawton, J.W. Cereal Chem. 2002, 79: 1-18
9. Cocchini, F.; Nobile, MR. Rheol. Acta 2003, 42: 232-242.
10. Cocchini, F.; Nobile, MR. Rheol Acta 2001, 40: 111-119.
11. Oom, A.; Pettersson, A.;Taylor, J.; Stading, M.; J. Cereal Sci. 2008, 47: 109-116
12. Da silva, L.S.; Taylor, J.R.N.; Cereal Chem. 2004, 81, 322-327
13. Byaruhanga, Y.B.; Erasmus, C.; Taylor, J.R.N. Cereal Chem. 2005, 82, 565-573.
14. (26) Eggleton, C; Tsai,, T.M.; Stebe, K. Phys. Rev. Lett.. 2001, 87.
15. Coleman, C.E.; Larkins, B.A.; The prolamins of maize. In: Shewry PR, Casey R. Eds.; Seed Proteins. Kluwar Academic Publishers, Dordrecht, 1999; pp.109-139.
16. Swarup, S.; Timmermans, M.C.; Chaudhuri, S.; Messing, J. Plant J 1995, 8: 359-368
17. Marcellino, LH; Bloch, C.; Gander, E.S. Protein and Peptide Letters 2002, 9: 237-244
18. Naren, A.P.; Virupaksha, T.K.; Cereal Chem. 1990a, 67: 32-34.
19. Naren, A.P.; Virupaksha, T.K.; Cereal Chem. 1990b, 67: 136-138.