Material Properties of Zein, Kafirin and Avenin Films

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ABSTRACT

Films from the cereal proteins zein (from maize), kafirin (from sorghum) and avenin (from oats) were manufactured and analyzed with respect to glass transition temperature, stress at break and strain at break.

INTRODUCTION

A great deal of the materials used today are based on petroleum products. Since oil is not a renewable resource the use of this resource runs counter the values of sustainability. A possible alternative to oil as the source for materials is proteins from cereals. Prolamins are cereal storage proteins, quite hydrophobic, and can be extracted from by-products, such as those from biofuel production and brewing.

The prolamin of maize, sorghum and oats are zein, kafirin and avenin respectively. All of these three cereals have their own characteristics which make them interesting. Maize is one of the two largest crops in the world. Sorghum is staple crop in southern Africa and resistant to drought. Avenin is mostly grown in the northern hemisphere and is more resistant to rain than other crops. Just like zein, kafirin is considered to be safe for those with celiac disease, while some, however very few, react on avenin.

In this study films from zein, kafirin and avenin have been manufactured and analyzed with respect to some material properties of interest from a mechanical of view. The films were plasticized by a mixture of polyethylene glycol, glycerol and lactic acid (zein and kafirin) or glycerol (avenin) to reduce brittleness. The different choice of plasticizer depends on differences in hydrophilicity; avenin is more hydrophilic and a more hydrophilic plasticizer is therefore needed for compatibility. Glass transition temperature (T_g), stress at break (σ_b), strain at break (ε_b) were measured at three different levels of plasticizer content.

MATERIALS AND METHODS

Protein Preparations

Commercial zein (Z3625; Sigma-Aldrich, Schnelldorf, Germany) was defatted as described by Oom et al.[1]. Kafirin was kindly provided by Ms S. Buchner of the Council for Scientific and Industrial Research, South Africa. Kafirin was extracted from decorticated, condensed tannin-free red sorghum grain using aqueous ethanol plus sodium metabisulphite at an elevated temperature, by means of a procedure similar to the industrial process described for zein by Shukla and Cheryan[2]. The kafirin was defatted as described by Oom et al.[1]. Avenin was extracted from decorticated, milled Swedish oats using aqueous ethanol plus sodium metabisulphite at an elevated temperature, also by means of a procedure similar to the industrial process described for zein by Shukla and Cheryan[2]. However, the protein concentrate was refrigerated at 2.5°C before acidification to pH 5.0 with 1 M hydrochloric acid and was subsequently refrigerated again before filtration (using a 2-µm filter cloth). The filtrate was centrifuged at 3020 *g* for 10 min, after which the sediment was collected and air dried at 18–25°C along with the filter cake recovered after filtration. Avenin was defatted as described by Oom et al. [1]. To reduce contamination from beta-glucans, the avenin was subject to the following additional purification procedure. Avenin and pH 6.5 phosphate buffer were mixed to form a slurry. Lichenase (beta-glucan degrading enzyme, see e.g. Roubroeks[3], E-LICHN; Megazyme, Wicklow, Ireland) was added (95 U enzyme/g protein). The slurry was vigorously stirred for 20 h, whereupon it was centrifuged at 2080 *g* for 1 h. The pellets were collected and mixed with aqueous ethanol (45% w/w); the mixture was then stirred and held at 70°C for 1 h, after which it was filtered under vacuum. The supernatant was collected and reduced by volume through rotary evaporation under vacuum. When a more easily manageable amount of mixture was obtained, it was freeze dried until the weight reduction was negligible. The protein content of zein was 92% (w/w), kafirin 82%, and avenin 87% respectively, determined using the Dumas method and multiplying the nitrogen content by 6.25 for zein and 6.67 [4] for avenin.

Film Casting

Kafirin and zein films were cast by mixing 1.76 g of kafirin (82% purity, w/w) or 1.56 g of zein (92% purity, w/w) with 9.0 g of 70% (w/w) ethanol, plasticizer (1:1:1 w/w/w PEG:glycerol:LA mixture), and a magnetic stirrer in 100-mL Erlenmeyer flasks, which were sealed with aluminium foil. Avenin films were cast by mixing 1.60 g of avenin (87% purity, w/w) with 10.0 g of 45% (w/w) ethanol, glycerol, and a magnetic stirrer in a 50-mL Erlenmeyer flask, which was sealed with aluminium foil. Total plasticizer levels were 20%, 30%, and 40% (w/w) for kafirin and zein, but 23%, 34%, and 44% (w/w) for avenin (for simplicity, the avenin result bars are sorted according to 20%, 30%, and 40% plasticizer contents). The total masses of the flasks were weighed. The kafirin and zein mixtures were stirred and heated at 70°C for 10 min; the avenin mixture was stirred for 15 min at the same temperature. During heating, a frozen cooling block was put on top of the sealing foil to reduce the evaporation of the solvent. After the heating, the flasks were reweighed and absolute ethanol was added until the flasks had returned to their original weights. After briefly stirring, 4 g of kafirin solution, 4 g of zein solution, and 5 g of avenin solution, were put into three preheated 9-cm-diameter polystyrene Petri dishes. The dishes were placed, level, in a 50°C oven (avenin with $2.6\cdot10^{-4}$ m^3/s of forced draft, kafirin and zein with no forced draft) overnight. The typical film thickness for all materials was 100 µm and was determined individually using a Mitutoyo IDC-112CD micrometer (Mitutoyo Corporation, Kawasaki, Japan). The cast films were stored in a climate chamber under constant 23°C and 50% RH conditions.

Thermomechanical Analysis

Thermomechanical properties were measured using DMA analysis using a Rheometrics RSAII rheometer (Rheometrics Scientific, Piscataway, NJ, USA). All samples were conditioned for at least 48 h at 23°C and 50% RH before testing. Film strips were 4 mm wide and the original gauge length was approximately 22.5 mm. The testing temperature range was –40°C to 100°C with a ramping speed of 1°C/min. T_g was determined as the temperature of the intersection of the E' linear fits, which indicated the drop of E'. Between three and seven samples were analysed for each plasticizer content.

Mechanical Properties Analysis

Mechanical properties were measured using an Instron 5542 single-column universal materials testing machine (Instron, Norwood, MA, USA) in accordance with the ASTM D882-91 standard, using 4 mm sample widths, a crosshead speed of 0.24 mm/s, and an original gauge length of 24–40 mm; between 4 and 13 samples were evaluated for each plasticizer content. Rubber padded grips were used. All samples were conditioned at 23°C and 50% RH for at least 48 h before testing. The instrument recorded force and strain. Young's modulus, σ_b, and ε_b were calculated from the original gauge length and cross-sectional area.

RESULTS AND DISCUSSION

Thermomechanical Analysis

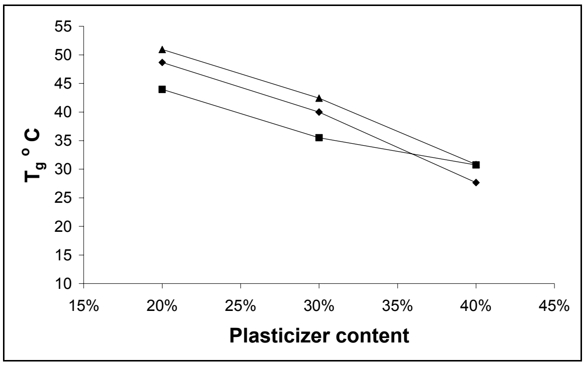

Figure 1. T_g of zein (triangles), kafirin (diamonds) and avenin (boxes) films with different plasticizer contents. Data from [5].

The T_g values of both zein, kafirin and avenin decreased with increased plasticizer content. Results, presented in figure 1, show that all three of the prolamin materials display equivalent T_g values at corresponding plasticizer amounts. Kafirin results match those reported by Emmambux et al.[6] It has been stated that avenin resembles gluten on a molecular level, which has been reported to have a T_g value of −5°C (plasticized by 40% glycerol, w/w)[7]. Avenin obviously did have higher T_g values, as did the other prolamins.

Mechanical Properties Analysis

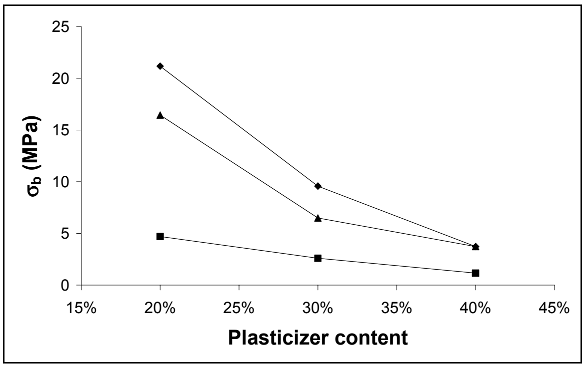

Figure 2. Stress at break of zein (triangles), kafirin (diamonds) and avenin (boxes) films with different plasticizer contents. Data from [5].

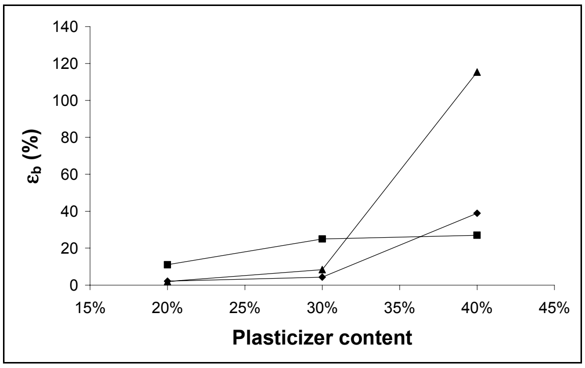

Figure 3. Strain at break of zein (triangles), kafirin (diamonds) and avenin (boxes) films with different plasticizer contents. Data from [5].

The mechanical properties σ_b and ε_b are shown in figure 2 and 3 respectively. Increased plasticizer levels decreased σ_b but increased ε_b. This was in line with the measured T_g values. Avenin is much weaker than the other two materials, and does also display higher extensibility at lower plasticizer contents. At higher plasticizer contents zein was the far most extensible material. As avenin is supposed to be molecularly similar to gluten[8], which has shown significant extensibility[9], it is noteworthy that avenin does not seem to share this mechanical quality.

CONCLUSION

It stands clear that these prolamins have equivalent T_g when containing the same amount of plasticizer, although the plasticizers differ. Besides avenin's supposed similarity to gluten, the results match those available in the literature. If looking for an alternative to today's petroleum based packaging materials, it is interesting to compare the qualities of these biopolymeric materials to those of synthetic polymers. It can generally be said that the strength of biopolymeric materials is comparable to that of synthetic polymers, but the extensibility is far less among these and other biopolymers.

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REFERENCES

1. Oom, A., Pettersson, A., Taylor, J. R. N., et al. (2008), "Rheological Properties of Kafirin and Zein Prolamins", *J. Cereal Sci.*, **47**, 109.

2. Shukla, R. and Cheryan, M. (2001), "Zein: The Industrial Protein from Corn", *Ind. Crop. Prod.*, **13**, 171.

3. Roubroeks, J. P., Mastromauro, D. I., Andersson, R., et al. (2000), "Molecular Weight, Structure, and Shape of Oat (1->3),(1->4)-ß-D-Glucan Fractions Obtained by Enzymatic Degradation with Lichenase", *Biomacromolecules*, **1**, 584.

4. Kim, S. I., Charbonnier, L., and Mosse, J. (1978), "Heterogeneity of Avenin, the Oat Prolamin. Fractionation, Molecular Weight and Amino Acid Composition", *Biochim. Biophys. Acta*, **537**, 22.

5. Gillgren, T. and Stading, M. (2008), "Mechanical and Barrier Properties of Avenin, Kafirin, and Zein Films", *Food Biophys.*, **IN PRESS**

6. Emmambux, M. N., Stading, M., and Taylor, J. R. N. (2004), "Sorghum Kafirin Film Property Modification with Hydrolysable and Condensed Tannin", *J. Cereal Sci.*, **40**, 127.

7. Cherian, G., Gennadios, A., Weller, C., et al. (1995), "Termomechanical Behaviour of Wheat Gluten Films: Effect of Sucrose, Glycerin, and Sorbitol", *Cereal Chem.*, **72**, 1.

8. Chesnut, R. S., Shotwell, M. A., Boyer, S. K., et al. (1989), "Analysis of Avenin Proteins and the Expression of Their Mrnas in Developing Oat Seeds", *Plant Cell*, **1**, 913.

9. Gennadios, A., Weller, C. L., and Testin, R. F. (1993), "Property Modification of Edible Wheat, Gluten-Based Films", *Transactions of the ASAE*, **36**, 465.