

SOME POTENTIAL APPLICATIONS FOR BREWERS SPENT GRAINS THE PROTEIN-RICH CO-PRODUCT, FROM SORGHUM LAGER BEER BREWING

Dr Janet Taylor, Prof John R N Taylor

Department of Food Science, University of Pretoria, Pretoria 0002, South Africa

Janet.taylor@up.ac.za

ABSTRACT

Brewing of lager beer from sorghum is becoming more widespread throughout Africa. It generates large quantities of the co-product, brewers spent grain, which is rich in protein but high in moisture. This material is of low value and is currently sold for animal feed. To improve the cost effectiveness of sorghum lager beer brewing, methods of adding value to co-products such as brewers spent grain are needed. Kafirin, the prolamin protein of sorghum is not produced commercially, despite its interesting properties and its uniquely high hydrophobicity. At the laboratory scale, aqueous ethanol at elevated temperatures is used to extract the kafirin from sorghum grain. Alternative and more efficient, ethanol-free methods of kafirin extraction have been sought. High yields of kafirin can be obtained with a glacial acetic acid extraction following a sulphur dioxide pre-soak. The application of this extraction technique to brewers' spent grains will require optimisation. The kafirin extracted could then be used to make bio-plastic materials such as films, which are biodegradable and made from a sustainable, renewable resource. These films may have potential at least in part, as replacements for non-biodegradable, synthetic packaging. Kafirin bio-plastic films are found to be stronger and as extensible as those made from commercial zein, maize prolamin protein, which is commercially available. Another application for kafirin is the preparation of microparticles (microspheres). These are small, (1-10 μ m), mainly spherical particles, with internal holes or vacuoles, resulting in a very large surface area. Kafirin microparticles can be used to make very thin (<15 μ m) bio-plastic films. Kafirin microparticle films are relatively strong but not extensible, with better water barrier properties and lower protein digestibility than conventionally cast kafirin films. An alternative application for kafirin microparticles is as encapsulating agents. Antioxidants, catechin and sorghum condensed tannins have been encapsulated within kafirin microparticles and the antioxidant release profiles, under simulated gastric conditions, were investigated. Over a period of four hours, catechin and sorghum condensed tannin encapsulated kafirin microparticles released approximately 70% and 50% respectively, total antioxidant activity. Thus, encapsulation of catechin and sorghum condensed tannins in microparticles has potential as an effective way to deliver dietary antioxidants and enhance potential health benefits, by controlled release of antioxidant activity within the stomach and gastrointestinal tract. Further, manipulation of the preparation conditions of kafirin microparticles results in microparticles with differing characteristics, which may have additional applications in the food, biomedical and pharmaceutical industries.

Keywords : waste, sorghum, kafirin, bioplastic, microparticles

INTRODUCTION

Brewing of lager beer from sorghum is becoming more widespread throughout Africa. It generates large quantities of the co-product, brewers spent grain, which is rich in protein but high in moisture. This material is considered of low value and is currently sold for animal feed. To improve the cost effectiveness of sorghum lager beer brewing, methods of adding value to co-products such as brewers spent grain are needed. Due to volatile oil prices and increasing concern over environmental pollution caused by plastic packaging material, there is renewed interest in plant biopolymers, not only to supply energy (bioethanol and biodiesel) but also to produce bioplastics. Kafirin, the sorghum grain prolamin storage protein, is potentially an excellent choice for making edible bioplastic films and coatings for food, feed and pharmaceutical applications. It is one of the most hydrophobic plant proteins and with wet-heating it cross-links strongly by disulphide bonding, reducing its digestibility⁶. It is also not known to be allergenic⁴. Despite its interesting properties and potential in these applications, kafirin, is not produced commercially.

Extraction of kafirin

Effective extraction of kafirin was believed to require the use of aqueous ethanol at elevated temperature or aqueous tert-butanol (a higher alcohol) at ambient temperature, or extraction of total grain proteins in basic buffer containing sodium dodecyl sulphate followed by precipitation of the non-kafirin proteins with aqueous tert-butanol^{9,10,13}. There are problems with these processes for industrial and food use. Ethanol is flammable, needs a government license for its use and is unacceptable by some religious communities. Tert-butanol is classified as not completely harmless in the Swiss poison class classification and so is not edible¹¹. Various food-compatible solvents for kafirin have been identified¹². Glacial acetic acid was found to be a good primary solvent for kafirin at ambient temperature and 55% aqueous isopropanol a good binary solvent.

High yields of kafirin can be obtained from whole grain sorghum flour with a glacial acetic acid extraction following a sulphur dioxide pre-soak (**Table I**). If the grain was pre-soaked 16hr in 0.5-1.0% metabisulphite, the recovery of kafirin was between 80 and 90% with a single extraction based on the estimate of sorghum grain kafirin content of 68-73%⁹. Steeping of maize in sulfur dioxide is known to cleave disulphide bonds and so disrupt intermolecular aggregates in native zein¹. It is suggested that pre-soaking the sorghum grain in sulphur dioxide prior to extraction with glacial acetic acid would act similarly on the sorghum matrix proteins, causing their partial disruption.

The application of this extraction technique to brewers' spent grains will require optimisation. Preliminary data using sorghum brewers spent grain obtained from the SAB Miller Technical Training Centre in South Africa (30% protein, dwb, 75% moisture), shows that approximately 40% of the total protein can be extracted with a single extraction into glacial acetic acid after a 24hr pre-soak in 0.5% sodium metabisulphite (**Table II**). Although the extraction efficiency is not as high as using whole grain sorghum, because the brewers spent grain has a higher protein content (30% dwb) than sorghum whole grain (approximately 9%), more protein is extracted. In addition, the protein that is extracted from brewers spent grain is probably purer kafirin as the water soluble proteins would be removed by the brewing process. The isolated protein from

brewers' spent grain was approximately 85% pure before defatting, which is higher than the purity of kafirin obtained when extracted from whole grain sorghum with either aqueous alcohols or glacial acetic acid after a metabisulphite pre-soak.

TABLE I
Effect of Different Extractants on the Yield and Recovery of Kafirin

Extractant	Protein extraction Yield (% of Total Grain Protein)	Recovery of Kafirin (%) ^a
Control: 70% ethanol + 0.5% sodium metabisulphite + 0.35% NaOH at 70°C	54.3	74.4-79.8
Control: 55% isopropanol + 0.5% sodium metabisulphite+ 0.35% NaOH at 40°C	55.3	75.8-81.3
Glacial acetic acid at 25°C	25.0	34.2-36.8
Glacial acetic acid + 0.5% sodium metabisulphite at 25°C	25.0	34.2-36.8
Pre-soak (1 hr) 0.5% sodium metabisulphite, glacial acetic acid at 25°C	52.8	72.3-77.6
Pre-soak (16 hr) 0.5% sodium metabisulphite, glacial acetic acid at 25°C	59.3	81.2-87.2
Pre-soak (16 hr) 1.0% sodium metabisulphite, glacial acetic acid at 25°C	61.0	83.6-89.7

^a Values calculated according to estimates of the kafirin content of sorghum grain (68-73%) made by Hamaker *et al* (1995)

TABLE II
Effect of Raw Material on Protein Extraction Yields

Raw material	Protein content (dwb)	Protein extraction yield (% of total grain protein)	Grams protein/100g raw material	Protein purity
Whole grain sorghum	9	59.3	5.3	68.0
Brewers spent grain	30	40.6	12.2	84.7

Kafirin Bioplastics

Buffo, Weller and Gennadios (1997) were the first to find that cast plastic films could be made from kafirin². When plasticised with glycerol and polyethylene glycol (PEG) 400, kafirin films had similar tensile and water vapour barrier properties to films made from commercial zein (the maize prolamin protein) plasticised in the same way. These authors extracted the kafirin from sorghum gluten, a by-product of starch wet milling, similar to the distillers dried grain (DDG) by-product of cereal grain bioethanol production.

In laboratories, the authors have shown kafirin bio-plastic films to be stronger (2.9-4.6N kafirin, 1.7N zein) and almost as extensible under certain conditions (26.7-91.7% kafirin, 133.5% zein), with similar water vapour barrier properties (WVP) (0.5-0.7gmm/m²hkPa kafirin, 0.5gmm/m²hkPa zein) as those made from zein, maize prolamin protein, which is commercially available (**Table III**).

TABLE III				
Effect of Extractant on the Thickness, Tensile Properties Water Vapour Barrier Properties of Kafirin Films Compared with those made from Commercial Zein				
Protein and Extractant	Thickness mean (µm)	Strength (N)	Extensibility (%)	WVP (gmm/m²hkPa)
Kafirin: 70% ethanol + 0.5% sodium metabisulphite + 0.35% NaOH at 70°C	84	3.1	26.7	0.5
Kafirin: 55% isopropanol + 0.5% sodium metabisulphite 0.35% NaOH at 40°C	118	2.9	35.0	0.6
Kafirin: Pre-soak (16hr) 0.5% sodium metabisulphite, glacial acetic acid at 25°C	142	4.6	91.7	0.7
Commercial zein-Extractant not known	94	1.7	133.5	0.5

The functional properties of kafirin films can be modified by cross-linking (tanning) the kafirin using hydrolysable or condensed tannins, the latter actually being extracted from tannin-type sorghum grain⁷. Strength was increased by 50-100% and extensibility decreased three to four-fold with increasingly level of tannin modification, up to 20% tannin relative to kafirin. Modification with tannins also reduced oxygen permeability by more than 50% but unfortunately did not improve water vapour permeability (WVP). The modification of film properties using natural components from plants such as tannins seems to be appropriate for the 'green' image of bioplastics.

In addition kafirin film properties can be modified by heat. Heating kafirin to 90-96°C using microwave energy greatly increases the strength and substantially reduces extensibility of cast kafirin films made from the kafirin. Importantly, the moisture barrier properties (WVP) of the films were also improved by the thermal treatment³. The film functional property improvements were thought to be a result of intermolecular cross-linking of the kafirin monomers as a result of heating.

Kafirin Microparticles and Potential Applications

Another application for kafirin is the preparation of microparticles (microspheres). These are small (1-10µm), mainly spherical particles, with internal holes or vacuoles, resulting in a very large surface area (**Fig. 1**).

The structure of the microparticles can be manipulated in such a way that the microparticles can be used for different applications in the food and pharmaceutical industries and for biomedical applications.

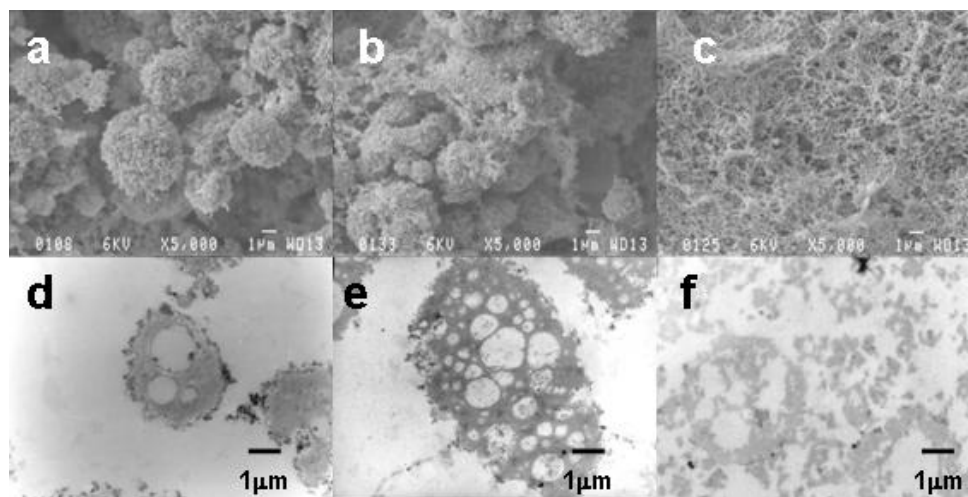


Fig. 1 Scanning Electron Micrographs (a-c) and Transmission Electron Micrographs (d-f) of kafirin microparticles:
a) d-microparticles for encapsulation;
b) e-microparticles for film formation;
c) f-microparticle matrices

Fig. 1a shows the structure of kafirin microparticles which can be used as encapsulating agents. Here advantage is taken of the large surface area of the microparticles. Antioxidants, catechin and sorghum condensed tannins have been encapsulated within kafirin microparticles and the antioxidant release profiles under simulated gastric conditions, investigated. Over a period of four hours, catechin and sorghum condensed tannin encapsulated kafirin microparticles released approximately 70% and 50%, respectively, total antioxidant activity. Thus, encapsulation of catechin and sorghum condensed tannins in microparticles has potential as an effective way to deliver dietary antioxidants and enhance potential health benefits by controlled release of antioxidant activity within the stomach and gastrointestinal tract.

Fig. 1b shows the structure of kafirin microparticles which can be used to form free-standing bioplastic films and coatings. Films made from kafirin microparticles were very thin (<15μm), smooth and transparent, having the appearance of commercial cling film. They were relatively strong (2.4-8.5N/mm²) but not extensible (1.15-2.5%), with better water barrier properties and lower protein digestibility than kafirin films of the same protein content cast from glacial acetic acid (**Table IV**).

TABLE IV Functional Properties of Kafirin Films		
Functional properties	Film type	
	Microparticle film	Conventional kafirin films
Thickness (μm)	14.0	30.0
WVP (gmm/ m ² hkPa)	0.22	0.50
Stress at break (N/m m ²)	3.72	5.39
Elongation (%)	2.53	2.99
Protein Digestibility (%)	65.7	89.0

Fig. 1c shows that the structure of kafirin microparticles can be further manipulated to form a matrix material, which may have applications as tissue scaffolds or as matrices for cell culture. This has not been investigated for kafirin. However, zein microparticles have been used to form scaffolds and film matrices with good biocompatibility and good properties for adhesion and cell proliferation^{5,14,8}.

Experiments have shown that kafirin microparticles can be made directly from an acetic acid extract of brewers spent grains after a metabisulphite presoak. The structure of these microparticles is the same as microparticles made from isolated kafirin as shown by light microscopy. Further, kafirin microparticles made directly from an acetic acid extract of brewers spent grains after a metabisulphite presoak have also been used to make free-standing kafirin bioplastic films and coatings.

CONCLUSIONS

A method of improving the cost effectiveness of sorghum lager beer brewing, is to add value to co-products such as brewers spent grain by extracting the prolamin protein, kafirin. Kafirin has great potential for use as bioplastics and microparticle production, with many possible high value applications in the food, pharmaceutical and bio-medical areas. Kafirin can be extracted directly from brewers spent grain and the extract can be used directly to make microparticles.

REFERENCES

1. Boundy, J.A., Turner, J.E., Wall, J.S., Dimler, R.J. (1967). Influence of commercial processing on composition and properties of corn zein. *Cereal Chemistry* 44, 281-287.
2. Buffo, R.A., Weller, C.L., Gennadios, A. (1997). Films from laboratory-extracted kafirin. *Cereal Chemistry* 74: 473-475
3. Byaruhanga, Y.B., Erasmus, C., Taylor, J.R.N. (2005). Effect of microwave heating of kafirin on the functional properties of kafirin films. *Cereal Chemistry* 82, 565-573.
4. Ciacci, C., Maiuri, L., Caporaso, N., Bucci, C., Del Giudice, L., Massardo, D.R., Pontieri, P., Fonzo, N., Bean, S.R., Ioerger, B., Londei, M. (2007). Celiac disease: In vitro and in vivo safety and palatability of wheat-free sorghum food products. *Clinical Nutrition* 26, 799-805.
5. Dong, J., Sun, Q., Wang, J.Y. (2004). Basic study of corn protein, zein, as a biomaterial in tissue engineering, surface morphology and biocompatibility. *Biomaterials* 25, 4691-4697.
6. Duodu, K.G., Taylor, J.R.N., Belton, P.S., Hamaker, B.R. (2003). Factors affecting sorghum protein digestibility. *Journal of Cereal Science* 38, 117-131.

7. Emmambux, M.N., Stading, M., Taylor, J.R.N.(2004). Sorghum kafirin film property modification with hydrolysable and condensed tannins. *Journal of Cereal Science* 40, 127-135.
8. Gong, S., Wang, H., Sun, Q., Xue, S.T., Wang, J.Y. (2006). Mechanical properties and in vitro biocompatibility of porous zein scaffolds. *Biomaterials* 27, 3793-3799.
9. Hamaker, B.R., Mohamed, A.A., Habben, J.E., Huang, C.P., Larkins, B.A. (1995). Efficient procedure for extracting maize and sorghum kernel proteins reveals higher prolamin contents than the conventional method. *Cereal Chemistry* 72, 583-588.
10. Johns, C.O., Brewster, J.F. (1916). Kafirin, an alcohol soluble protein from kafir, andropogon sorghum. *The Journal of Biological Chemistry* 28, 59-65.
11. Merck Chemical Database. (2005). ChemDat, Merck KGA D 6427, Darmstadt.
12. Taylor, J., Taylor, J.R.N., Dutton, M.F., De Kock, S., 2005. Identification of kafirin film casting solvents. *Food Chemistry* 32, 149-154.
13. Taylor, J.R.N., Novellie, L., Liebenberg, N., VdW. (1984). Sorghum protein body composition and ultrastructure. *Cereal Chemistry* 61, 69-73.
14. Wang, H.J., Lin, Z.X., Liu, X.M., Sheng, S.H., Wang, J.Y. (2005). Heparin-loaded zein microsphere film and hemocompatibility. *Journal of Controlled Release* 105, 120-131.