

PROPERTIES OF HEAT PROCESSED SORGHUM KAFIRIN AND MAIZE ZEIN

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INTRODUCTION

Sorghum (*Sorghum bicolor* L. Moench.) is an indigenous crop to the African continent. It plays an important role for food security in rural areas in the southern Africa as it is very drought tolerant. Sorghum is generally eaten as cooked porridge, the major staple food for people in Southern Africa. Thus, sorghum is an important nutrient source when the sorghum porridge is a major food in the diet. Like other cereals, the protein content of sorghum is low, about 7.3-15.6 % (Hulse *et al.*, 1980). The *in vitro* protein digestibility of sorghum is about 60 % and the digestibility decreases when sorghum is wet cooked (Duodu *et al.*, 2002). This is an important nutritional constraint. It has been hypothesized that α -, γ -kafirin and β - kafirin prolamin storage proteins at the periphery of the sorghum endosperm protein bodies can undergo extensive crosslinking by disulphide bonding during wet cooking (reviewed by Duodu *et al.*, 2003). This may impede proteolytic enzyme access to the major α -kafirin storage protein for digestion.

This study determines the effect of different cooking methods (boiling and pressure cooking) on the properties of kafirin in comparison to zein, the very similar prolamin storage protein of maize.

EXPERIMENTAL

Treatments

One g samples of kafirin and zein were suspended in 5 ml distilled water and then placed in a boiling water bath for 30 min or pressure cooked for 10 min. Kafirin (from white tan plant cultivars PANNAR PEX 202 & 606) and zein (commercial maize meal) were extracted according to Taylor *et al.* (1984) using aqueous tertiary butanol. The prolamin extracted from sorghum and zein was, respectively, 94 and 92 % (db) protein.

Methods

In vitro protein digestibility

In vitro protein digestibility was assayed as described by Hamaker *et al.* (1986) and modified by Duodu *et al.* (2002). Fifty mg protein samples were used instead of 200 mg.

Soft gel size exclusion chromatography (SEC)

SEC was performed on the kafirin and zein. Sample (100 mg) was dissolved in 1.5 ml of a solvent system consisting of 1 M urea and 0.02 M sodium phosphate buffer at pH 3 in a solution of 5 parts ethanol: 2 water: 3 lactic acid (w/w). The solution was then transferred in a glass column (1 cm internal diameter \times 65 cm long) containing SuperoseTM 6B (Pharmacia Fine Chemicals, Uppsala, Sweden). The sample was then eluted with the same solvent system at a rate of 2ml/h. The eluent containing the fractionated proteins was collected in 2 ml aliquots. The fractions were then run through a spectrophotometer

at 280 nm to create a chromatography profile. The proteins from specific peak fraction were recovered by 10 x dilution with distilled water, evaporation of the ethanol followed by cold precipitation at pH 4.5 and analysed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE).

SDS PAGE

SDS-PAGE was performed under reducing and non reducing conditions using a Nupage® mini 4-12 % gradient gel system (Invitrogen™ life technologies, Carlsbad, CA). About 10 µg protein was loaded per sample well. Electrophoresis was carried out at constant 220 V with a current of 80 mA. The gels were stained with Coomassie blue R-250, destained and scanned.

Fourier Transform Infrared Spectroscopy (FTIR)

The spectra of treated and untreated kafirin and zein were measured using a Perkin Elmer Spectrum GX FTIR System (Perkin Elmer, Waltham, MS, USA) equipped with a ATR (attenuated total reflectance) crystal. The sample was placed on the ATR crystal to cover the crystal surface. The FTIR spectra were Fourier deconvoluted using resolution enhancement factor of 1.5 and full height band width of 15 cm⁻¹.

RESULTS AND DISCUSSION

Kafirin had an *in vitro* protein digestibility of about 76 %. When wet cooked in boiling water for 30 min, digestibility reduced to about 58 % (a 24 % decrease). Pressure cooking reduced the protein digestibility of extracted kafirin to about 64 %. The zein had a high digestibility, about 99 %. A relatively small decrease in digestibility was noted when the zein was wet cooked in boiling water and pressure cooked, to 94 and 95 %, respectively.

SEC of extracted and untreated kafirin showed three peaks (Fig. 1a). The first peaks represent high molecular weight and the last peaks represent the lowest molecular weight compounds. SEC showed the same peaks for boiled and pressure cooked kafirin, but peak 1 increased and peak 2 decreased in intensity. Extracted and untreated zein showed only 2 peaks with a small shoulder at fraction before fraction 10 (Fig. 1b). When zein was heat treated in boiling water, the shoulder before fraction 10 increased, and the intensity of peak 2 decreased. Pressure cooked zein showed a peak before fraction 10 instead of a shoulder and the peak 2 further decreased in intensity.

SDS-PAGE (results not shown) under non-reducing conditions showed peak 1 to be mainly oligomers, dimers and polymers. Peak 2 was mainly kafirin monomers (β-, α1-, α2-, and γ-kafirin) and some dimers, but almost no oligomers. Peak 3 did not precipitate for recovery, suggesting that it did not contain kafirin proteins. Under reducing conditions, most of oligomers and dimers were reduced to monomeric proteins.

Deconvoluted FTIR spectra of sorghum kafirin from the frequency range 1750-1450 cm⁻¹ showed 2 main regions (Fig. 2a). The region from about 1700 to 1600 cm⁻¹ is known as the amide I and the region 1575-1475 cm⁻¹ is known as amide II (Duodu *et al.*, 2001; Gao *et al.*, 2005; Byaruhanga *et al.*, 2006). Aqueous tertiary butanol extracted and untreated kafirin had one main peak at 1650 cm⁻¹ with a shoulder at 1625 cm⁻¹ in the amide I region (Fig. 2a). The peak at 1650 cm⁻¹ is attributed to α-helical conformation (Duodu *et al.*, 2001; Gao *et al.*, 2005; Byaruhanga *et al.*, 2006). Kafirin cooked in boiling water showed

a peak at 1625 cm^{-1} . This peak is attributed to β -sheet structures (Duodu *et al.*, 2001; Gao *et al.*, 2005; Byaruhanga *et al.*, 2006). Pressure cooked kafirin also showed a peak at 1625 cm^{-1} , but the intensity of the peak was lower than when kafirin subjected to boiling. In strong contrast, zein subjected to cooking did not show any change in the amide I region in comparison to untreated in the amide I region (Fig. 2b).

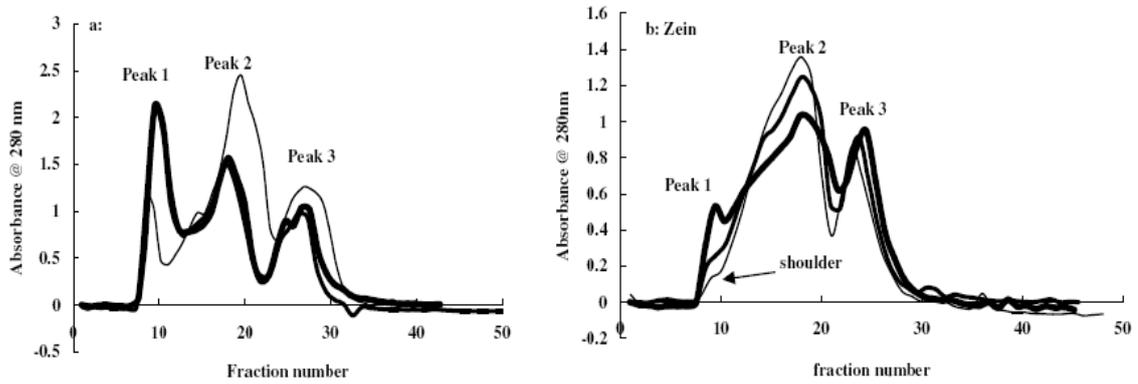


Figure 1. SE-chromatography of kafirin and zein (— control, — boiled, — pressure cooked).

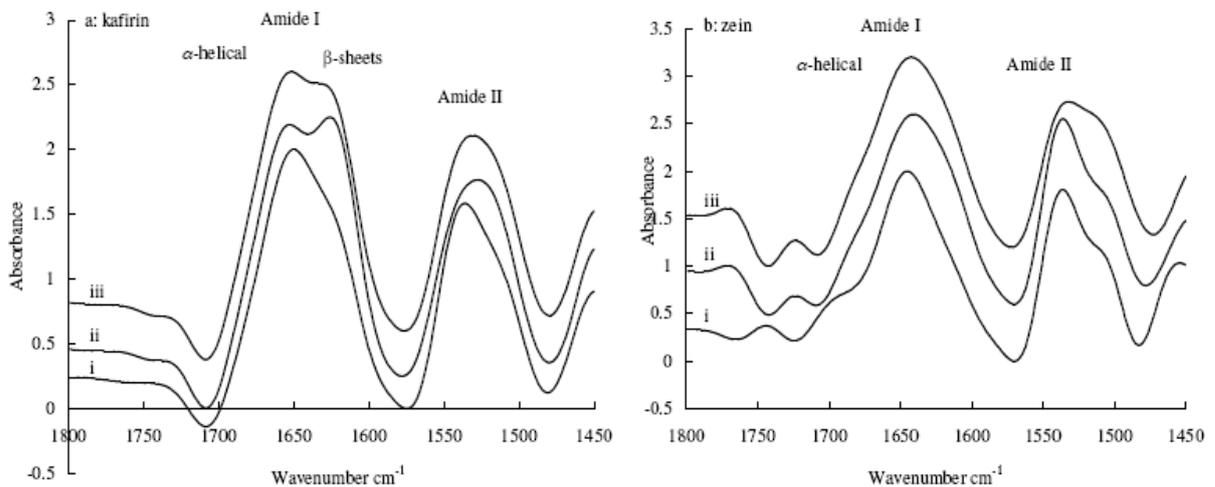


Figure 2. Deconvoluted FTIR spectra of kafirin and zein (i control, ii boiled, iii pressure cooked)

The reduced *in vitro* digestibility of kafirin protein during wet cooking is in agreement with other documented research (Hamaker *et al.*; 1986, Oria *et al.*, 1995; Duodu *et al.*, 2002). However, the work report here is specifically on kafirin, whereas other documented research is mostly on sorghum proteins. The major reasons for decreased digestibility have been reported to be a change in protein conformation by disulphide bonding (Duodu *et al.*, 2002) and a change from α -helical to β -sheet structures (Duodu *et al.*, 2001). Disulphide induced protein crosslinking can increase the molecular weight of the protein. In fact, the above results show that kafirin increases in molecular weight by disulphide bonding when kafirin was wet cooked. In addition, β -sheets are also formed

during wet cooking. These samples were low in protein digestibility. Polymeric proteins and β -sheet formation can thus change the protein conformation to possibly inhibit proteolytic enzyme access for digestion. Unexpectedly, pressure cooking (a severe heating process) significantly ($P < 0.05$) increased digestibility of kafirin when compared with wet cooking in boiling water. This severe heat treatment produced less β -sheet in comparison to boiling. However, the SEC showed occurrence of similar amount of polymeric protein. Thus, the improvement in protein digestibility by pressure cooking seems to be related to the decrease in β -sheet formation.

Maize zein did not show a significant increase in polymeric kafirin and formation/increase in β -sheets structures in comparison to sorghum kafirin during wet cooking. These differences seem to be responsible for the low kafirin digestibility in comparison to maize zein when wet cooked. This is in agreement with results reported by Duodu *et al.* (2002). It is worth noting that extracted kafirin seemed to have more polymeric kafirin than extracted zein. This may either suggest that kafirin can polymerise at low temperature (25 °C) or that kafirin also occurs as polymeric protein in the grain endosperm as compared to zein protein. The occurrence of polymeric kafirin in sorghum was reported by El Nour *et al.* (1998).

CONCLUSIONS

Wet cooking in boiling water and pressure cooking reduce the protein digestibility of kafirin, possibly as a result protein polymerisation by disulphide bond formation and β -sheet formation. These changes occur at a very low extent in maize zein. Pressure cooking as a severe heating treatment may improve kafirin digestibility by decreasing formation of β -sheets structure.

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