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# Characteristics and Functional Properties of Sorghum Protein (Kafirin)

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## ABSTRACT

Kafirin, the major sorghum storage protein, is alcohol-soluble protein. It is an alternative source for zein in the preparation of edible film. In this research, characteristics and properties of -kafirins from different sorghum cultivars were studied. They include yield and protein content of extraction, protein composition, microstructure, as well as color, water- and oil-binding capacity of protein concentrate. After extraction, 100 g of sorghum flour yielded 4.65-5.64 g of protein. The protein concentrate contained 77.48-83.13% protein. By using SDS-PAGE, the protein concentrate consisted of α-, β-, and γ-forms of kafirin. The results showed that α-kafirin had the highest proportion among groups. Protein bodies containing kafirin had spherical shape and hold tightly together. Moreover, the ones from KU 439 had larger size than those of KU 804. In addition, the color of sorghum protein concentrates from KU 804 was more yellowish and brighter than KU 439. Water- and oil- binding capacities were decreased with increasing protein concentration.


**Key words:** sorghum, kafirin, edible films

## INTRODUCTION

Sorghum (*Sorghum bicolor* L. Moench) is in the fifth among cereal crop with total annual yield ranging from about 55–60 million tones (Chamba *et al*., 2005). It could survive in the semi-arid condition. Sorghum is mostly used as feed rather than human food due to its limited properties in food processing and digestibility. Recent year, the environmental concerns are growing; therefore, the researches to develop edible and biodegradable films from natural renewable sources have accelerated. Edible films with the quality of renewability, degradability, compostability, and edibility could make such films particularly appealing for food and nonfood packaging applications. Nowadays, zein is used as edible film for several products such as coated on fruits or meat to keep them fresh and reduce water loss (Trezza and Vergano, 1994). The major protein in sorghum is prolamin or called kafirin (Mazhar and Chandrashekar, 1995). Kafirin is similar to zein in its molecular weight, solubility, structure and amino acid composition (Da Silva and Taylor, 2005). It can be extracted in 70% aqueous ethanol with reducing agent (Hamaker and Bugusu, 2003). Kafirin can be classified into three groups based on their molecular weight, γ (27-28 kDa), α (22-28 kDa) and β (19-20 kDa) (Mazhar and Chandrashekar, 1995). Kafirin has the potential to be an alternative source for zein in film production. It is more hydrophobic and has more

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disulfide bonds than zein; therefore it could make a better barrier and stronger films (Taylor *et al*., 2005). In order to develop an edible film from kafirin, it is necessary to characterize kafirin properties (physical, chemical and physicochemical properties). There are many factors known to affect the functional properties of protein film such as protein type, purity, structure of protein, water-binding capacity, oil-binding capacity, color, concentration, denaturation temperature and environmental factors. This research was focused on the study of chemical and physical properties of kafirins to understand and be able to use kafirin as an edible film,

## MATERIALS AND METHODS

### 1. Kafirin preparation

Kafirin was extracted from dry milling of decorticated grain (KU 439 and KU 804 varieties). Sorghum flour was extracted for 1 hr with 70% aqueous ethanol (w/w) containing 0.5% sodium metabisulphite (w/w) and 0.35% sodium hydroxide (w/w) at 70°C with constant stirring. The extract was separated by centrifugation at 0.82×1000g for 5 min. The supernatant was poured into a shallow open tray placed in a fume cupboard and the solvent is allowed to evaporate overnight at room temperature. The protein was then washed with a minimal amount of cold distilled water (<10°C) and the pH was adjusted to approximately pH 5. The protein was recovered by filtration and then freeze dried. The kafirin were defatted with hexane at room temperature at a protein to solvent ratio of 1:10 (w/w) (Taylor *et al*., 2005).

### 2. Yield and recovery of kafiirin

Kafirin were analyzed in triplicate for protein by Kjeldahl method (A.O.A.C., 2000).

### 3. Scanning Electron Microscope (SEM)

The sorghum flour and kafirin were prepared for scanning electron microscopic observation as follows. Dried sample was sprinkled on an aluminum stub (with double-stick tape on it) and coated thoroughly with gold and viewed with a JEOL JSM 5600LV scanning electron microscope at an accelerating voltage at 10 kV for flour and 15 kV for kafirin.

### 4. SDS-PAGE analysis of kafirin composition

Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) was used to verify kafirin composition. SDS–PAGE was prepared by using 12% separating gel and 4% stacking gel. A Mini-protein II Electrophoretic cell were used. Sample was prepared by dissolving the protein concentrate in 0.5 Tris-HCl pH 6.8, 10% glycerol, 5% β-Mercaptoethanol and 1% bromophenol blue, then heated in a boiling water bath for 3 min and placed in the sample well. The electrophoresis was conducted at 150 mV for 1 hr. A broad range molecular weight standard (MW 6.5-66 kDa) (from Sigma Company) was used. Gel was stained with 0.1% Coomassie Brilliant Blue R250 in 40% methanol and 10% acetic acid and de-stained with 10% methanol in 7.5% acetic acid.

### 5. Color determination

Color of kafirin was determined by Minolta CM-3500d in CIELAB system ($L^*$, $a^*$ and $b^*$scales)

### 6. Water-binding capacity (WBC) and Oil-binding capacity (OBC)

Water-binding capacity and oil-binding capacity were determined by following Beuchat (1977). The protein sample was suspended in a mixer (in concentration of 6% and 9%) for an hour. Then the suspension was centrifuged at 0.6×1000g for 15 min and supernatant was discarded. The precipitate was measured for the sample weight.

**7. Statistical analysis**

Statistical analyses of kafirin characteristics and properties were carried out by SPSS version- 10 and the analysis of variance was carried out by ANOVA test at 5 % confidence and differences among means were differentiated by Duncan's Multiple Range Test.

## RESULTS AND DISCUSSION

**Kafirin yield and recovery**

Two types of sorghum grains, KU 804 and KU 439 were milled and then extracted for protein. KU 804 yielded 4.65% protein, while KU 439 yielded 5.64% protein from 100 g flour. It was found that the percent of protein content of KU 804 (83.24%) was higher than that of KU 439 (77.49%), the extracted protein was called protein concentrate because the protein content about 60-90%. The lower protein extractability may be due to the formation of complexes between protein components (albumins, globulins and kafirin) with tannin (Chibber *et al.*, 1978; Taylor *et al.*, 1984).

**Kafirin morphology**

Figure 1C and 1D showed the morphology of kafirin from KU 439 and KU 804. Extracted kafirin has spherical shape and hold tightly together. Kafirin from KU 439 is bigger than those from KU 804. Kafirin was in the form of protein bodies (arrow point). In the sorghum endosperm, non-kafirin proteins form a coating around the protein bodies that effectively "glued" them into a matrix that surrounding the starch granules of the vitreous endosperm portion (Figure 1A and 1B). Sorghum proteins must form structures with themselves or with other constituents during processing and/or cooking to

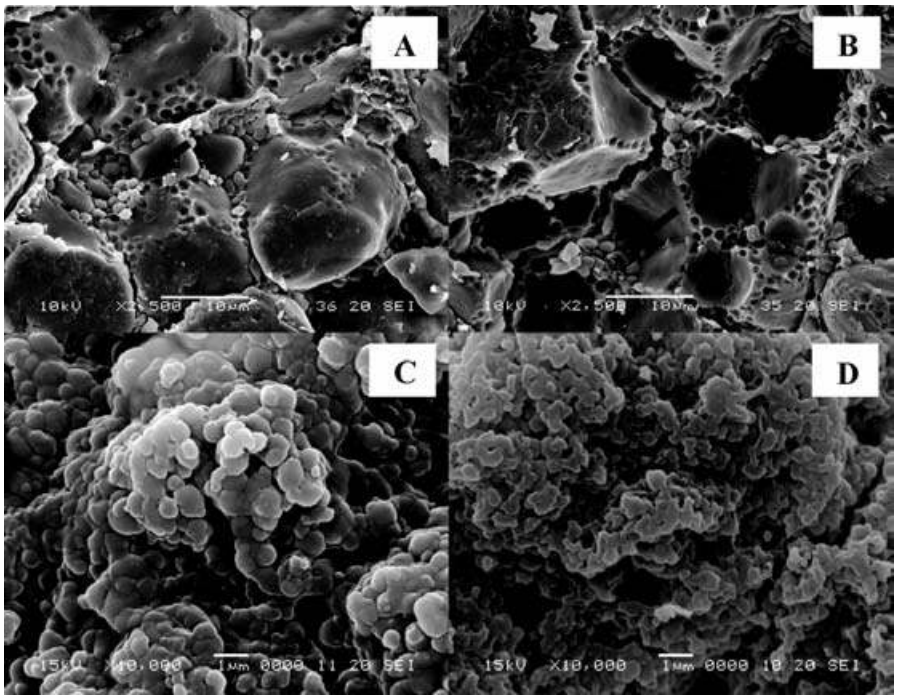


**Figure 1** Scanning electron photomicrograph of flour and kafirin from KU 439 and KU 804 A: flour from KU 439 (2500x), B: flour from KU 804(2500x), C: kafirin from KU 439 (10000x), D: kafirin from KU 804 (10000x).

directly impact functional properties and quality of edible films of kafirin (Hamaker and Bugusu, 2003).

**Kafirin composition**

SDS-PAGE of kafirin (Figure 2) showed bands which were classified into 3 groups named α-, β-, and γ-kafirin. Track M is the molecular weight makers (6.5-66 kDa). α-kafirin consisted of two polypeptides of $M_r$ 20 kDa and 24 kDa (track 3, 4, 5 and 6). These proteins comprised 66-71% and 80-84% of the total kafirin in the opaque and vitreous kernel sections, respectively (Watterson *et al.*, 1993). β-kafirin had three polypeptides of $M_r$ 19 kDa, 16 kDa and 14 kDa (track 3, 4, 5 and 6). γ-kafirin consisted of a polypeptide of $M_r$ 36 kDa (track 3, 4, 5 and 6), it comprised of 9-12% of the total kafirin (Shull *et al.*, 1991). α- prolamin was major storage protein in zein (track 1 and 2) and sorghum (track 3, 4, 5 and 6) (Hamaker *et al.*, 1995), of which bands manifested on slab gel and had broader bands than the others. The α-prolamin is found in the central part of the protein bodies; whereas, the β-, and γ-prolamin are found either at the protein bodies periphery or in dark-staining inclusions within protein bodies (Hamaker *et al.*, 1995). The solution plus 5% 2-ME (Figure 2, track 2, 4 and 6) is shown to contain similar molecular weight as the reduced extracted kafirin, yet in different proportions compare with the ones in the absence of 2-ME (Figure 2, track 1, 3 and 5). In particular, the quantity of $\alpha_2$-kafirin appeared to be lower, whereas that of β-kafirin was greater in the protein extracted directly when the of reducing agent was present.

**Color characteristics of kafirin**

Color characteristics of proteins may become important factors for consumer acceptance in edible film application. The kafirin powder from different sorghum cultivars, differed in their color (Table 1). The results show that kafirin from KU 804 had higher $L^*$(whiter) value, lower $a^*$(less

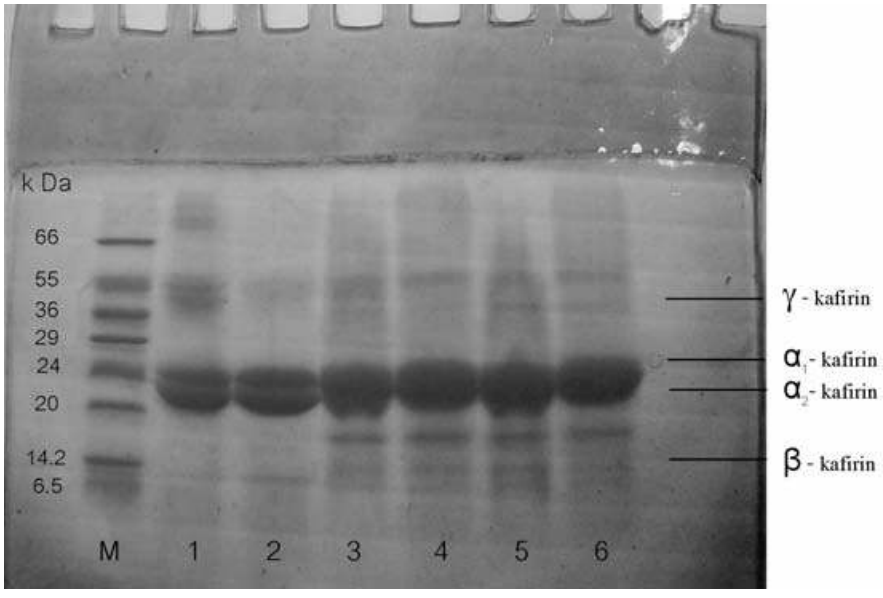


**Figure 2** SDS-PAGE of kafirin. Tracks: M: Molecular weight marker, 1: zein, 2: zein with 2-ME, 3: kafirin from sorghum KU 439, 4: kafirin from sorghum KU 439 with 2-ME, 5: kafirin from sorghum KU 804, 6: kafirin from sorghum KU 804 with 2-ME.

reddish) value and higher b*(more yellowish) value than kafirin from KU 439 (Table1). Kafirin from KU 804 had lower content of pigments than kafirin from KU 439. It is possible that KU 804 flour had simple sugars lower than KU 439 flour; therefore during the extraction process, flour from KU 439 might be readily undergone Maillard reaction to the more extent than the flour from KU 804. (Watson, 1984)

**Water-and oil-binding capacity**

Water-binding capacity is an interaction between the water molecules and hydrophilic groups of the protein side chains occured via hydrogen bonding (Hutton and Campbell, 1981). Binding of water to protein is related to the polar hydrophilic groups, such as imino, amino, carboxyl, and sulfhydryl groups (Hutton and Campbell, 1981). Figure 3A showed that water binding capacity of kafirin from KU 439 and KU 804 decreased with increasing protein concentration (Figure 3A). Furthermore, the oil-binding capacity (OBC) of kafirin from KU 439 and KU 804 decreased with increasing protein concentration (Figure 3B). From the results, it could indicate that kafirin from both cultivars could interact with oil slightly more than water and have more hydrophobic properties.

**Table 1** Color[a] of flour and kafirin from KU 439 and KU 804.

| | | Flour | Kafirin |
|---|---|---|---|
| **L*** | KU 439 | $80.1^{a}_{a} \pm 0.11$ | $87.19^{b}_{a} \pm 0.16$ |
| | KU 804 | $79.35^{a}_{b} \pm 0.10$ | $87.65^{b}_{a} \pm 0.26$ |
| **a*** | KU 439 | $3.10^{a}_{b} \pm 0.04$ | $1.59^{b}_{a} \pm 0.03$ |
| | KU 804 | $3.34^{a}_{a} \pm 0.03$ | $1.35^{b}_{b} \pm 0.00$ |
| **b*** | KU 439 | $11.46^{a}_{a} \pm 0.19$ | $6.85^{b}_{b} \pm 0.06$ |
| | KU 804 | $10.67^{a}_{b} \pm 0.06$ | $7.14^{b}_{a} \pm 0.01$ |

[a] L (100-lightness,0-darkness); +a to –a, increasing red to increasing green, +b to –b increasing yellow to increasing blue.
Mean value in the same row with different superscripts are significantly different ($p \leq 0.05$).
Mean value in the same column with different subscripts are significantly different ($p \leq 0.05$)

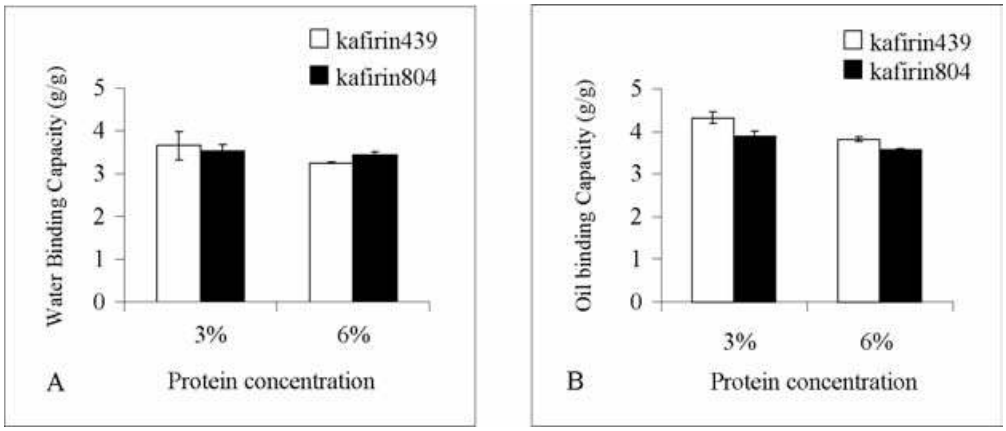


**Figure 3** Water-binding capacity (A) and Oil-binding capacity (B) of kafirin 439 and kafirin 804.

## CONCLUSION

Yield of kafirin from KU 439 was higher than KU 804 but protein content of KU 804 was higher. Morphology of protein bodies had round shape and hold tightly together in which kafirin from KU 439 had bigger size than KU 804. Kafirin was composed of α-, β-, and γ- kafirin. α-kafirin was the major form of kafirin. Color of protein concentrate from KU 804 was whiter, less reddish and more yellowish than from KU 439. Water-binding capacity and oil-binding capacity decreased with increasing protein concentration. From the result, kafirin has potential to cast film and may give good barrier and strong film.

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