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Characterization of Kafirin and Zein Oligomers by Preparative Sodium Dodecyl Sulfate−Polyacrylamide Gel Electrophoresis

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Quantitative and qualitative analysis of uncooked zein and kafirin fractions were performed through sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE) and electrophoretic profiles. Kafirins and zeins present the same oligomer and monomer compositions with the exception of a 66 kDa oligomer that is only present in kafirins. The quantitative analysis showed differences between zein and kafirin. The composition of each oligomer was established via preparative SDS−PAGE. Part of the cooked oligomers resists reduction; the presence of those oligomers could be related to the decrease on protein digestibility with the cooking process.

KEYWORDS: Sorghum; maize; zein; kafirin; preparative electrophoresis

INTRODUCTION

Sorghum bicolor (L.) Moench is a cereal that grows in areas of reduced content of water and extreme temperatures, and it is a staple food in many regions of Africa and India.

The endosperm is the major component of the sorghum grain (82.3%). The outermost layer of cells in the endosperm is the aleurone. The endosperm cells are directly beneath the aleurone cells and contain high concentration of protein and starch granules. Protein bodies are found in all mature cells, and in the case of sorghum, they persist into maturity (*1*).

The major protein component of the protein bodies are the prolamins (*2*). The prolamins are the most abundant proteins in sorghum, making up 70−80% of the total endosperm proteins and having no other known function apart from storage. They are synthesized during endosperm development and then they are accumulated in protein bodies (*3*) as cross-linked aggregates, whose formation is promoted by maturation and drying of the seed (*4*).

There is a matrix protein that surrounds the protein bodies and holds the starch granules in place. Seckinger and Wolf (*2*) concluded that these proteins are mainly glutelins. However, Hamaker and Bugusu (*5*) refer to these proteins as nonkafirins.

Shull et al. (*6*) proposed a nomenclature for kafirins (sorghum prolamins) that is based on the similarities in molecular weight, solubility, and structure between kafirins and zeins (maize prolamins). These similarities were confirmed by SDS−PAGE electrophoresis, differential solubility, and immunocytochemical tests. Therefore the kafirins are named γ (28 kDa), α (23 and 25 kDa), and β (20 kDa).

According to Shull et al. (*7*) α-kafirins are placed in the center region of the protein body and the γ- and β-kafirins are in a peripheral layer surrounding them.

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It is well-known that these proteins are organized in oligomers. One study reports the oligomer composition of uncooked flour samples (*8*) without referring to the composition of those oligomers in cooked flour. It is known that wet cooking may promote main structural changes in the protein fraction (*9*).

Therefore, study of the aggregates of cooked flour is important for comprehending the decreased protein digestibility of sorghum flour when it is wet-cooked.

MATERIALS AND METHODS

Biological Material. Cereal samples (African cultivars) consisted of one maize (PAN 6043) and four sorghum varieties (NK283, KLW, KAT369, and PAN8564).

Cereal Sample Preparation. Whole-grain samples were ground with a coffee mill to pass through a 0.4 mm screen.

Cooked Samples. To obtain cooked samples, 1 part of ground flour was mixed with 5 parts of water and the mixture was placed in a boiling water bath for 20 min. After cooking, the samples were freeze-dried and were ground again. The cooked samples are prepared under conditions of no mass loss.

Prolamin Extraction. Samples (100 mg) of uncooked and cooked flours were extracted with 0.5 mL of *t*-butanol 60%, with mechanical stirring. The extraction time of the uncooked samples and maize cooked sample was 1 h. An efficient protein recovery of kafirins from cooked samples is only possible with a 24 h extraction.

SDS−PAGE. Electrophoresis was carried out on 15% (w/v) polyacrylamide gels. A Mini-Protean II electrophoretic cell (Bio-Rad) was used and the electrophoresis was conducted at 150 V for 1 h until the tracking dye, bromophenol blue, reached the bottom of the resolving gel. For SDS−PAGE, 20 μL of *tert*-butyl alcohol protein extract was dried under N_2 atmosphere and the obtained residue was dissolved in electrophoretic sample buffer [2% (w/v) SDS, 0.0625 M Tris, 10% (v/v) glycerol, and 0.01% (w/v) bromophenol blue, pH 6.8]. To observe the oligomers, nonreducing conditions were applied. When reducing conditions were needed, 5% (w/v) 2-ME was used in the sample buffer. The samples were heated in a boiling water bath for 5 min. The gels were stained with Coomassie Blue R (Pharmacia) (*10*).

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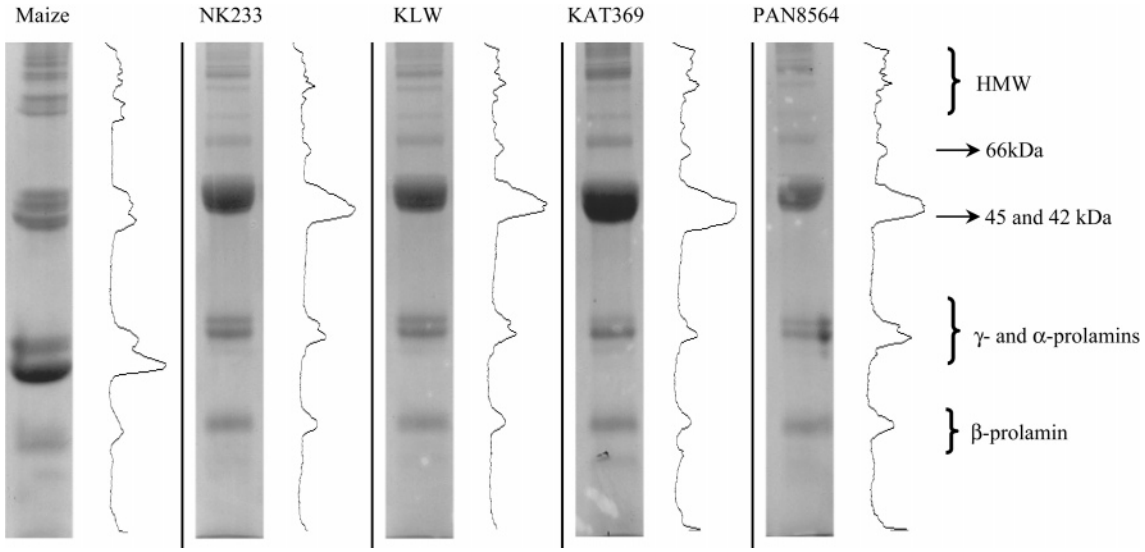

Figure 1. Electrophoretic gels and electrophoretic profiles of the prolamin fractions of maize and sorghum uncooked samples run under nonreducing conditions.

Table 1. Quantitative Analysis of Sorghum and Maize Prolamin Constituents from Unreduced and Reduced Uncooked Prolamin Fractions[a]

	uncooked nonreduced samples					uncooked reduced samples				
	maize	NK283	KLW	KAT369	PAN8569	maize	NK283	KLW	KAT369	PAN8569
HMW	23.7 ± 0.1	15.3 ± 0.9	19.8 ± 0.3	22.9 ± 0.5	22.6 ± 1.2					
66 kDa		3.2 ± 0.6	3.7 ± 0.4	4.2 ± 0.4	3.0 ± 0.2					
45 kDa	21.6 ± 0.2	50.3 ± 0.2	45.9 ± 0.5	53.9 ± 1.2	37.0 ± 1.2	2.7 ± 0.5	2.4 ± 0.1	1.5 ± 0.2	3.0 ± 0.2	2.0 ± 0.1
$\gamma + \alpha$	46.0 ± 0.1	20.4 ± 0.0	20.7 ± 0.2	11.1 ± 0.1	25.4 ± 0.8	84.6 ± 0.1	86.0 ± 0.1	86.7 ± 0.2	88.1 ± 0.3	86.7 ± 0.2
β	8.3 ± 0.3	10.7 ± 0.1	9.9 ± 0.2	7.9 ± 0.1	12.0 ± 0.5	12.7 ± 0.6	11.6 ± 0.0	11.9 ± 0.1	9.1 ± 0.1	11.2 ± 0.1

[a] Values are expressed as a percentage.

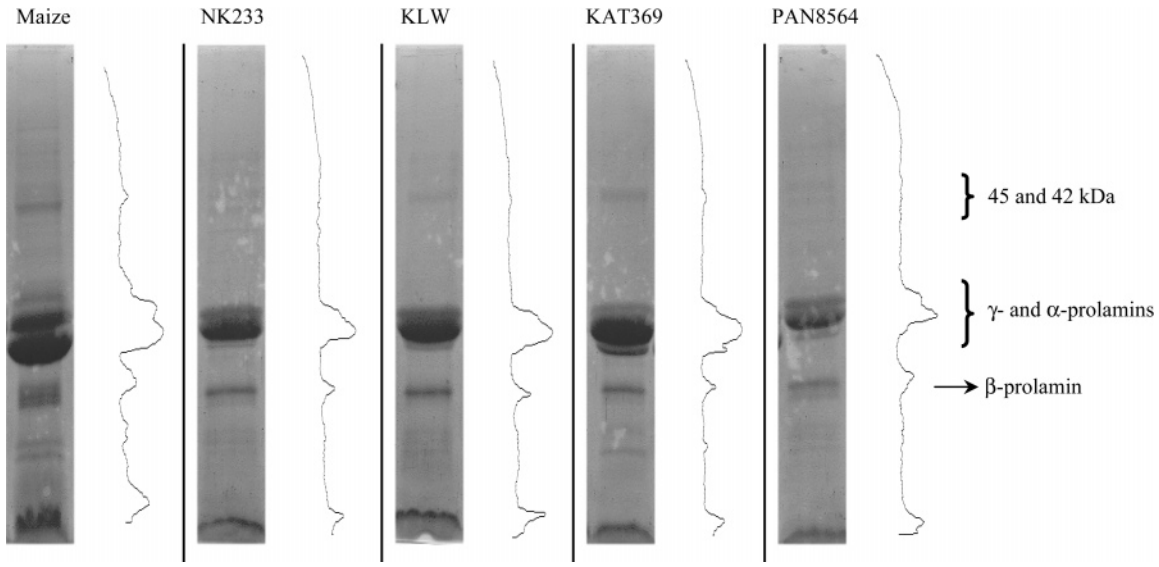

Figure 2. Electrophoretic gels and electrophoretic profiles of the prolamin fractions of maize and sorghum uncooked samples run under reducing conditions.

Preparative SDS−PAGE. Following electrophoresis of six gels (run under nonreducing conditions), the stained bands of interest were cut from the gels and destained with 50% (v/v) methanol and 10% (v/v) acetic acid until no more Coomassie blue color was visible. The protein was extracted from the gel by grinding the piece into the elution buffer [0.1% (w/v) SDS, 0.5 M Tris, 1 mM ethylenediaminetetraacetic acid (EDTA), and 5 mM dithiothreitol (DTT)] and left for24 h before the protein solution was separated from the gel pieces (*11*). Each extract was mixed with reducing electrophoretic sample buffer, boiled for 5 min, and analyzed by SDS−PAGE. The amount of samples used was dependent on the concentration of each extract. Due to the small quantities of the protein in the samples, the obtained gels were silver-stained (*12*).

Analysis of SDS−PAGE Images. Electrophoretic gels were digitalized, without previous drying, in a Hewlett-Packard ScanJet 3600C scanner. The acquired image was converted into a matrix representative of the different color intensities (color-coded images). Each of the electrophoretic lanes was split out and separately submitted to a mathematical treatment based on joint density probability estimation. The mathematical treatment acts as a filter that enhances protein

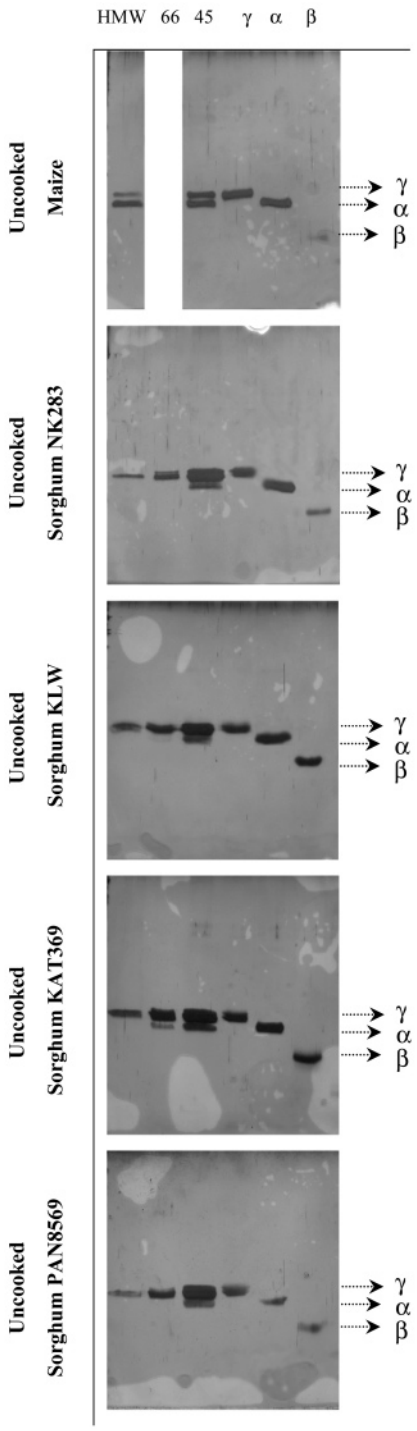

Figure 3. Electrophoretic gels, run under reducing conditions, of the HMW aggregates, oligomers, and monomers obtained by preparative SDS–PAGE from uncooked sorghum and maize.

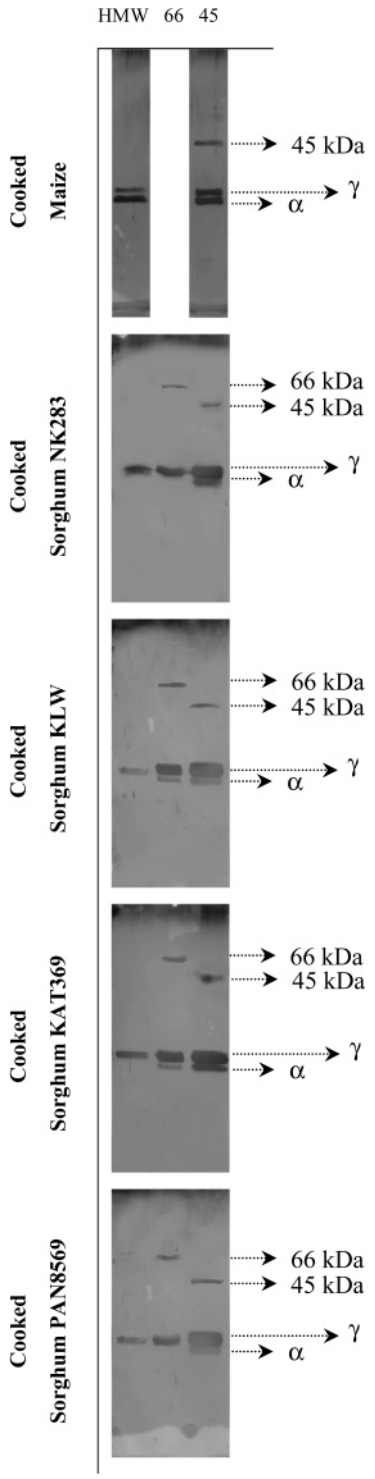

Figure 4. Electrophoretic gels, run under reducing conditions, of the HMW aggregates, oligomers, and monomers obtained by preparative SDS–PAGE from cooked sorghum and maize.

Table 2. Composition of Prolamin HMW Aggregates and Oligomers from Uncooked and Cooked Samples

	uncooked			cooked		
	HMW	66 kDa	45 kDa	HMW	66 kDa	45 kDa
maize	$\gamma+\alpha$		$\gamma+\alpha$	$\gamma+\alpha$		45 kDa + $\gamma+\alpha$
NK283	γ	γ	$\gamma+\alpha$	γ	66 kDa + γ	45 kDa + $\gamma+\alpha$
KLW	γ	$\gamma+\alpha$	$\gamma+\alpha$	γ	66 kDa + $\gamma+\alpha$	45 kDa + $\gamma+\alpha$
KAT369	γ	$\gamma+\alpha$	$\gamma+\alpha$	γ	66 kDa + $\gamma+\alpha$	45 kDa + $\gamma+\alpha$
PAN8569	γ	γ	$\gamma+\alpha$	γ	66 kDa + γ	45 kDa + $\gamma+\alpha$

concentration determination (*10*). All electrophoretic profile regions corresponding to electrophoretic spots were submitted to area estimation by the program Origin, Microcal Software Inc. Values are the mean of three replicates and are expressed as a percentage.

RESULTS AND DISCUSSION

SDS−PAGE Characterization of Kafirins and Zeins. The 15% polyacrylamide homogeneous SDS−PAGE gels showed the composition of the prolamin extracts and allowed qualitative and quantitative analysis. **Figure 1** shows the electrophoretic gels, and respective electrophoretic profiles, of the uncooked samples under nonreducing conditions.

In the maize sample, HMW aggregates with molecular weights between 200 and 80 kDa were found. Oligomers, at 45 and 42 kDa, were also present together with the monomers: γ (28 kDa), α (24 kDa), and β (19 kDa).

The molecular masses found for the kafirins were 200−80 kDa for HMW, 66 and 45 kDa for the oligomers, and 31, 29, and 17 kDa respectively for the γ, α, and β monomers (**Figure 1**).

Apart from a slight difference in molecular weights observed on the gels, all sorghum samples have a similar composition as maize, with the exception of the 66 kDa oligomer, which is not present in maize.

Table 1 shows the relative percentage of each kind of protein according to the electrophoretic profile. As can be seen, in uncooked nonreduced samples, the percentage clearly changes from maize to sorghum and from variety to variety. The most abundant zein component are the $\gamma + \alpha$ monomers. In the case of kafirins, the most abundant component is the 45 kDa oligomer. The minor component of kafirins is the 66 kDa oligomer. For zeins, the minor component is the β monomer.

According to the electrophoretic gels run under reducing conditions (**Figure 2**), as the S−S bonds were cleaved, the oligomers were disrupted and their constituents were revealed. **Table 1** also shows that, for all samples, a massive increase of the monomers (γ, α, and β) can be observed and only a small amount of the 45 and 42 kDa oligomers persists in the gels after reduction. The relative composition regarding the monomers is reasonably similar for all samples.

The reducing conditions approach confirms that the oligomers are in fact built by monomers establishing S−S bonding. However, this electrophoretic technique is not suitable to establish the composition of each single oligomer, because all of them (HMW and 66, 45, and 42 kDa) are reduced simultaneously. The electrophoretic profiles from the cooked samples are very similar to those of the uncooked samples (results not shown).

Preparative SDS−PAGE of Kafirins and Zeins. Preparative SDS−PAGE was used to establish the composition of each reduced oligomer independently. **Figure 3** presents the electrophoresis of the extracted and then reduced oligomers from the uncooked samples showing their composition in monomers. **Table 2** summarizes the results for maize and all sorghum varieties. HMW aggregates of maize are composed of $\gamma + \alpha$ monomers and the HMW aggregates of sorghum samples are composed only of γ monomers.

The 66 kDa oligomer, present only in sorghum samples, has a different composition according to the variety: in KLW and KAT369 it is formed by $\gamma + \alpha$, and in NK283 and PAN8569 it is formed only by the γ monomer.

The 45 kDa oligomer in maize and all sorghum varieties is formed by the $\gamma + \alpha$ monomers. β Monomer was not detected, either in the composition of the HMW or in the composition of the oligomers (66 and 45 kDa). These findings are in disagreement with results that report the presence of the β monomer in the composition of HMW aggregates (*8*). The β monomer quantity remains constant in the electrophoretic gel run under reducing conditions, confirming the absence of β monomer in the composition of the HMW aggregates and oligomers (**Figure 2** and **Table 1**).

Figure 4 presents the results of the preparative SDS−PAGE of the cooked samples.

According to these results (also summarized in **Table 2**) the HMW aggregates have the same composition of the corresponding uncooked samples: $\gamma + \alpha$ monomers in the case of zeins and γ monomers in the case of kafirins.

A fraction of the 66 kDa oligomer (present only in sorghum samples) is not susceptible to reduction, since an electrophoretic spot remains detectable at this molecular weight. However, part of the oligomer is reduced to γ-kafirin, in the case of NK283 and PAN8569, and to $\gamma + \alpha$, in the case of KLW and KAT369. The cooking procedure may have caused structural change of the protein that did not allow a complete S−S bond cleavage.

The 45 kDa oligomers behave similarly to the 66 kDa ones. Part of this oligomer suffers reduction to $\gamma + \alpha$ monomers and a fraction remains visible at 45 kDa. The electrophoretic studies were repeated with higher concentration of 2-ME and with DTT (stronger reducing agent) and the results were not different.

Summarizing, it was found that the composition of the oligomers depends, in the case of sorghum, on the variety. It is interesting to notice that in the sorghum varieties whose digestibility is more affected by cooking, namely, KLW and KAT369 (*9*), the 66 kDa oligomer is formed by the monomers $\gamma + \alpha$. To our knowledge this fact represents the first report on differences between kafirins of sorghum varieties.

In the present conditions of extraction (*tert*-butyl alcohol 60%), the β monomer was only detected in the free form and was not found to be part of the HMW aggregates, as proposed before (*8*).

The study of the oligomers of the prolamins extracted from cooked samples shows that the composition of the oligomers does not suffer major changes by cooking. However, cooking promotes the formation of 45 kDa oligomers (in both sorghum and maize) and of 66 kDa oligomers (only in sorghum) that are not affected by the treatment with reducing agents. Attention should be paid to the eventual role of this 66 kDa oligomer in the digestibility of cooked sorghum.

ABBREVIATIONS USED

2-ME, 2-mercaptoethanol; DTT, dithiothreitol; HMW, high molecular weight; M_r, protein molecular weight; SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; *tert*-butyl alcohol, 2-methylpropan-2-ol.

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