# ROLE OF GRAIN ORGANISATIONAL STRUCTURE IN SORGHUM PROTEIN DIGESTIBILITY

BY

**KWAKU GYEBI DUODU**

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I declare that the dissertation herewith submitted for the degree of PhD (Food Science) at the University of Pretoria, has not previously been submitted by me for a degree at any other university or institution of higher education.

# ABSTRACT

## ROLE OF GRAIN ORGANISATIONAL STRUCTURE IN SORGHUM PROTEIN DIGESTIBILITY

by

Kwaku Gyebi Duodu

Promoter: Prof JRN Taylor
Co-promoter: Prof PS Belton
Department: Food Science
Degree: PhD (Food Science)

Sorghum (*Sorghum bicolor* (L.) Moench) is a drought-tolerant basic food cereal in many parts of Africa and Asia. Wet cooking decreases the digestibility of sorghum proteins significantly and this is a limitation to the use of sorghum as food. Uncooked sorghum protein digestibility is also considered lower than other cereals.

*In vitro* protein digestibility of uncooked and cooked condensed-tannin-free sorghum varieties and a white maize variety was examined at four levels of grain organisational structure: whole grain, endosperm, protein body preparations and isolated proteins. The possibility of polyphenols and gelatinised starch having an effect on *in vitro* protein digestibility of sorghum and maize was also examined. Fourier transform infrared (FTIR) and solid-state $^{13}C$ NMR spectroscopic methods were used to investigate changes in protein secondary structure on thermal processing. Electrophoresis (SDS-PAGE) was used to investigate the possibility of protein crosslinking on thermal processing by examining molecular weight differences.

Uncooked and cooked sorghum protein digestibility improved from whole grain, through endosperm to protein body preparations. However, uncooked and cooked maize protein digestibility was essentially the same at these levels. Uncooked sorghum protein digestibility was lower than uncooked maize at the whole grain level, same as maize at the endosperm level and higher than maize at the protein body-enriched level. Isolated kafirins and zeins from sorghum and maize had similar uncooked protein digestibility. Total polyphenol content

of sorghum decreased from whole grain to endosperm and increased from endosperm to protein body preparations.

As expected, cooking reduced protein digestibility of sorghum whole grain, endosperm, protein body preparations and extracted kafirins. Cooked sorghum whole grain and endosperm had similar protein digestibilities but cooked protein body preparations were more digestible. There was overall improvement in sorghum protein digestibility with change in organisational level. Protein digestibilities of uncooked and cooked maize were essentially the same at all the organisational levels.

Treating cooked whole grain and endosperm samples of sorghum and maize with alpha-amylase before pepsin digestion improved protein digestibility. In the protein body preparations where the proportion of starch was lower, such treatment had no effect on protein digestibility.

SDS-PAGE under non-reducing and reducing conditions of uncooked and cooked protein body preparations from normal sorghum, maize and sorghum mutants (of known high protein digestibility) showed oligomers of $M_r$ 45, 66 and >66 kDa and monomeric kafirins and zeins. Sorghum had more 45-50 kDa oligomers than maize. In comparison with maize, more of these oligomers were resistant to reduction in cooked normal sorghum.

SDS-PAGE also showed that residues of the protein body preparations remaining after pepsin digestion consisted mainly of α-zein (uncooked and cooked maize) or α-kafirin (uncooked normal sorghum), whilst cooked normal sorghum had in addition, β- and γ-kafirin and reduction-resistant 45-50 kDa oligomers.

FTIR and solid-state $^{13}C$ NMR spectra of normal sorghum, maize and sorghum mutants indicated a change in protein secondary structure from α-helical to antiparallel intermolecular β-sheet conformation on cooking. The extent of secondary structural change seemed to be greater in sorghum than in maize.

Grain organisational structure does influence sorghum protein digestibility. Interfering factors in the grain outer layers, namely pericarp and germ may be responsible. The decrease in

sorghum total polyphenol content from whole grain to endosperm accompanied with an increase in uncooked and cooked sorghum protein digestibility suggests that polyphenols may affect sorghum whole grain protein digestibility. In contrast to earlier reports, uncooked sorghum protein digestibility may not always be lower than that of maize. It depends on the nature of the material being assayed. Gelatinised starch, probably by reducing accessibility of pepsin to protein, reduced digestibility of sorghum and maize whole grain and endosperm.

It appears that cooking reduces protein digestibility in sorghum by unravelling of prolamin polypeptides in the α-helical conformation which re-associate either through disulphide or non-disulphide crosslinks to form the antiparallel intermolecular β-sheet conformation. This conformation may be less digestible due to restricted enzyme access to the protein. Such crosslinking may occur to a greater extent in sorghum than in maize perhaps due to subtle differences in prolamin tertiary structure between the two cereals, contributing to the worse digestibility of cooked sorghum proteins.

**Do not be afraid of walking slowly**

**Be afraid only of standing still**

**- Old Chinese proverb**

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# TABLE OF CONTENTS

## LIST OF TABLES

## LIST OF FIGURES