Protein digestibility of sorghum and maize flours and porridges as affected by gamma-irradiation

By

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DEDICATION

To My Family

My parents, Peter N. Fombang and Ruth N. Fombang

My elder brother, Emmanuel Tadoh Fombang, for his exemplary character.

My junior siblings, Kenneth, Mccpowell, Raymond, Christabel, Cyrille and Lina.

You are each adorable for different reasons.

DECLARATION

I declare that the thesis which I hereby submit for the degree PhD (Food Science) at the University of Pretoria is my own work and has not previously been submitted by me for a degree at another University or institution of higher education.

Edith Nig Fombang
July 2005

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TO GOD BE THE GLORY.

ABSTRACT

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Sorghum foods contribute significantly to the protein intake of millions of people in developing countries. One limitation to sorghum's use as a protein source is that its proteins become less digestible on wet cooking, primarily through the formation of disulphide-linked enzymatically resistant protein polymers. Irradiation of foods can modify bonds involved in stabilizing protein structure, resulting in changes in the protein. The effects of irradiating sorghum cultivars BR7 and Madjeri, and maize cultivar PAN 6043 flours under mild (10 kGy dry) and severe (50 kGy dry and 10 and 50 kGy wet) conditions, followed by cooking into porridge on the digestibility, solubility and some molecular properties of their proteins, were investigated. Pepsin and multienzyme methods of determining protein digestibility (PD) were compared.

As expected, pepsin PD of sorghum decreased more with cooking alone (12-18%) compared to maize (4%). Sorghum porridges had more disulphide-bonded prolamin dimers than maize as shown by SDS-PAGE under non-reducing conditions. However, the amounts of disulphide bonds in both porridges appeared similar. Prolamin extractability (PE) decreased more with cooking in sorghum compared to maize. There was no significant correlation between the pepsin and multienzyme methods, suggesting the latter may not simulate *in vivo* PD that has been reported to correlate positively with pepsin PD.

Mild and severe irradiation of sorghum flour before cooking alleviated somewhat the reduction in sorghum PD on cooking. Maize porridge digestibility was unaffected by prior irradiation of dry flour but decreased with irradiation of wet flour. Mild irradiation of sorghum alleviated the reduction in PD with cooking most, almost to the level of uncooked flour. The alleviation in PD coincided with alleviation in the reduction in PE. With severe irradiation, the alleviation in PE was not consistent. Pepsin PD was positively correlated with PE for sorghum BR7 ($r=0.83$, $p<0.01$) and Madjeri ($r=0.75$, $p<0.05$), but not for maize.

With increasing irradiation severity, disulphide bond concentration decreased, while free sulphydryl groups increased in sorghum porridges from irradiated compared to unirradiated flour. This suggests breakdown of disulphide bonds to free sulphydryls. SDS-PAGE under non-reducing conditions showed lower dimer concentrations in sorghum porridges from irradiated compared to unirradiated flour. Disulphide bonds in maize were not significantly affected by irradiation and cooking. Dimers in maize porridge only decreased with severe irradiation. Nitrogen solubility index (NSI) did not change significantly with irradiation and cooking in BR7, whereas in Madjeri and maize, NSI increased slightly or not at all with mild irradiation, but with severe irradiation, some decreases occurred. Albumin and globulin (AG) solubility decreased more with irradiation alone in BR7 compared to Madjeri and maize. The differences in NSI and AG solubility in BR7 with Madjeri and maize were attributed to the presence of polyphenols in BR7 that may have showed some antioxidant activity during irradiation. Sorghum and maize porridges became darker in colour with irradiation suggesting Maillard browning.

It is proposed that irradiation of sorghum flour before cooking alleviated the reduction in PD by causing a change in protein structure. Irradiation cleaved disulphide and hydrogen bonds that stabilize kafirin protein structure giving a more open protein network, exposing additional peptide bonds to proteolysis. Under severe conditions of irradiation, covalent non-disulphide bonds may be formed, and may cause closing up of the protein structure, resulting in masking of some previously exposed peptide bonds. Irradiation is a potentially useful technique that can improve protein digestibility and nutrient density of sorghum porridge, with a potential to also reduce microbial load and improve safety.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1 INTRODUCTION AND LITERATURE REVIEW

1.1 STATEMENT OF PROBLEM

Sorghum (*Sorghum bicolor* (L.) Moench) and maize (*Zea mays* L.) are staple food crops for many of the world's poorest people and constitute a major source of energy and proteins for millions of people in Africa and Asia (Taylor & Belton, 2002; Eckhoff, Paulsen & Yang, 2003). Sorghum and maize can therefore be considered as significant contributors to ensuring food security in these regions.

Sorghum contains about 9-14% proteins (Rooney & Serna-Saldivar, 2003) and maize 8-12% (Eckhoff *et al*., 2003). Sorghum and maize proteins are limiting in the essential amino acid lysine (Rooney & Serna-Saldivar, 2003; Wilson, 1983). However, sorghum is more limiting in its use as a protein source, in that its proteins become less digestible when cooked into porridge. This has been demonstrated in both *in vitro* and *in vivo* studies (reviewed by Duodu, Taylor, Belton & Hamaker, 2003). The reduction in the digestibility of sorghum proteins with cooking is attributed to the formation of proteolytic enzyme resistant protein polymers, through disulphide bonding of β- and γ-kafirins at the periphery of the protein body with themselves and with matrix proteins, which restricts digestion of α-kafirin at the interior of the protein body (Oria, Hamaker & Shull, 1995b). This suggests that the conformation of sorghum proteins could be an important factor influencing its digestibility, as this may affect enzyme access to the proteins.

The poor protein digestibility of sorghum porridges could perhaps be related to the prevalence of malnutrition in sorghum consuming areas. It was estimated that in 2000 33% (182 million) children (<5 year) in developing countries were stunted, with 70% of these children in Asia, 26% in Africa and 4% in Latin America and the Caribbean (de Onis, Frongillo & Blössner, 2000). In Cameroon, in the early 1990s, malnutrition in children under the age of 5 years was estimated to be at 24% (Balépa, Fotso & Barrère 1992). The situation was most acute in the three northern provinces of Cameroon where malnutrition was estimated at 30%, and infant mortality rates in these regions was 2.5 times higher than elsewhere in the country (Balépa *et al*., 1992). Sorghum, together with

pearl millet and maize are the staple foods in these areas, and are consumed mainly as porridges. Given that protein digestibility of sorghum decreases substantially when cooked into porridge, it is therefore not unlikely that this could contribute to the higher prevalence of malnutrition in these regions. More digestible proteins in sorghum porridges may assist towards reducing the incidence of malnutrition.

Irradiation is a technology used in food processing. In cereal grains, it is recommended for grain disinfestations to eliminate spoilage microorganisms (Institute of Food Science and Technology, IFST, 1998). As radiation energy passes through foods, it interacts with food molecules converting them to free radicals that can then react with each other or other unchanged molecules in the food. These reactions can bring about changes in the food components. Radiation energy can cleave disulphide bonds, hydrogen bonds and other bonds involved in stabilizing protein structure (Garrison, 1987; Di Simplicio, Cheeseman & Slater, 1991; Köksel, Sapirstein, Celik & Bushuk, 1998). This could lead to denaturation or breakdown of the proteins to smaller peptides (Davies, Delsignore & Lin, 1987a; Garrison, 1987; Kempner, 1993). However, with increasing irradiation dose, the denatured proteins or smaller peptides may polymerise to form high molecular weight protein polymers (Kempner, 1993; Cieśla, Roos & Głuszewski, 2000). This notwithstanding, some proteins have been reported to become more susceptible to proteolysis after irradiation (Davies, 1987; Davies, Lin & Pacifici, 1987b).

Disulphide bonds occur in mature sorghum (Oria, Hamaker & Shull, 1995a) and maize (Larkins, Pedersen, Marks & Wilson, 1984) and as indicated before, their, additional formation during cooking is associated with the lower digestibility of wet cooked sorghum (Hamaker, Kirleis, Butler, Axtell & Mertz, 1987; Rom, Shull, Chandrashekar & Kirleis, 1992). Considering that irradiation has the potential to break disulphide and other bonds, and consequently to modify protein structure, both of which are important factors influencing sorghum wet cooked protein digestibility, it was important to investigate the effects of irradiation of sorghum flour before cooking into porridge on its protein digestibility, in comparison with maize.

1.2 LITERATURE REVIEW

This review will examine the concept of protein digestibility, relevant enzymatic methods of determining protein digestibility in sorghum and maize together with protein interactions that affect their digestibility. It will also examine sorghum and maize proteins and the effect of wet cooking on their digestibility. The effect of irradiation on proteins and in particular cereal proteins and how this could affect the digestibility of sorghum and maize proteins will also be reviewed.

1.2.1 Protein Digestibility

The protein digestibility of a food is one of the determinants of its nutritional quality. Protein quality in essence refers to the ability of proteins to meet human nutritional requirements for essential amino acids (Walker, 1983). Before proteins can be utilized by man, they must be digested by the gastric, pancreatic and intestinal proteolytic enzymes in the digestive tract to yield small peptides and amino acids which can then be absorbed into the bloodstream to be utilized by the consuming organism (Swaisgood & Catignani, 1991). To obtain information about the digestibility and nutritional quality of a protein intended for human consumption, it would be ideal to feed it to humans. However, ethical issues involved with using humans for such experiments limit their use. Also, *in vivo* determination of protein digestibility is time-consuming and costly (Pederson & Eggum, 1983). To overcome these shortcomings, rapid *in vitro* assays, which use enzymes similar to those found in the human intestinal tract and carried out under conditions that simulate physiological conditions, have been developed (Walker, 1983). The following section will briefly consider some relevant *in vitro* enzymatic methods that have been used to determine the protein digestibility of cereals, with particular reference to sorghum and maize, and their relationship to *in vivo* digestibility.

1.2.1.1 In Vitro Enzymatic Methods of Determining Protein Digestibility

In vitro enzymatic assays for determining protein digestibility usually require treating the sample with proteolytic enzymes, either singly or in combination. Protein digestibility is then measured as a change in protein solubility, amino nitrogen or free amino acids (Phillips, 1997). These assays either measure the initial rate of hydrolysis, which is then extrapolated to obtain total digestibility, or they measure maximal digestibility values (Boisen & Eggum, 1991).

In single enzyme systems the sample is incubated with one enzyme, usually pepsin, trypsin, pronase, papain or rennin (Boisen & Eggum, 1991). Single enzyme systems using pepsin have been widely used to determine *in vitro* protein digestibility in sorghum and maize (Chibber, Mertz & Axtell, 1980; Mertz, Hassen, Cairns-Whittern, Kirleis, Tu & Axtell, 1984; Hamaker, Kirleis, Mertz & Axtell, 1986; Duodu, Nunes, Delgadillo, Parker, Mills, Belton & Taylor, 2002) as well as of barley (Bhatty & Whitaker, 1987). Digestibility in this case is measured as the amount of nitrogen solubilized by pepsin over a given period and expressed as a percentage of the total nitrogen in the sample (Mertz *et al*., 1984). However, *in vitro* results can only be of practical significance if they correlate with *in vivo* results. In the case of sorghum, Axtell, Kirleis, Hassen, D'Croz-Mason, Mertz & Munck (1981) using *in vitro* pepsin assay, showed that the protein digestibility of cooked sorghum gruel (50%) was similar to that obtained by MacLean, Lopez de Romana, Placko & Graham (1981) using children (47%). Significant correlations have also been obtained between *in vitro* pepsin (Mertz *et al.* 1984) and *in vivo* (MacLean, Lopez de Romana, Gastanaduy & Graham, 1983) protein digestibility with extruded sorghum, and with barley (Bhatty & Whitaker, 1987).

Incubation with two enzymes, trypsin-chymotrypsin in one step has also been used to determine changes in protein digestibility in sorghum (Chibber *et al*., 1980; Hamaker *et al*., 1986) and maize (Hamaker *et al*., 1986) with processing. Chibber *et al*. (1980) investigated the effect of sequential decortication of condensed tannin sorghum on its protein digestibility, whereas Hamaker *et al*. (1986) studied the effect of wet cooking on protein digestibility. In both studies, the digestibility of proteins using these two enzymes

was compared with pepsin digestibility, and it was found that more proteins were solubilized using pepsin enzyme alone than when a combination of trypsin and chymotypsin was used. Chibber *et al*. (1980) concluded that pepsin was more effective in solubilizing sorghum proteins than trypsin and chymotrypsin. Pepsin preferably cleaves bonds containing hydrophobic residues (Huang & Tang, 1968), and has been reported to cleave peptide bonds between Phe-Val, Phe-Thr, Leu-Met, Asp-Tyr, Val-Ala, Leu-Val, Ala-Leu, Leu-Tyr, Tyr-Leu, Phe-Phe, Phe-Tyr, Glu-His, Glu-Ala, Gly-Phe residues (Ryle & Porter, 1959; Huang & Tang, 1968; Etherington & Taylor, 1971). Trypsin cleaves peptide bonds at residues of arginine and lysine, whereas chymotrypsin cleaves peptide bonds at aromatic amino acid residues such as tyrosine, phenylalanine and tryptophan (Brody, 1994). In sorghum and maize, the predominant proteins are the hydrophobic prolamins (Guiragossian, Chibber, Van Scoyoc, Jambunathan & Mertz, 1978; Wilson, 1983; Taylor, Novellie & Liebenberg, 1984b). This could explain why Chibber *et al.* (1980) and Hamaker *et al.* (1986) observed a higher protein digestibility in sorghum and maize using pepsin alone compared to using a combination of trypsin and chymotrypsin. However, a predigestion with pepsin followed by trypsin-chymotrypsin solubilized more proteins, giving a higher digestibility than when either pepsin or trypsin-chymotrypsin was used alone (Hamaker *et al.,* 1986). This could probably be due to the fact that trypsin and chymoytypsin cleaved additional peptide bonds not cleaved by pepsin. Notwithstanding, pepsin used alone, or trypsin and chymotrypsin used together, or a combination of these three enzymes, were all able to differentiate between uncooked and cooked protein digestibility in sorghum and maize

Protein digestibility in cereals has also been determined using a multi-enzyme assay with three enzymes: namely, trypsin, chymotrypsin and peptidase (Pederson & Eggum, 1983; Weaver, Hamaker & Axtell, 1998; Aboubacar, Axtell, Huang & Hamaker, 2001). This assay monitors the rate of peptide release over a 10 min period, and correlates this to the protein digestibility of the sample. The rate of peptide release is determined either by a drop in pH, the pH drop method (Hsu, Vavak, Satterlee & Miller, 1977), or by measuring the amount of sodium hydroxide needed to maintain the pH constant over the assay period, the pH stat method (Pederson & Eggum, 1983). The pH drop method was used to estimate protein digestibility in barley (Bhatty & Whitaker, 1987), but it did not seem to

be as sensitive as the pepsin method, being unable to distinguish differences in protein digestibility between barley genotypes, which the pepsin method did. The inability of the multienzyme assay to differentiate between barley genotypes was attributed to a strong buffering effect by the barley meal, which may have affected pH changes. Rombo (2002) determined protein digestibility in maize porridge prepared from irradiated flour using both the pepsin and pH drop methods. The methods gave opposite results at 2.5 kGy irradiation, with pepsin showing an increase in digestibility and the pH drop multienzyme a reduction in protein digestibility. A low multienzyme digestibility implies that the pH of the sample decreased only slightly under the test conditions. This could suggest that there was some buffering of the pH by the sample. However, the reasons for these differences in digestibility between pepsin and pH drop assays in barley and maize are not quite clear and need to be investigated further.

The pH stat method is less influenced by the buffering capacity of the test material compared to the pH drop method (Pederson & Eggum, 1983), and could therefore be better suited for determining protein digestibility in barley and maize, since it appears that these cereals have a strong buffering capacity that may hinder the determination of their digestibility using the pH drop method. Aboubacar *et al*. (2001) used the pH stat and pepsin assays to determine uncooked protein digestibility in normal and highly digestible sorghum lines and found a strong correlation ($r = 0.86$) between the two methods. Weaver *et al*. (1998) used the pH stat assay together with the pepsin assay to determine differences in digestibility between uncooked normal and high-lysine sorghum and maize. Both methods showed differences between the more digestible high-lysine mutants and the less digestible normal ones. It shows that the pH stat assay can be used and may indeed be better than the pH drop assay in determining digestibility differences of uncooked sorghum flours. It is therefore likely that it could also discriminate between digestibility of uncooked and cooked sorghum and maize proteins. This, however, still needs to be investigated.

1.2.1.2 Protein Interactions that Affect Digestibility

While cooking or heating of food generally denatures proteins and improves their digestibility (Mostafa, 1987; Farag, 1999), these thermal treatments sometimes result in the formation of protein crosslinks that are less digestible and consequently reduce the digestibility of the protein (Erbersdobler, 1989).

When protein foods are heated in the presence of carbohydrates, such as reducing sugars, Maillard reactions can occur between the ∈-amino group of lysine and the carbonyl group of the reducing sugar (Baltes, 1982; Rizzi, 1994). The participation of lysine in Maillard browning results in the destruction of lysine (Mauron, 1982). Products of Maillard reactions are resistant to proteolysis (Öste, Dahlqvist, Sjöström, Norén & Miller, 1986; Öste, Miller, Sjöström & Norén, 1987), and may result in reduced digestibility of proteins in which such crosslinks are present. Other amino acids such as cysteine and leucine may be affected through their reaction with products from the Maillard reaction to form enzyme-resistant crosslinks (Hurrell, 1984). Sorghum and maize are poor in lysine, which means they may be less likely to be affected by Maillard reactions. High-lysine mutants, on the other hand, may be a cause for concern because of their higher lysine content (Mertz, 1992; Weaver *et al.,* 1998). Maillard reactions can also occur during irradiation of foods (Krumhar & Berry, 1990; Cunha, Sgabieri & Damasio, 1993). This will be discussed in the section on irradiation (1.2.5).

Severe heating and/or alkaline treatment of proteins can destroy some essential amino acids through racemization, which is the conversion of amino acids from the L- to the D-form (Hurrell, 1984; Friedman, 1999a, 1999b). The D-form in addition to not being easily hydrolysed by enzymes, is absorbed more slowly and even when absorbed is not utilised by humans (Hurrell, 1984; Cheftel, Cuq & Lorient, 1985). The amino acids most likely to undergo racemization include aspartic acid, tyrosine, glutamic acid, alanine, lysine and leucine (Erbesdobler, 1989). Sorghum and maize prolamin proteins contain high levels of asparagine, glutamine, alanine and leucine (Taylor & Schüssler, 1986; Wilson, 1983) and can therefore be expected to undergo racemization during cooking. Cheftel *et al.* (1985), however, stated that the heat and alkali treatments employed during domestic cooking of

foods, such as solubilization of vegetable proteins and cooking of corn in lime, would result in the formation of only small amounts of lysinoalanine and its derivatives. Racemised amino acids can subsequently undergo β-elimination to form dehydroalanine derivatives, which can then crosslink with the ∈-amino group of lysine residues to form the isopeptide, lysinoalanine (Liardon & Hurrell, 1983; Friedman, 1999a). Other amino acids that can be destroyed by forming isopeptides include cysteine, ornithine, and histidine (Friedman, 1999a).

Amino acids of the same type within a food may crosslink with one another during processing to form less digestible complexes. Cysteine residues within a protein food can become oxidised and crosslink with one another through disulphide bonds (Gerrard, 2002). Heat treatment can enhance formation of disulphide linkages, as observed in sorghum during wet cooking, which in turn would produce less digestible protein polymers (Hamaker *et al*., 1987; Duodu *et al*., 2002) (see below, 1.2.3). Tyrosine amino acids may also become linked through a phenolic coupling of two or three phenoxy radicals of tyrosine to form di- or tri-tyrosine (Otterburn, 1989; Brady, Sadler & Fry, 1996). Crosslinking of tyrosine amino acids with each other has been reported in irradiated foods (Mezgheni, D'Aprano & Lacroix, 1998). More details are provided in the section on irradiation (1.2.5).

1.2.2 Sorghum and Maize Proteins

Sorghum and maize proteins, like other seed proteins, may be classified according to solubility as proposed by Osborne into the albumins, globulins, prolamins and glutelins (Landry & Moureaux, 1970; Guiragossian *et al*., 1978; Taylor, Schüssler & Van der Walt, 1984a).

1.2.2.1 Albumins and Globulins

The albumins and globulins are the salt-soluble proteins in sorghum and maize. They are usually the first fraction to be extracted in a sequential extraction. The proteins extracted

in salt solution contain low molecular weight nitrogen compounds including small peptides and free amino acids, in addition to the albumins and globulins (Wilson, 1983; Taylor *et al* 1984a). To eliminate the salt and low molecular weight nitrogen components in this extract, it is recommended to dialyse them against water (Taylor *et al*., 1984a).

The albumins and globulins together make up about 23% of total proteins in sorghum whole grain flour (Taylor *et al*., 1984a; El Nour, Peruffo & Curioni, 1998) and 19% of total protein in maize whole grain flour (Landry & Moureaux, 1970). However, the proportion of these proteins is much lower in the endosperm, about 7.5% in sorghum (Watterson, Shull & Kirleis, 1993) and about 3.5% in maize (Yau, Bockholt, Smith, Rooney & Waniska, 1999). This is because the albumin and globulin proteins are abundant in the germ (Taylor & Schüssler, 1986; Wilson, 1987), which is removed during the decortication and degerming processes to produce endosperm flour (Table 1.1). The extent of reduction in albumin and globulin content in endosperm flour will therefore depend on the extent of removal of the germ. This fraction is nutritionally important, being rich in the essential amino acid lysine, which happens to be the first limiting amino acid in these cereals (Guiragossian *et al*., 1978; Van Scoyoc, Ejeta & Axtell, 1988; Yau *et al*., 1999).

The nutritional importance of albumins and globulins derives from the fact that the nutritional quality of a protein depends among other factors on its ability to meet human needs for essential amino acids (Walker, 1983). The enhanced lysine content of high-lysine mutant types results primarily from an increase in relatively lysine-rich glutelins plus albumin and globulins and a decrease in the lysine-deficient prolamin proteins (Guiragossian *et al*., 1978; Van Scoyoc *et al.,* 1988; Yau *et al.,* 1999). This makes them nutritionally superior to the normal lines. Albumin and globulin proteins in sorghum consist of a mixture of low molecular weight proteins of M_r 15 and 18 k, and high molecular weight proteins with M_r 38 and 44 k on sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) (Watterson *et al*., 1993).

Table 1.1. The distribution of different protein fractions in the different anatomical parts of normal sorghum and maize types (% of total protein)

Protein fractions	Whole grain		Endosperm		Germ		Pericarp	
	Sorghum[1]	Maize[2]	Sorghum[1]	Maize[2]	Sorghum[1]	Maize[2]	Sorghum[1]	Maize
Albumins + globulins	18.3	12	5.8	6	33.3	52	10.4	N/A
Prolamins	44.0	52	68.3	60	8.7	5	11.6	N/A
Glutelins[3]	18.5	25	14.0	26	6.9	18	18.9	N/A

[1]Average values for 2 sorghums (Taylor & Schüssler, 1986) (% of total protein)

[2]Wilson, 1987 (% of total N)

[3] Extracted glutelins

N/A Not available

1.2.2.2 Prolamins

The prolamins (aqueous-alcohol soluble proteins) are the major protein fraction in sorghum and maize. They are called kafirins in sorghum and zeins in maize, where they are found as protein bodies within the starchy endosperm (Taylor *et al*., 1984b, Lending & Larkins, 1989). Their main function is as nitrogen reserve for the plant (Taylor & Schüssler, 1986; Fukushima, 1991; Shewry, 2002). The prolamins in sorghum and maize cannot all be extracted by aqueous alcohol, alone as some prolamins exist crosslinked with themselves and other proteins and thus require a reducing agent to cleave these crosslinks and assist in their efficient extraction (Esen, 1987; El Nour *et al*., 1998). This gives rise to the uncrosslinked prolamins (prolamin I) extracted with aqueous alcohol alone and the crosslinked prolamin (prolamin II) requiring in addition a reducing agent. The combination of these two fractions makes up the total prolamins. The prolamin I is rich in monomeric α-prolamin with some oligomeric and polymeric components, whereas

prolamin II is rich in β- and γ-prolamins as well as in δ-zeins in maize (Watterson *et al.,* 1993; Shewry, 2002).

Hamaker, Mohamed, Habben, Huang & Larkins (1995) using aqueous alcohol plus reducing agent together with SDS as extracting solvent, reported total zein and kafirin contents of about 50-56% and 68-73% respectively in maize and sorghum whole grain flour. El Nour *et al.* (1998) and Taylor *et al*. (1984a), however, reported lower total prolamin protein contents (50.8 and 48% respectively) for sorghum whole grain flour using a sequential extraction with alcohol without and with reducing agent. The differences in prolamin contents reported by these authors could result from the differences in the extraction procedures used. The addition of SDS probably allowed for a more complete extraction of the prolamins, hence the higher values reported by Hamaker and co-workers. With endosperm flour, Taylor & Schüssler (1986) reported kafirin contents of 67-69% for sorghum, while (Yau *et al*., 1999) obtained zein contents of 52-61% for maize. Prolamin contents of endosperm flour are higher than those from whole-grain flour. This is so, because the removal of the germ entails loss of protein material mostly albumin and globulins, but not prolamins which, as stated, are located in protein bodies in the starchy endosperm (Taylor *et al*., 1984b; Lending & Larkins, 1989). This would then give a higher concentration of prolamin when it is expressed as a percentage of total protein. The prolamins generally contain a high proportion of the amino acids proline, asparagine, glutamine, leucine and alanine, but are low in lysine (Table 1.2) (Wilson, 1983; Taylor & Schüssler, 1986; Larkins, Lending & Wallace, 1993).

Zeins have been separated into α-, β-, γ- (Esen, 1987) and δ-zeins (Esen & Stetler, 1992) based on their solubility differences, amino acid composition, electrophoretic, chromatographic and immunological properties (Table 1.3). The kafirins, based on their similarity to zein (DeRose, Ma, Kwon, Hasnain, Klassy & Hall, 1989) and their solubility differences have been separated into the α-, β- and γ-kafirins (Shull, Watterson & Kirleis, 1991). Delta-kafirin homologues have recently been reported by Chandrashekar & Venkatesha (2001). Table 1.3 summarizes the solubility, sequence features and molecular weight properties of the different prolamin fractions (α-, β-, γ- and δ-) in maize and sorghum proteins.

Table 1.2. Amino acid content (mole % of amino acid) of the different kafirins and zeins

Amino acid	Total zein[1]	α-zein[2]	β-zein[2]	γ-zein[2]	Total kafirin[3]	α-kafirin[2]	β-kafirin[2]	γ-kafirin[2]
Asparagine (N)	4.1	5.3	2.5[a]	0	4.8	6.0	3.3[a]	0
Aspartic acid (D)	N/A	0	N/A	0	N/A	0.4	N/A	0
Threonine (T)	3.1	2.8	2.5	4.4	2.8	4.0	4.6	4.7
Serine (S)	6.5	6.9	5.0	3.9	4.7	6.0	4.6	5.2
Glutamine (Q)	16.4	20.7	18.1[b]	14.7	20.0	24.6	17.8[b]	11.9
Glutamic acid (E)	N/A	0.8	N/A	1.0	N/A	0.4	N/A	1.0
Proline (P)	10.7	8.9	8.8	25	11.2	7.7	9.7	23.3
Glycine (G)	2.5	0.8	8.8	6.4	2.7	1.6	6.8	8.8
Alanine (A)	14.5	13.8	13.8	4.9	15.6	14.9	13.4	5.7
Cysteine (C)	1.0	0.4	4.4	7.4	0.7	0.4	4.9	7.8
Valine (V)	4.0	6.9	1.9	7.4	5.6	4.4	5.2	6.2
Methionine (M)	1.5	2.0	11.3	0.5	1.7	0.8	5.7	1.0
Isoleucine (I)	3.7	4.5	0.6	2.0	4.1	5.6	2.3	2.6
Leucine (L)	19.4	17.1	10.0	9.3	15.4	15.3	12.0	8.3
Tyrosine (Y)	3.6	2.8	8.8	2.0	3.0	2.8	3.0	2.1
Phenylalanine (F)	5.6	3.3	0	1.0	4.7	2.4	1.9	1.6
Histidine (H)	1.1	1.2	0	7.8	1.6	1.2	0.9	7.8
Lysine (K)	0.1	0	0	0	0.2	0	0.5	0
Arginine (R)	1.3	1.6	3.1	2.5	1.2	0.8	2.7	2.1
Tryptophan (W)	N/A	0	N/A	0	N/A	0.4	N/A	0

[1] Esen, Bietz, Paulis & Wall. (1985)

[2] Taylor & Belton (2002)

[3] Evans Schüssler & Taylor (1987)

[a] Asparagine + Aspartic acid are expressed as Asparagine

[b] Glutamine + Glutamic acid are expressed as Glutamine

N/A Not available

Table 1.3. Characterisation of the protein species of zein and kafirin

Name	Molecular weight (k)	Optimum solvent for extraction[a]	Consensus repeat motifs / sequence features[b]	% of total prolamin protein
Maize[1,2,3,4]				
α- zein	21-25	50-95% propan-2-ol	AALQQFPAQL	75-85
β-zein	17, 18	30-85% propan-2-ol + 2-ME	Methionine-rich	10-15
γ-zein	27	Water plus reducing agent and up to 80% propan-2-ol plus reducing agent	8 repeats of PPPVHL; cysteine-rich	5-10
δ-zein	10	Water plus reducing agent and up to 80% propan-2-ol plus reducing agent	No repeats	N/A
Sorghum[4,5,6,7]				
α-kafirin	23, 25	40-90% t-butanol + 2-ME	AALQQFPAQL ANSYLQQ	80
β-kafirin	20, 18, 16	10-60% t-butanol + 2-ME		7-8
γ-kafirin	28	Water plus reducing agent or 10-80% t-butanol plus reducing agent	4 repeats of PPPVHL; cysteine-rich C-terminal domain	9-12
δ-kafirin	15	N/A	Methionine-rich	N/A

[a] 2-ME, 2-mercaptoethanol ; N/A not available

[1] Esen (1987), [2] Esen & Stetler (1992), [3] Lending & Larkins (1989)

[4] Shewry (2002), [5] Shull *et al*. (1991), [6] Watterson *et al*. (1993) [7] Chadrashekar & Venkatesha (2001)

[b] Refer to Table 1.2 for the single letter abbreviation for the amino acids

The amino acid composition of zein and kafirin are very similar; as are those of α-, β-, and γ-zein to their kafirin homologues α-, β-, and γ-kafirin (Table 1.2) (Wilson, 1983; Taylor & Belton, 2002). Detailed sequence analysis of the sorghum kafirin and maize zein using cDNA has revealed extensive sequence homology between the two proteins (DeRose *et al*., 1989) indicating structural similarity between them. Argos, Pederson, Marks & Larkins (1982) studied the M_r 19 and 22 k α-zein proteins using circular dichroism and found them to consist mainly of α-helical and β-turn structures with no β-sheet structure. They proposed a model for the structure of zein based on circular dichroism data and amino acid sequences. In this model (Fig. 1.1), it is proposed that zein consists of a collection of nine helical rods packed in a capsular shaped array with the end pieces of the capsule consisting of a glutamine repeat sequence, which serve to join the capsules together by hydrogen bonding. The helical structures themselves are held together by hydrogen bonds and Van der Waals interactions. However, only the α-zeins were considered in this model, and possibly explains why only α-helical structures were observed. Given the similarities between zein and kafirin proteins (DeRose *et al*., 1989), this model could be applied to the α-kafirin species as well.

Duodu, Tang, Grant, Wellner, Belton & Taylor (2001) using Fourier Transform Infra Red (FTIR) and solid state ^{13}carbon nuclear magnetic resonance (^{13}C NMR) spectroscopy with protein body-enriched flour samples showed that the prolamin proteins in both sorghum and maize as well as high-lysine sorghum mutants contained an abundance of α-helical structures together with β-sheet structures. Alpha-helical structures represented about 55-59% of secondary structure in these samples. These results agree with earlier reports from circular dichroism (CD), optical rotatory dispersion (ORD) and infra red (IR) spectroscopy (Wu, Cluskey & Jones, 1971), which indicated that kafirins had an abundance of α-helical and unordered structures with some β-turns. The kafirins studied by Wu *et al.* (1971) were dissolved in 60% *t*-butanol without a reducing agent, which could mean that mainly the α-kafirins were solubilized (Shull, Watterson & Kirleis, 1992). As stated, α-zein was shown to consist mainly of α-helices and β-turns (Argos *et al*., 1982), and this could explain the observation of only α-helices and β-turns in the kafirins analysed by Wu *et al.* (1971). The α-helical and β-sheet structures reported by Duodu *et al*. (2001) would appear to be more representative of the secondary structure

composition of sorghum and maize prolamin proteins in general, since all the different types of prolamins were taken in to account through the use of protein body enriched samples. Recent studies by Forato, Doriguetto, Fischer, Mascarenhas, Craievich & Colnago (2004), analysed the conformation of Mr 19 zein polypeptide using FTIR and reported 46% α-helix, 22% β-sheets, 23% β-turns and other undefined structures making up 13% of this protein.

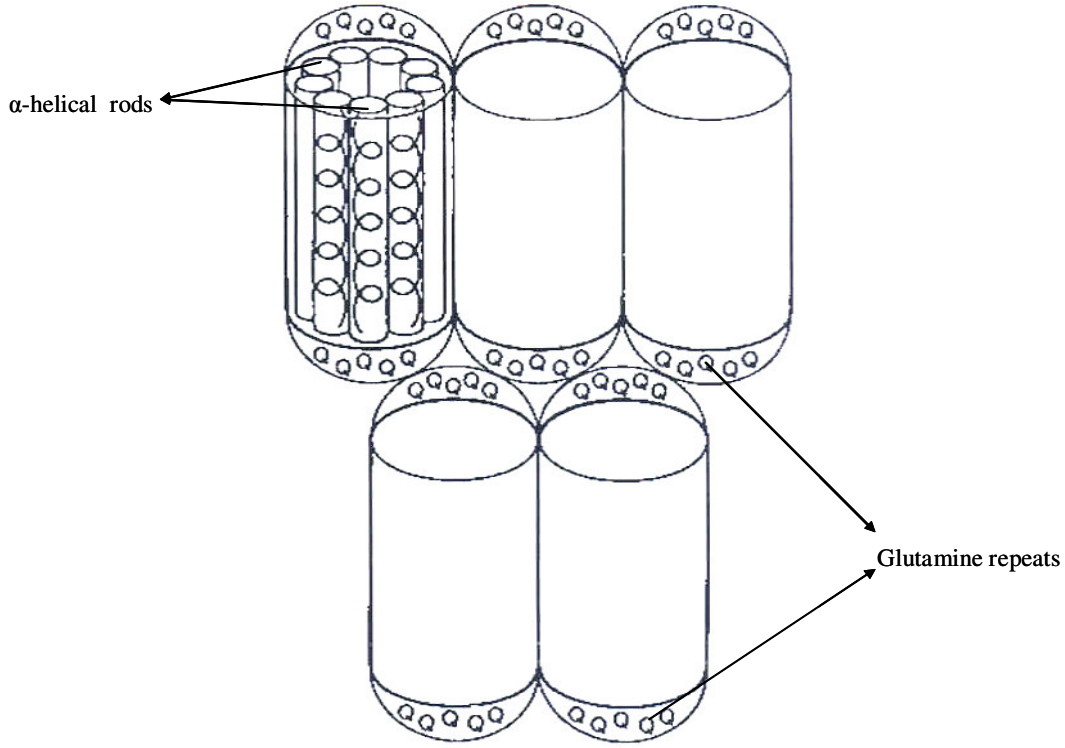

Figure 1.1. Structural model for maize zein (Argos *et al*., 1982)

1.2.2.2.1 Alpha-prolamins

Alpha-kafirin has high concentrations of glutamine together with the hydrophobic amino acids phenylalanine, leucine and isoleucine (Table 1.2) compared to β- and γ-kafirins

which explains the need for higher concentrations of alcohol for their extraction (Shull *et al*., 1992). It is, however, low in sulphur-containing amino acids and lysine. It occupies the central portion of the protein body (Shull *et al*., 1992). The alpha-zeins like their kafirin homologues are located at the centre of the protein body and are rich in proline, glutamine, and in the non-polar amino acids alanine and leucine (Lending & Larkins, 1989; Dombrink-Kurtzman & Bietz, 1993; Shewry, 2002). Alpha-kafirins are the most digestible of all the kafirins when isolated, but in intact protein bodies they are the least digested, because of their central location. This is so because protein bodies are digested from the outside inwards (Oria *et al*., 1995b).

1.2.2.2.2 Beta-prolamins

The β-kafirins compared to the α-kafirins, have low amounts of the hydrophobic amino acids phenylalanine, leucine and isoleucine but have a high content of the hydrophilic amino acid glycine (Table 1.2), which makes them soluble in low concentrations of alcohol (10-60% *tert*-butanol plus 2-mercaptoethanol) (Shull *et al.,* 1992). The β-kafirins and β-zeins are rich in methionine and cysteine with some of the cysteine residues forming interchain disulphide bonds. The latter explains the need for a reducing agent to efficiently extract them (Esen, 1987; Shull *et al*., 1992; Shewry, 2002). Unlike the α-kafirins, the β-kafirins do not contain repeated sequences or have a clear domain structure, but the methionine residues tend to be clustered (Shewry, 2002). Esen *et al*. (1985) found no N-terminal sequence homology between β-zeins and the other zeins and suggested that they may have a unique primary structure. Of all the zein proteins, β-zein is lowest in proline, valine, isoleucine, histidine and phenylalanine, but has high levels of methionine, glycine, alanine, tyrosine and arginine with four repeats each of Ala-Gly and Gly-Leu (Esen *et al.,* 1985). β-kafirins (Shull *et al.,* 1992) and β-zeins (Ludevid, Torrent, Martinez-Izquierdo, Puigdomenech & Palau, 1984) are located at the periphery of the protein body and also occur as inclusions within the protein body. Beta-kafirins in intact protein bodies are more digestible compared to α-kafirins, because of their peripheral location in the protein body that exposes it to proteolytic enzymes before α-kafirin (Oria *et al*., 1995b).

1.2.2.2.3 Gamma-prolamins

They are rich in the hydrophilic amino acids glycine and histidine (Table 1.2) and this could be the reason why they are extractable in low concentrations of alcohol (Esen, 1987; Shull *et al*., 1992) or even in water containing a reducing agent (Esen, 1986; Taylor, Von Benecke & Carlsson, 1989). Gamma-kafirin contains high levels of cysteine and proline with low levels of lysine and aspartic acid (Watterson, Shull, Mohamed, Reddy & Kirleis, 1990). The high amounts of cysteine in both γ-zein (Esen, 1987) and γ-kafirin (Shull *et al*., 1992) makes them prone to forming intermolecular disulphide bonds, hence the need for a reducing agent during their extraction. Gamma-kafirins contain the highest levels of disulphide crosslinked compounds compared to α- and β-kafirin (Oria *et al*., 1995b). The γ-prolamins differ from the other prolamins in being soluble in water when reduced, although a reducing agent is required for efficient extraction (Esen, 1986; Watterson *et al.,* 1993). They contain an N-terminal domain comprising highly conserved hexapeptide repeats (PPPVHL) with eight copies being present in γ-zein and four in γ-kafirin (Shewry, 2002). Gamma-kafirin (Shull *et al.,* 1992) and γ-zein (Ludevid *et al*., 1984) are found at the periphery of the protein bodies, together with the β-prolamins, and also as inclusions within the protein body. Gamma-kafirin, like β-kafirin, is more digestible than α-kafirins in intact protein bodies, also due to its peripheral location (Oria *et al.,* 1995b).

1.2.2.2.4 Delta-prolamins

Delta-zein comprises polypeptides with M_r of about 10 k and may be present at the protein body core interspersed with α- and γ-zein (Esen & Stetler, 1992). Delta-kafirin on the other hand has a reported M_r of about 15 k, and is rich in methionine. Its amino acid sequence consists of 134 amino acids with no repeat sequence (Chandrashekar & Venkatesha, 2001). Delta-zeins also do not contain repeated amino acid sequences but resembles the β-zeins in its amino acid composition (Shewry, 2002). It shows extensive immunological cross reactivity with α- and γ-zein (Esen & Stetler, 1992).

1.2.2.3 Glutelins

The glutelins are classified as proteins extractable in dilute alkali or the residue left after extraction of the salt soluble and aqueous alcohol soluble proteins (Wilson, 1983). Glutelins have been quantified as the amount of proteins remaining after sequential extraction with salt and aqueous alcohol without and with a reducing agent and found to represent less than 30% of total proteins in sorghum whole grain flour (Taylor *et al*., 1984a) and 24–29 % in sorghum endosperm flour (Taylor & Schüssler, 1986; Watterson *et al.*, 1993). The glutelins account for about 30-40% of maize proteins (Landry & Moureaux, 1970). They are the second largest protein fractions in sorghum after the prolamins (Taylor & Schüssler, 1986). The endosperm glutelins are high molecular weight proteins and are primarily located in the matrix surrounding the protein bodies (Taylor *et al*., 1984b). These glutelin polymers are crosslinked by intermolecular disulphide bonds (Wall, 1971; Wilson, 1983). Their amino acid pattern is similar to that of the albumins and globulins, both of which are high in lysine and thus are of good nutritional quality (Wilson, 1983).

1.2.3 Effect of Wet Cooking on Sorghum and Maize Protein Digestibility

Sorghum in the uncooked state has been reported to have a lower protein digestibility when compared to maize and other cereals such as rice and wheat (Axtell *et al*., 1981; Mertz *et al*., 1984; Hamaker *et al*., 1987; Duodu *et al*., 2002). The lower digestibility of sorghum proteins in the uncooked state is thought to be due to the formation of less digestible disulphide bonded protein polymers at the later stages of grain development up until maturity (Oria *et al*., 1995b). Duodu *et al*., (2002) however, stated that uncooked sorghum protein digestibility may not always be lower than that of uncooked maize but depends on the origin and physical form of the uncooked sorghum fractions. They observed a progressive increase in protein digestibility of uncooked sorghum flour as its composition varied from whole grain, through endosperm to protein body enriched flour (Table 1.4).

Table 1.4. Effect of wet cooking on the *in vitro* protein digestibility of sorghum and maize

Flour Sample	Protein Digestibility (%)		Enzyme Assay	Reference
	Uncooked	Cooked		
Sorghum whole grain	80.7	64.8	Pepsin	Hamaker *et al*. (1986)
	72.7	57.9	TC[1]	Hamaker *et al*. (1986)
	87.6	70.5	P-TC[2]	Hamaker *et al*. (1986)
Maize whole grain	81.5	81.9	Pepsin	Hamaker *et al*. (1986)
	79.4	87.7	TC	Hamaker *et al*. (1986)
	88.3	90.7	P-TC	Hamaker *et al*. (1986)
Sorghum whole grain	80.8	56.3	Pepsin	Hamaker *et al*. (1987)
Maize whole grain	83.4	79.3	Pepsin	Hamaker *et al*. (1987)
Sorghum whole grain	79.0	58.0	Pepsin	Rom *et al*. (1992)
Sorghum decorticated	69.2	43.6	Pepsin	Oria *et al*. (1995b)
Sorghum whole grain	73.2	55.2	Pepsin	Oria *et al*. (1995a)
Red sorghum whole grain	59.1	30.5	Pepsin	Duodu *et al*. (2002)
Red sorghum endosperm	65.7	35.9	Pepsin	Duodu *et al*. (2002)

Table 1.4 continues on next page

Table 1.4 continued

Red sorghum protein body preparation	72.8	44.2	Pepsin	Duodu *et al*. (2002)
White sorghum whole grain	55.8	36.6	Pepsin	Duodu *et al*. (2002)
White sorghum endosperm	67.4	39.4	Pepsin	Duodu *et al*. (2002)
White sorghum protein body preparation	74.3	63.5	Pepsin	Duodu *et al*. (2002)
Maize whole grain	66.6	62.0	Pepsin	Duodu *et al*. (2002)
Maize endosperm	67.4	63.6	Pepsin	Duodu *et al*. (2002)
Maize protein body preparation	68.8	67.4	Pepsin	Duodu *et al*. (2002)
Red sorghum whole grain	68.6	32.7	Pepsin	Nunes *et al*. (2004)
Maize whole grain	66.0	64.5	Pepsin	Nunes *et al*. (2004)
High lysine sorghums				
P721Q decorticated	86.7	71.9	Pepsin	Weaver *et al*. (1998)
P851171 decorticated	88.3	72.5	Pepsin	Weaver *et al*. (1998)
P850029 decorticated	89.4	75.1	Pepsin	Weaver *et al*. (1998)

[1]TC, Trypsin Chymotrypsin; [2]P-TC, Pepsin, Trypsin, Chymotrypsin

Upon wet cooking, as in porridge making, the digestibility of sorghum proteins decreases substantially compared to that of maize. This observation has been made both in human studies (Kurien, Narayanarao, Swaminathan & Subrahmanyan, 1960; MacLean *et al*., 1981, 1983) and *in vitro* protein digestibility studies (Axtell *et al*., 1981; Hamaker *et al.*, 1986, 1987; Oria *et al.,* 1995a, 1995b; Duodu *et al*., 2002). Kurien *et al*. (1960) observed a progressive reduction in digestibility of rice diets of schoolboys aged 10-11 years with increasing sorghum substitution. Digestibility decreased from 74.9% in an all rice diet to 55.2% in an all sorghum diet. In another study with Peruvian children MacLean *et al*. (1981) reported low *in vivo* digestibility (46%) for gruels made from sorghum, whereas rice, maize and wheat gruels had a higher digestibility, 66, 73 and 81% respectively. In *in vitro* studies using pepsin, wet cooking brought about a 24.5% reduction in protein digestibility of sorghum, while digestibility of maize only decreased by 4.1% (Hamaker *et al*., 1987). Numerous *in vitro* studies have established the reduced protein digestibility of wet cooked sorghum. Table 1.4 summarises several studies showing the effect of wet cooking on sorghum and maize protein digestibility.

The reduction in protein digestibility of sorghum on wet cooking has generally been attributed to the formation of disulphide linked protein polymers during cooking, which are resistant to proteolysis and as such could lead to a reduction in digestibility (Hamaker *et al*., 1987; Rom *et al.,* 1992; Oria *et al*., 1995b; Duodu *et al.,* 2003). Oria *et al*. (1995b) postulated that during wet cooking, β- and γ-kafirins could crosslink with themselves or with matrix proteins through disulphide bonds to form polymers that are resistant to proteolytic digestion.

Since the majority of β- and γ-kafirin proteins are found at the periphery of the protein body (Shull *et al.,* 1992), crosslinks between them could form a barrier around the protein body which could in turn restrict access of proteolytic enzymes to α-kafirins which are located within the protein body. The α-kafirins in isolation are more digestible than the β- and γ-kafirins (Oria *et al.,* 1995b) and represent the bulk (80%) of the kafirins (Watterson *et al.,* 1993). If the digestibility of α-kafirin is impaired, it could therefore result in a reduction in protein digestibility of sorghum. Pepsin indigestible residues from cooked sorghum comprised mainly α-kafirin (Oria *et al.,* 1995b) together with β- and γ-kafirin

(Oria *et al.,* 1995b; Nunes, Correia, Barros & Delgadillo, 2004), indicating that the reduced digestibility of wet cooked sorghum proteins may indeed be related to poor digestion of α-kafirin.

It has been demonstrated in uncooked sorghum, using scanning electron microscopy (SEM), that protein bodies are digested by pitting from the outer surface towards the centre (Rom *et al.,* 1992) as has been observed during germination using transmission electron microscopy (TEM) (Taylor, Novellie & Liebenberg, 1985). This would imply that β- and γ-kafirin at the protein body periphery would be degraded before the α-kafirins at the interior. Using TEM, Oria *et al.* (1995b) observed that in cooked sorghum the majority of the protein bodies remained undigested after treatment with pepsin. As stated, it has been postulated that during cooking β- and γ-kafirin located at the periphery of the protein body form less digestible disulphide bonded protein polymers with themselves and matrix proteins to form a protective coat around the protein body which is resistant to digestive enzymes (Oria *et al.,* 1995b). The TEM observations by Oria *et al.* (1995b) are therefore consistent with the formation of disulphide bonds as proposed by Hamaker *et al.* (1987).

A problem with this theory is that disulphide bonds are also formed in maize during wet cooking, but the digestibility of maize is not reduced to the same extent as that of sorghum (Hamaker *et al.*, 1987; Duodu *et al.*, 2002). It, however appears, that more disulphide-bonded oligomers are formed in sorghum during cooking compared to maize, which may in part explain the lower digestibility of sorghum proteins (Duodu *et al.*, 2002). However, the small differences in the amount of disulphide bonds formed cannot completely explain the differences in reduction in digestibility in sorghum and maize on wet cooking (Duodu *et al.*, 2003). The possibility of non-disulphide crosslinks participating in reducing protein digestibility of wet cooked sorghum has been suggested. Alkylation of uncooked kafirin and zein points to the possible formation of non-disulphide crosslinks during cooking (Duodu, 2000). In the alkylation process, free thiol groups are reacted with an alkylating agent such as iodoacetamide or iodoacetate to prevent their oxidation to form disulphide bonds (Hollecker, 1997). Alkylated uncooked and cooked kafirin and zein samples were found to be more digestible compared to

unalkylated samples (Duodu, 2000). However, alkylated and cooked kafirin had a much lower digestibility than alkylated uncooked kafirin, suggesting that non-disulphide crosslinks may also be involved in reducing protein digestibility of wet cooked sorghum. High molecular weight kafirin oligomers of M_r 45–60 k (Duodu *et al.,* 2002; Nunes *et al.,* 2004) have been identified in wet cooked sorghum, and in the pepsin indigestible residues from cooked sorghum. These oligomers were not completely cleaved by a reducing agent, also suggesting that disulphide bond formation may not be the only reason for the reduction in sorghum protein digestibility with wet cooking.

The kafirins and the zeins are known to be hydrophobic proteins, but the kafirins appear to be slightly less soluble, hence more hydrophobic than the zein (Wall & Paulis, 1978). The slightly hydrophobic nature of kafirin could also contribute to the difference in its protein digestibility compared to zein, since enzymes function in an aqueous environment. Therefore, if kafirins are more hydrophobic than zeins, they may be less accessible to the enzymes and consequently less digestible (Duodu *et al*., 2003). In fact, α-zein and α-kafirin have the same level of hydrophobicity, whereas it is the γ-kafirin that is slightly more hydrophobic than γ-zein (Duodu *et al*., 2002). Considering that the γ-kafirin and γ-zein are located primarily at the periphery of the protein bodies and come in contact with the enzyme first, a more hydrophobic γ-kafirin could compound the problem of an already low digestible disulphide bonded γ-kafirin (Oria *et al*., 1995b), and hence, reduce the digestibility of the protein even further.

Duodu *et al*. (2001) studied the changes brought about in the secondary structure of sorghum and maize proteins by wet cooking using FTIR and ^{13}C NMR. They found that during wet cooking the proteins assume a more antiparallel intermolecular β-sheet structure, accompanied by a reduction in α-helices. It is possible therefore that the change to β-sheet conformation during cooking could encourage the formation of disulphide crosslinks between polypeptides in close proximity, resulting in a rigid, less digestible structure (Duodu *et al*., 2001). The same structural changes have been observed in high lysine sorghums and also in popped sorghum (Duodu *et al*., 2001). However, the maize, high lysine sorghum and popped sorghum were more digestible than the normal sorghum

when cooked (Parker, Grant, Rigby, Belton & Taylor, 1999; Duodu *et al*., 2001; 2002). The changes in protein secondary structure, as with the reduction in digestibility, occurred to a slightly greater extent in normal sorghum than in maize, high-lysine sorghum and popped sorghum. It is possible therefore, that the formation of β-sheet structure could be related to the decrease in protein digestibility of wet cooked sorghum and maize. Notwithstanding this, it is difficult to believe that these small structural differences could alone account for the large decreases in sorghum protein digestibility. In spite of all the possible reasons advanced for the observed differences between sorghum and maize cooked protein digestibility, the exact reasons for this are still not known.

Besides wet cooking, the condensed tannins present in some sorghum cultivars can bind the sorghum proteins and render them less digestible (Butler, Riedl, Lebryk & Blutt, 1984). The kafirin (Emmambux & Taylor, 2003) together with the salt-soluble proteins (Jambunathan & Mertz, 1973; Chibber, Mertz & Axtell, 1978) are the ones most affected by tannin binding. Cell walls may also form a barrier restricting access of proteolytic enzymes to proteins within the endosperm cells (Duodu *et al*., 2002) contributing to reducing digestibility.

In high-lysine sorghum mutants, higher uncooked and cooked protein digestibility (Table 1.4) was found to be a result of more rapid digestion of the main sorghum storage protein α-kafirin (Weaver *et al*., 1998). In these sorghum mutants (Fig. 1.2), the protein body structure is not spherical but highly invaginated and α-kafirin is exposed at the surface of the protein body with γ-kafirin located at the base of the invaginations (Oria, Hamaker, Axtell & Huang, 2000). The more digestible α-kafirin is thus directly exposed to proteolytic enzymes during digestion, accounting for the higher protein digestibility in these mutants. It therefore appears that the structure of sorghum protein bodies influences the access of digestive enzymes to the kafirin proteins and accordingly its digestibility. However, one drawback with these high-lysine mutants is that their endosperms of are of a floury type (Mertz, 1992; Weaver *et al*., 1998) making them more prone to insect damage (Mertz, 1992).

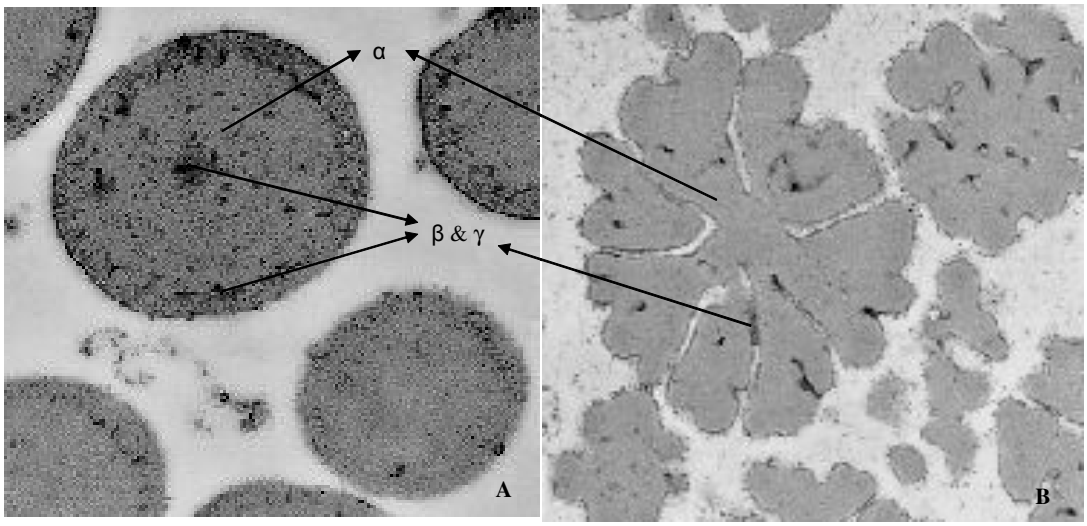

Figure 1.2. Sorghum protein bodies in a protein matrix. (A) Spherical protein body of a normal sorghum. (B) Protein body of a high digestibility sorghum mutant with invaginations (Oria *et al*., 2000)

1.2.4 Effect of Some Processing Methods on Sorghum and Maize Protein Digestibility

The reduction in protein digestibility obtained with wet cooking in sorghum has not been observed when dry heat treatments were applied to sorghum. Popping is a dry heat treatment that expands the starch granules in the grain to produce an expanded grain (Parker *et al*., 1999). *In vitro* protein digestibility of sorghum and maize grains was not reduced by popping (Parker *et al*., 1999). Popping induces fragmentation of the cell walls that will in turn allow proteolytic enzymes greater access to the proteins, resulting in a high digestibility (Parker *et al*., 1999).

Attempts have been made to improve wet cooked digestibility of sorghum through processing with varying degrees of success. When the reducing agents, sodium

metabisulphite, ascorbic acid or L-cysteine (El Khalifa, Chandrashekar, Mohamed & El Tinay, 1999), sodium bisulfite (Rom *et al*., 1992; Arbab & El Tinay, 1997), and 2-mercaptoethanol, sodium bisulphite, dithiotheitol or L-cysteine (Hamaker *et al*., 1987) were mixed with sorghum flour before cooking they prevented the reduction in protein digestibility observed on wet cooking. Protein digestibility of sorghum cooked under reducing conditions was 25% higher than the digestibility of sorghum cooked in water alone (Hamaker *et al.,* 1987). When maize flour was cooked with reducing agent, protein digestibility increased by only 5% (Hamaker *et al*., 1987). According to these authors, the fact that reducing agents could prevent a reduction in sorghum protein digestibility on wet cooking supports the proposition that the formation of disulphide bonds during cooking plays a major role in making sorghum gruels less digestible.

Extrusion cooking has been found to improve cooked sorghum protein digestibility (MacLean *et al*., 1983; Mertz *et al*., 1984; Fapojuwo, Maga & Jansen, 1987; Hamaker, Mertz & Axtell, 1994). In fact, the proteins of extruded sorghum were found to be well digested *in vitro* (79%) (Mertz *et al*., 1984) and *in vivo* (81%) (MacLean *et al*., 1983) compared to the wet cooked sorghum where *in vitro* protein digestibility was only 57% (Mertz *et al*., 1984). The extrusion cooking process employs heat and shearing action that could disrupt the structure of the protein bodies, possibly breaking disulphide and other bonds. This would in turn facilitate access by digestive enzymes to the more digestible α-kafirin at the centre of the protein body, hence the increase in digestibility (Hamaker *et al*., 1994).

Fermentation gives some minimal improvement in cooked sorghum protein digestibility (Au & Fields, 1981; Kazanas & Fields, 1981; El Khalifa & El Tinay, 1995; Hassan & El Tinay, 1995; Taylor & Taylor, 2002). A modification in protein structure rather than breakdown of proteins to smaller components was proposed as the mechanism by which fermentation improved protein digestibility (Taylor & Taylor, 2002).

From the foregoing it appears that the hindering of α-kafirin digestion by crosslinking involving the γ- and β-kafirins with themselves and with matrix proteins in conjunction with their peripheral location in the protein body is the most important factor influencing

protein digestibility in sorghum. It would appear therefore, that, if kafirin structure is modified to impede crosslinking of γ- and β-kafirins with themselves and matrix proteins, this could allow proteolytic enzymes better access to α-kafirins and other proteins, and contribute towards enhancing cooked sorghum protein digestibility.

1.2.5 Food Irradiation

Food irradiation involves treating foods with ionising radiation (γ-rays, X-rays and electron beam) (WHO, 1988). It is called ionising radiation because it removes electrons from atoms and molecules and in the process converts them into ions (Thakur and Singh, 1994). However, not all kinds of ionising rays may be used with foods. The α–particles for example are not used because of their low penetrating power, whereas high-energy electrons and X–rays generated above certain energy levels may induce radioactivity in the irradiated food. In view of these constraints a joint FAO/IAEA/WHO expert committee meeting recommended the following types of ionising radiation for use in foods (FAO/IAEA/WHO, 1981):

Gamma rays from ^{60}Co and ^{137}Cs at energy levels of 5 MeV (1 eV = 1.6 x 10^{-19}J)

X–rays generated from machine sources at or below energy levels of 5 MeV

Electrons generated from machine sources at or below an energy level of 10 MeV.

1.2.5.1 Mechanism of Action of Ionizing Radiation

When ionising radiation penetrates food, the atoms and molecules in the food absorb its energy. Depending on the amount of energy absorbed these atoms and molecules could either become excited (I), lose an electron (II) or dissociate (III) to form ions or free radicals. These ions and excited species are unstable and chemically reactive. They can react with each other or with other components in the food to form stable products and in the process modify food components (Urbain, 1986, Thakur & Singh, 1994).

$RH \rightarrow {}^{\cdot}RH$ Excitation (I)

$RH \rightarrow {}^{\cdot}RH^{+} + e^{-}$ Ionization (II)

$RH \rightarrow {}^{\cdot}R + {}^{\cdot}H$ Dissociation (III)

In dry food systems, the ions and excited species formed are not completely free to move about and thus combine with free radicals and ions in their vicinity to form stable products (Thakur & Singh, 1994). In such systems therefore, food molecules would be modified mostly through direct interaction between ionising radiation and the food molecule (Garrison, 1987; Kempner, 1993; Thakur & Singh, 1994).

On the other hand, in moist systems, the water molecules are ionised to produce reactive species that can move about and react with other ions and free radicals causing extensive modification in food macromolecules (Kempner, 1993; Thakur & Singh, 1994). The effects of irradiation in foods will therefore be expected to be more pronounced in moist than in dry systems given the additional effects of water radiolysis products (Kempner, 1993) and the increased accessibility of the free radicals (Cho, Yang & Song, 1999).

Radiolysis of water

$H_2O \rightarrow {}^{\cdot}OH + e^{-}_{aq} + {}^{\cdot}H + H_2O_2 + H_3O^{+}$ (Thakur & Singh, 1994)

1.2.5.2 Effect of Irradiation on Proteins

Proteins are made up of amino acids linked together by peptide bonds (Cheftel *et al.*, 1985). Changes in proteins brought about by irradiation will therefore be reflected as changes in their primary, secondary and tertiary structures (Kempner, 1993).

Some amino acids such as the sulphur containing and aromatic amino acids are reported to be more susceptible to radiation damage than the others (Khattak & Klopfenstein, 1989; Diehl, 1990; Swallow, 1991). When maize grain was irradiated at 5 kGy methionine and cysteine contents both decreased by about 18% (Khattak & Klopfenstein, 1989). In another study by Hooshmand & Klopfenstein (1995) maize grains were surface

sterilised in 80% aqueous methanol, followed by 2% sodium hypochlorite (NaOCl), washed with distilled water and air dried. The dried grains were then milled into flour and irradiated. At low doses of 5 kGy there was no significant difference in cysteine and methionine contents. When the irradiation dose was increased to 20 kGy, methionine content decreased by about 18%, whereas cysteine content was not significantly affected. The reason for the differences in the effects of irradiation on cysteine and methionine contents in maize in these two studies is not clear, but could be related to variability that occurs in the amino acid analysis (Finley, 1982). In both studies the proteins were hydrolysed with *p*-toluenesulphonic acid and amino acids determined chromatographically using an amino acid analyser. As explained earlier, the β- and γ-prolamin proteins of sorghum and maize that have been proposed to form disulphide crosslinks during cooking and cause the reduction in protein digestibility in wet cooked sorghum (Hamaker *et al*., 1987) contain high levels of cysteine (Shewry, 2002). If this amino acid is affected by irradiation, then these proteins will also be affected and this may influence digestibility.

Destruction of amino acids during irradiation can occur through dimerisation, which is the combination of two amino acid radicals to form dimers or trimers (Swallow, 1991). An example is bityrosine, a covalently bound biphenol, produced by the reaction of two tyrosyl radicals or a tyrosyl radical and a tyrosine molecule (Davies *et al.,* 1987a; Tuce, Janata, Radojčić & Milosavljević, 2001). Irradiation of caseinates to produce edible film enhanced crosslinking of tyrosine residues (Mezgheni *et al.,* 1998). Bityrosine formation in these films increased with increasing radiation dose from 8 to 128 kGy. Intermolecular bityrosine formation has been implicated in the crosslinking of proteins during irradiation (Davies, 1987; Davies & Delsignore, 1987). Such crosslinks may impair the digestibility of food proteins (Davies *et al*., 1987b). Cysteine molecules in foods could also crosslink during irradiation to form disulphide crosslinks, which leads to polymerisation of proteins (Swallow, 1991). As discussed, disulphide crosslinks are the major cause of reduced cooked protein digestibility in wet cooked sorghum. If such crosslinks are formed during irradiation of sorghum, it may further compound the problem of reduced digestibility when sorghum is wet cooked.

Aggregation of proteins is common when food proteins are irradiated in the absence of oxygen. However, in oxygenated systems, irradiation of proteins yields mainly fragmentation products (Schuessler & Schilling, 1984; Puchala & Schuessler, 1993; Tuce *et al*., 2001). Using bovine serum albumin (BSA) as a model protein, Schuessler & Schilling (1984) proposed a mechanism for the fragmentation of proteins in oxygenated systems. This begins with an attack on the protein by the $OH^{\cdot}$ radical to form a protein radical (1), which then reacts with oxygen to form a peroxy radical (2). Two peroxy radicals can combine to form an oxyradical (3), which can later decompose into fragments (F) (4). If oxygen is absent the protein radicals can combine to form aggregates also known as protein polymers or protein crosslinks.

$$PH + OH^{\cdot} \rightarrow P^{\cdot} + H_2O \qquad (1)$$

$$P^{\cdot} + O_2 \rightarrow PO_2^{\cdot} \qquad (2)$$

$$2PO_2^{\cdot} \rightarrow 2PO^{\cdot} + O_2 \qquad (3)$$

$$PO^{\cdot} \rightarrow F_1 + F_2 \qquad (4)$$

However, in practice foods will not normally be irradiated under oxygen or nitrogen. In practical food irradiation therefore the effects of oxygen may be minimal because the oxygen present in the system becomes depleted as irradiation proceeds (Swallow, 1991). This appears to suggest that at higher doses of irradiation aggregation of proteins may be favoured because of the absence of oxygen, if the oxygen in the system gets used up and is not replenished. However, before either fragmentation or aggregation can take place the proteins first have to be denatured, as the unfolding of the proteins facilitates both fragmentation and aggregation (Davies & Delsignore, 1987). Thus, at low doses of irradiation fragmentation of proteins is more likely to occur, whereas as the dose is increased the unfolded or fragmented proteins may then aggregate to form high molecular weight protein polymers. This has been demonstrated with irradiated (0.5-10 kGy) solutions of egg white proteins (ovalbumin, ovomucoid, ovotransferrin) (Kume & Matsuda, 1995; Moon & Song, 2000), BSA and lactoglobulin solutions irradiated at 0.5-10 kGy (Cho *et al*., 1999) and in irradiated (1-10 kGy) bovine and porcine plasma protein solutions (Lee, Lee & Song, 2003). In these studies, the formation of high molecular

weight polymers was negligible at low doses, but increased with increasing irradiation dose. Fragmentation and aggregation of proteins may lead to changes in protein structure. Such changes in proteins have been shown to influence their susceptibility to proteolysis (Davies, 1987; Davies *et al*., 1987b).

Proline residues have been identified as possible targets for peptide chain scission because tertiary amide bonds involving proline are easier to oxidise than secondary amide bonds that are formed by the other amino acids (Schuessler & Schilling, 1984). Gamma-prolamin proteins of sorghum and maize are high in proline and could be fragmented by irradiation. Fragmentation of proteins would perhaps expose more peptide bonds to proteolytic attack. Splitting of disulphide bonds could also cause fragmentation of the proteins (Di Simplicio *et al*., 1991).

Lysine is a limiting amino acid in most cereals including sorghum and maize (Van Scoyoc *et al*., 1988; Yau *et al*., 1999) and it may be destroyed by irradiation. Lysine content decreased by 7 and 13% respectively, when maize and wheat flours were irradiated at 7.5 kGy (Hooshmand & Klopfenstein, 1995). Irradiation (5 kGy) of maize and wheat grains reduced lysine content by 12 and 11%, respectively (Khattak & Klopfenstein, 1989). On the other hand, the lysine contents of isolated wheat gluten and free lysine content of wheat flour were not affected by irradiation at 10 kGy (Srinivas, Ananthaswamy, Vakil & Sreenivasan, 1972). The mechanism of lysine loss or destruction by irradiation is not certain but may be similar to what occurs when food is heated, given that the chemical effects of irradiation on foods are similar to those of heat treatment (Diehl, 1990; Kume & Matsuda, 1995). When protein foods are heated, they may undergo Maillard reactions in which the $\in$-amino group of lysine reacts with the carbonyl group of a reducing sugar resulting in destruction of lysine (Hurrell, 1984). Similar reactions may occur during irradiation of proteins and cause loss of lysine. Evidence of Maillard reactions occurring during irradiation of proteins has been found by Krumhar & Berry (1990) in BSA and bovine lactalbumin and also in beans by Cunha *et al.* (1993). Irradiation of maize flour (Rombo, Taylor & Minnaar, 2001) and of rice grains (Wootton, Djojonegoro & Driscoll, 1988) resulted in darkening of these products and was attributed to Maillard reactions.

In spite of the fact that irradiation may cause loss of some essential amino acids it has also been reported to improve absorption of some amino acids and proteins. Lysine availability in maize grains increased by 7.5% following irradiation at 5 kGy (Khattak & Klopfenstein, 1989). Irradiation (30-90 kGy) of coarsely ground barley and oats improved amino acid absorption and feed utilisation in chicks (Campbell, Classen & Ballance, 1986). It was suggested that increased feed absorption and utilisation in this case was not linked to a direct effect of irradiation on the amino acids or the proteins but rather to the depolymerisation of β-glucans that reduced the viscosity of the feed, thereby improving protein absorption by chicks. Apparently, the observed effects of irradiation on proteins in food systems, as opposed to pure systems, may not always be directly related to the effect of irradiation on the proteins themselves but could be a consequence of irradiation effects on different components of the food. As stated, some sorghums contain condensed tannins that can complex with proteins and render them less digestible (Butler *et al*., 1984). Reduction of tannins with irradiation has been reported in sorghum (Abu-Tarboush, 1998) and could improve the protein digestibility of condensed tannin sorghums.

Moon & Song (2000) used circular dichroism CD to measure changes in protein secondary structure of egg white proteins, ovalbumin, ovomucoid and ovotransferrin with irradiation up to 10 kGy. Ovalbumin and ovomucoid have mostly α-helical and β-sheet structures, respectively. When solutions of these proteins were irradiated at 10 kGy there was an increase in unordered structures and random coils, suggesting that the covalent bonds holding the proteins together in their native conformation had been cleaved leading to denaturation and unfolding of the proteins. Sorghum and maize proteins both have α-helical and β-sheet structures in the uncooked state (Duodu *et al*., 2001). Irradiation could have similar effects on these proteins (denaturation and unfolding), that may prevent disulphide bond formation during subsequent cooking and thus prevent the reduction in protein digestibility with wet cooking; this, together with the fact that irradiation can cleave disulphide bonds (Di Simplicio *et al.*, 1991; Köksel *et al*., 1998), which also occur in the proteins of mature sorghum and maize (Vivas-Rodriguez, Waniska & Rooney, 1992; Hamaker *et al*., 1994).

Glutenin proteins of wheat are polymeric proteins linked by intermolecular disulphide bonds (Stauffer, 1998). Srinivas *et al.* (1972) found that irradiation of wheat flour at 10 kGy resulted in a reduction in the glutenin chromatographic peaks, and an increase in the low molecular weight proteins, suggesting that disulphide bonds of glutenin may have been cleaved to fragment proteins. Similar observations have been made by Köksel *et al.* (1998) who irradiated wheat at 2.5-20 kGy and extracted the 50% 1-propanol insoluble glutenin proteins. This fraction consists of HMW-GS (high molecular weight-glutenin subunits) that are believed to be directly related to dough quality (Stauffer, 1998). Using CD, size exclusion HPLC and SDS-PAGE, Köksel *et al.* (1998) investigated the effects of gamma irradiation on the 50% 1-propanol insoluble glutenin and gliadin fractions. Wheat gliadins are single chain proteins with no crosslinks (Stauffer, 1998), and these were not affected by gamma irradiation. On the other hand, the concentrations of HMW-GS and LMW-GS (low molecular weight-glutenin subunits) in the 50% 1-propanol insoluble glutenin fraction were both reduced by irradiation at 10 and 20 kGy (Köksel *et al.*, 1998). The decrease was evident both on SDS-PAGE and HPLC. Irradiation, however, had a greater effect on the HMW-GS, which may be associated with the disruption of disulphide bonds. This seems to suggest that disulphide bonds are susceptible to irradiation damage. Working with BSA that has 17 disulphide residues per molecule, Di Simplicio *et al.* (1991) also demonstrated that irradiation could cleave disulphide bonds leading to fragmentation of these proteins. Other reports of radiation cleaving disulphide bonds appear in the literature (Doguchi, 1969; Garrison, 1987; Swallow, 1991)

1.2.6 Conclusions

Wet cooking causes a reduction in the digestibility of sorghum and maize proteins. The reduction is more acute in sorghum than in maize. The major cause for this reduction is believed to be the formation of disulphide bonded polymers between γ- and β-prolamin proteins. These disulphide bonded protein polymers are resistant to proteolysis, hence, the reduction in digestibility. Irradiation can cleave disulphide bonds in proteins (Di

Simplicio *et al.*, 1991; Köksel *et al.*, 1998). Information is, however, lacking about the effects of irradiation on sorghum and maize proteins. Considering that sorghum prolamin proteins like the glutenin proteins of wheat, contain disulphide bonds, and have α-helical and β-sheet secondary structures, and that the disulphide bonds in wheat are cleaved by irradiation (Srinavas *et al.*, 1972; Köksel *et al.*, 1998), it could be expected that irradiation might cleave the disulphide bonds in sorghum and maize prolamin proteins and cause changes in protein structure. Modification of the primary, secondary and tertiary structure of proteins by irradiation has been shown to improve susceptibility of proteins to proteolytic enzymes, possibly by exposing more peptide bonds to enzymatic hydrolysis (Davies, 1987; Davies *et al.*, 1987b). How these structural changes would affect protein digestibility of sorghum and maize is not known.

1.3 Objectives

The goal of this study was to establish whether irradiation could be used to alleviate the reduction in protein digestibility of sorghum proteins when cooked into porridge, in comparison with maize

The specific objectives were to:

Determine the effects of irradiation in wet and dry systems with and without cooking on the *in vitro* protein digestibility of sorghum and maize flours.

Compare the pepsin and multi-enzyme methods of determining *in vitro* protein digestibility in sorghum and maize.

Determine the effects of irradiation and cooking of sorghum and maize flours on disulphide bond concentration, and how this affects the extractability of their prolamins and their protein digestibility.

Determine changes brought about by irradiation and cooking on some molecular properties of sorghum and maize proteins using techniques such as sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and Fourier transform infrared spectroscopy (FTIR).

Determine the fundamental cause of the effects of irradiation on sorghum and maize protein digestibility.

1.4 Hypotheses

Irradiation will cleave disulphide bonds in sorghum and maize prolamin proteins, as has been observed with the disulphide bonds in wheat gluten proteins, resulting in a change in protein structure.

A change in protein structure brought about by irradiation can in turn improve susceptibility of the sorghum and maize proteins to proteolytic enzymes by exposing additional peptide bonds to proteolysis.

Irradiation in wet medium would generate more free radicals through the radiolysis of water, and as such bring about more changes in sorghum and maize proteins.

High dose of irradiation would also bring about greater changes in the proteins of sorghum and maize, since high doses generate more radiation energy that could produce more free radicals.

2 RESEARCH

The research chapter is divided into two sections to address the objectives stated in section 1.3. These are:

2.1: Use of gamma irradiation to alleviate the poor protein digestibility of sorghum porridge

2.2: Effects of irradiation and cooking of sorghum and maize flours on the structure of their proteins.

2.1 Use of Gamma Irradiation to Alleviate the Poor Protein Digestibility of Sorghum Porridge *

2.1.1 Abstract

One limitation to the use of sorghum as a food is that its proteins become more indigestible on wet cooking, primarily through the formation of disulphide linked enzymatically resistant protein polymers. Irradiation can modify bonds involved in protein secondary structure. The effect of irradiation (10 and 50 kGy), of dry and wet sorghum and maize flours on the digestibility and solubility of their proteins, when further cooked into porridge was investigated. Irradiation of sorghum flour followed by cooking alleviated the adverse effect of cooking on sorghum protein digestibility. Maize porridge digestibility was unaffected by irradiation of dry flour but decreased with wet irradiation. Increase in digestibility was not generally accompanied by an increase in nitrogen solubility index or in albumin and globulin protein solubility, suggesting that it was probably related to modification of protein structure allowing better access to proteolytic enzymes. Maillard reactions and protein polymerization at high doses negatively affected digestibility. Polyphenols influenced the effects of irradiation.

Keywords: Protein digestibility, sorghum, maize, porridge, irradiation, Maillard reactions, protein polymerization, polyphenols.

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2.1.2 Introduction

One of the limitations of using sorghum as a food crop is that its proteins become more indigestible on wet cooking compared to maize. *In vitro* (Mertz *et al.,* 1984; Hamaker *et al.,* 1987; Duodu *et al*., 2002) and *in vivo* (MacLean *et al.,* 1981; MacLean *et al.,* 1983) studies have shown that wet cooking of sorghum as in porridge making decreases its protein digestibility significantly. This, however, is not the case with maize where digestibility of its proteins is only minimally affected by wet cooking (Hamaker *et al*., 1987; Duodu *et al*., 2002). The reduction in the digestibility of sorghum protein is believed to result primarily from the formation of enzymatically resistant protein polymers during cooking through disulphide bonding of β- and γ-kafirins with themselves and with matrix proteins (Oria *et al.* 1995b). These disulphide crosslinks restrict access of proteolytic enzymes to the more digestible and abundant α-kafirin located at the centre of the protein body (Oria *et al*., 1995b), hence the reduction in digestibility (Hamaker *et al*., 1987; Oria *et al*., 1995b).

Protein digestibility of wet cooked sorghum has been improved using processes such as fermentation (El Khalifa & El Tinay, 1995; Taylor & Taylor, 2002), extrusion cooking (Mertz *et al*., 1984; Hamaker *et al*., 1994) and cooking with reducing agents (Hamaker *et al*., 1987; Rom *et al.,* 1992). This improvement is thought to occur through cleaving of disulphide bonds (Rom *et al*., 1992) and modification of protein structure (Taylor & Taylor, 2002) that prevents the formation of disulphide bonds during cooking, with the result that proteins are more accessible to proteolytic enzymes. It thus would appear that the conformation of sorghum β- and γ-kafirin proteins and the extent to which this conformation allows access of enzymes to the α-kafirin proteins is an important factor influencing protein digestibility in sorghum.

Irradiation is a processing technique that has been shown to affect protein structure. It can cleave disulphide bonds as was the case in wheat flour irradiated at 20 kGy (Köksel *et al.,* 1998) and 100 kGy (Doguchi, 1969), and other crosslinks (hydrogen bonds, ionic and hydrophobic interactions) involved in protein secondary and tertiary structure, leading to denaturation and fragmentation of proteins (Davies *et al.,* 1987b; Garrison, 1987;

Kempner, 1993). However, high doses of irradiation may result in crosslinking and/or aggregation of proteins (Garrison, 1987; Kempner, 1993; Cieśla *et al.,* 2000). In some cases, increased susceptibility of irradiated proteins to enzyme hydrolysis has been observed (Davies, 1987). Disulphide bonds occur in mature sorghum (Oria *et al.,* 1995a) and maize (Larkins *et al.,* 1984) prolamin proteins, and as indicated before their formation during cooking is associated with the lower digestibility of wet cooked sorghum (Hamaker *et al*. 1987; Rom *et al*., 1992).

The objective of this study was thus to determine the effect of irradiation of dry and wet sorghum and maize flours on the digestibility and solubility of their proteins, when further cooked into porridge.

2.1.3 Materials

The materials used in this study were two condensed tannin-free sorghum cultivars: BR7, a red cultivar with tan glume from South Africa and Madjeri, a white cultivar with purple glume from Cameroon, and a white maize hybrid PAN 6043 from South Africa.

2.1.4 Methods

2.1.4.1 Preparation of Flour Samples, Irradiation and Porridge Making

Grain samples were cleaned and then milled to whole grain flour in a laboratory hammer mill (Falling Number AB, Huddinge, Sweden) fitted with a 0.5 mm opening screen. Flour samples (300 g) were packaged in polyethylene bags. These samples were designated as dry flour, and had moisture contents ranging from 8 to 10%. Wet flour samples were prepared by mixing dry flour with distilled water at 30% solids content, packaged as above, and then refrigerated at 4°C. Both wet and dry flour samples were irradiated at target doses of 10 and 50 kGy using a ^{60}Co gamma irradiation source at the Isotron irradiation plant and a dose rate of 1.74 kGy/h (Isando, South Africa). Dry flour samples were irradiated at room temperature and wet flour samples at a temperature of about 4°C.

Wet flour samples were maintained at a temperature of about 4°C throughout the irradiation process by placing dry ice in the carrier buckets. The latter was done in order to prevent possible fermentation and microbial growth occurring during the irradiation process, (which required about 48 h to attain a dose of 50 kGy). Following irradiation, the wet irradiated samples were freeze-dried. Portions of all the samples (irradiated and non-irradiated) were cooked into porridges. Water (200 g) was brought to boil in a saucepan over a hot plate. Flour (150 g) was mixed with 150 g of water and added to the boiling water while stirring. Stirring was continued until bubbles were observed and then for a further 5 min. The porridge was poured into aluminium trays, frozen and freeze-dried. Freeze-dried samples were finely ground, packaged in gastight glass bottles and stored at 4°C until analysed.

2.1.4.2 Protein Content

Protein content (N x 6.25) was determined using the Dumas combustion method, American Association of Cereal Chemists, AACC (2000) method 46-30.

2.1.4.3 Amino Acid Composition

Amino acid composition was determined using the Pico.Tag method (Bidlingmeyer, Cohen & Tarvin, 1984). The flour samples were hydrolysed with 6 M hydrochloric acid, and the amino acids derivatized with phenylisothiocyanate (PITC) to produce phenylthiocarbamyl (PTC) amino acids. These amino acids derivatives were then analysed by reverse phase HPLC and detected at 254 nm against norleucine standard.

2.1.4.4 Pepsin Protein Digestibility

In vitro pepsin protein digestibility was determined using the pepsin method as described by Hamaker *et al*. (1987), with modification. Protein in the residue following digestion was determined by the spectrophotometric method of Devani, Shishoo, Shah & Suhagia (1989). The method is based on the reaction of ammonia with acetylacetone-

formaldehyde reagent in aqueous medium to give a yellow complex (3,5-diacetyl-1,4-dihydrolutidine), which has an absorption maxima at 412 nm.

2.1.4.5 Multienzyme Protein Digestibility

Multienzyme digestibility was determined using the pH stat method of Pederson & Eggum (1983). A multi-enzyme solution containing 22,704 units porcine pancreatic trypsin (type IX, Sigma), 186 units α-chymotrypsin from bovine pancreas (type II, Sigma) and 52 units porcine intestinal peptidase (Sigma) was prepared and the pH adjusted to 8.0 at 37°C using 0.1 M HCl or 0.1 M NaOH. Enzyme solutions were prepared fresh daily and stored on ice. Sodium caseinate was used as an external standard to test the activity of the enzymes. A 50 ml sample solution containing 1 mg N/ml was prepared and kept at 4°C for at least an hour before analyses. The pH was then adjusted to 8.0 in a 37 °C water bath using 0.1 M HCl or 0.1 M NaOH solutions. Multienzyme solution (5 ml) was added to the sample solution while stirring at 37°C, and the pH maintained constant at 7.98 for 10 min by automatic titration with 0.1 M NaOH. The amount of 0.1 M NaOH needed to maintain the pH constant at 7.98 over a 10 min period was recorded. Protein digestibility (PD) was calculated as follows:

$$PD = 76.14 + 44.77X \quad \text{(Pederson \& Eggum, 1983)}$$

Where X is the volume (ml) of 0.1 M NaOH needed to maintain the pH at 7.98 for 10 min.

2.1.4.6 Nitrogen Solubility Index

Nitrogen solubility index (NSI) was determined using the American Association of Cereal Chemists, AACC (2000) method 46-23.

2.1.4.7 Albumin and Globulins

Flour samples were extracted in 1.25 M NaCl (1:5 w/v) (Taylor *et al.,* 1984a) at 4°C for three consecutive 1 h periods. The extracts were dialysed against distilled water at 4°C for

24 h to remove the salt. Dialysis resulted in the loss of low molecular weight nitrogenous compounds. The extracts were freeze-dried and their nitrogen content determined using the Dumas combustion method.

2.1.4.8 Colour

Flour colour was measured using a Hunter Lab Color Quest (Hunter Associates, Reston, USA) tristimulus colorimeter using the L and b scales. The instrument was calibrated using black and white tile standards.

2.1.4.9 Polyphenols

Polyphenols in the flour samples were determined using a modified International Organization for Standardization (ISO, 1988) ferric ammonium citrate method. Polyphenols in the extract were determined by pipetting the following into a test tube in the following sequence with careful mixing after each addition: 5 ml distilled water; 1 ml carboxymethyl cellulose / EDTA reagent; 0.2 ml DMF (dimethyl formamide) extract or working standard; 0.2 ml ferric reagent and 0.2 ml alkali reagent (ethanolamine, 29% w/w). For sample blanks the ferric reagent was replaced with distilled water. After reacting for 10 min the absorbance was read at 525 nm. Results were expressed as tannic acid equivalents.

2.1.4.10 Antioxidant Activity

Antioxidant activity was determined using the TEAC (Trolox equivalents antioxidant activity) assay as described by Re, Pellegrini, Proteggente, Pannala, Yang & Rice-Evans (1999) with modification. Flour samples (0.3 g) were extracted in 10 ml solution of 1% HCl in methanol (v/v) for 2 h with vortexing every 5 min, and centrifuged at 3500 *g* for 8 min. Trolox (6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid; Sigma Aldrich) solutions (0-800 µM concentration) were used to prepare a standard curve. To 100 µl of standard and sample extracts was added 2900 µl of $ABTS^{\cdot+}$ (2,2'-azinobis 3-ethylbenzothiazoline 6-sulphonic acid) solution and the mixture allowed to react for 15

and 30 min for standards and sample extracts respectively. Absorbance was read at 734 nm against a water blank. Results were expressed as trolox equivalents (TE), mMTE /g of sample.

2.1.4.11 Statistical Analyses

Analysis of variance (ANOVA) was used to analyse the data for variation between samples and the means separated using the least significant difference test at the 5% level. Pearson's correlation was used to determine the relationship between all the parameters tested. Experiments were replicated twice and the samples analysed in duplicate. For amino acid analysis, samples from both experiments were pooled and analysed in duplicate.

2.1.5 Results

2.1.5.1 Protein Content

Irradiation alone in dry or wet medium had no significant effect ($p<0.05$) on protein content of flour samples (Table 2.1.1). However, when the irradiated and unirradiated samples were cooked into porridge, a small increase in protein content was observed.

2.1.5.2 Amino Acid Composition

The amino acid composition of sorghum BR7, sorghum Madjeri and maize PAN 6043, with irradiation alone or combined with cooking are presented in Tables 2 .1.2 a, b, and c respectively. Comparing the irradiation doses at which cooked protein digestibility of sorghum was improved most (10 kGy dry) and that at which it was least improved (50 kGy wet) with unirradiated samples, amino acid composition of sorghum BR7 (Table 2.1.2a) generally decreased with increasing irradiation dose both in the uncooked flours and in the cooked porridges, with the exception of methionine whose content increased in porridges from 10 kGy dry irradiated flour.

Table 2.1.1. Effects of irradiating wet and dry sorghum and maize flours, followed by cooking to make porridges, on their protein contents (g/100 g db)

Sample[3]	Sorghum BR7	Sorghum Madjeri	Maize PAN 6043
Unirradiated Flour	[1]9.02 [a] (0.10)[2]	8.50 [ab] (0.02)	9.80 [a] (0.06)
Irradiated dry flour (10 kGy)	9.10 [ab] (0.12)	8.47 [a] (0.01)	9.87 [b] (0.13)
Irradiated dry flour (50 kGy)	9.03 [a] (0.03)	8.47 [a] (0.03)	9.77 [a] (0.07)
Irradiated wet flour (10 kGy)	9.02 [a] (0.08)	8.49 [ab] (0.03)	9.81 [ab] (0.07)
Irradiated wet flour (50 kGy)	8.99 [a] (0.08)	8.51 [b] (0.05)	9.83 [ab] (0.02)
Porridge from unirradiated flour	9.12 [ab] (0.29)	8.66 [d] (0.04)	9.96 [c] (0.04)
Porridge from irradiated dry flour (10 kGy)	9.44 [d] (0.05)	8.55 [c] (0.01)	10.04 [d] (0.04)
Porridge from irradiated dry flour (50 kGy)	9.28 [cd] (0.04)	8.67 [d] (0.02)	10.02[cd] (0.03)
Porridge from irradiated wet flour (10 kGy)	9.09 [ab] (0.09)	8.59 [c] (0.01)	10.04 [d] (0.10)
Porridge from irradiated wet flour (50 kGy)	9.23 [bc] (0.18)	8.73 [e] (0.05)	9.95 [c] (0.05)

[1]Values in the same column with different letters are significantly ($p<0.05$) different from each other

[2]Values in parentheses are standard deviations of duplicate experiments (n = 4)

[3]The wet irradiated and porridge samples were freeze-dried

Table 2.1.2a. Effect of irradiating wet and dry sorghum BR7 flours, followed by cooking to make porridges on their amino acid composition (g/100g flour db)

Sample	Asp	Glu	Ser	Gly	His	Arg	Thr	Ala	Pro	Tyr	Val	Met	Iso	Leu	Phe	Lys
Unirradiated Flour	[1]0.57^{de}	1.75^{fg}	0.46^{cd}	0.32^{c}	0.19^{c}	0.40^{f}	0.35^{e}	0.81^{fg}	0.73^{ef}	0.30^{abcd}	0.43^{c}	0.13^{ab}	0.36^{e}	1.17^{e}	0.50^{ef}	0.20^{cd}
	[2](0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)
Irradiated dry flour (10 kGy)	0.54^{cd}	1.64^{d}	0.43^{bc}	0.30^{abc}	0.18^{abc}	0.33^{bcd}	0.32^{bcde}	0.77^{de}	0.69^{cd}	0.28^{ab}	0.39^{ab}	0.16^{b}	0.32^{bcd}	1.11^{d}	0.47^{cde}	0.19^{cd}
	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)
Irradiated dry flour (50 kGy)	0.57^{de}	1.76^{gh}	0.47^{d}	0.31^{bc}	0.19^{c}	0.37^{def}	0.34^{cde}	0.80^{fg}	0.73^{f}	0.32^{cde}	0.42^{bc}	0.14^{b}	0.34^{de}	1.20^{ef}	0.50^{f}	0.21^{d}
	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)
Irradiated wet flour (10 kGy)	0.54^{cd}	1.71^{ef}	0.43^{bc}	0.30^{abc}	0.18^{abc}	0.35^{cde}	0.33^{cde}	0.78^{efg}	0.70^{cde}	0.33^{de}	0.43^{c}	0.19^{c}	0.33^{cde}	1.10^{d}	0.45^{c}	0.18^{abc}
	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)
Irradiated wet flour (50 kGy)	0.48^{ab}	1.44^{a}	0.39^{a}	0.28a^{b}	0.15^{ab}	0.32^{b}	0.28^{a}	0.69^{a}	0.61^{a}	0.27^{ab}	0.36^{a}	0.11^{a}	0.27^{a}	0.93^{a}	0.38^{a}	0.15^{ab}
	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(00)	(0.02)	(0.02)	(0.02)	(0.02)
Porridge from unirradiated flour	0.58^{e}	1.79^{h}	0.46^{cd}	0.31^{bc}	0.19^{bc}	0.38^{ef}	0.35^{de}	0.81^{g}	0.74^{f}	0.35^{e}	0.44^{c}	0.14^{b}	0.36^{e}	1.21^{f}	0.49^{def}	0.19^{bcd}
	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)
Porridge from irradiated dry flour (10 kGy)	0.51^{bc}	1.62^{cd}	0.41^{ab}	0.29^{abc}	0.17^{abc}	0.32^{bc}	0.31^{abc}	0.73^{bc}	0.67^{bc}	0.31^{cd}	0.41^{bc}	0.20^{c}	0.30^{abc}	1.05^{c}	0.44^{bc}	0.19^{cd}
	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)
Porridge from irradiated dry flour (50 kGy)	0.54^{cd}	1.71^{e}	0.44^{bcd}	0.31^{bc}	0.17^{abc}	0.33^{bc}	0.33^{cde}	0.78^{ef}	0.72^{def}	0.31^{bcd}	0.43^{c}	0.21^{c}	0.33^{cd}	1.09^{d}	0.46^{cd}	0.18^{bcd}
	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)
Porridge from irradiated wet flour (10 kGy)	0.50^{ab}	1.60^{c}	0.42^{ab}	0.30^{abc}	0.16^{abc}	0.33^{bc}	0.32^{bcd}	0.74^{cd}	0.69^{cd}	0.29^{abc}	0.41^{bc}	0.14^{b}	0.31^{bc}	1.05^{c}	0.43^{bc}	0.19^{bcd}
	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)
Porridge from irradiated wet flour (50 kGy)	0.48^{a}	1.52^{b}	0.39^{a}	0.27^{a}	0.14^{a}	0.28^{a}	0.29^{ab}	0.70^{ab}	0.65^{b}	0.27^{a}	0.37^{a}	0.14^{b}	0.29^{ab}	1.00^{b}	0.41^{ab}	0.14^{a}
	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)

[1]Values in the same column with different letters are significantly ($p<0.05$) different from each other; [2]Values in parentheses are standard deviations for duplicate analyses (n = 2)

Table 2.1.2b. Effect of irradiating wet and dry sorghum Madjeri flours, followed by cooking to make porridges on their amino acid composition (g/100g flour db)

Sample	Asp	Glu	Ser	Gly	His	Arg	Thr	Ala	Pro	Tyr	Val	Met	Iso	Leu	Phe	Lys
Unirradiated Flour	[1]0.61^{e} [2](0.02)	1.51^{cd} (0.02)	0.41^{ab} (0.02)	0.30^{a} (0.02)	0.15^{a} (0.02)	0.36^{bcd} (0.02)	0.33^{ab} (0.02)	0.70^{bcd} (0.02)	0.66^{b} (0.02)	0.31^{cd} (0.02)	0.41^{d} (0.02)	0.11^{a} (0.0)	0.32^{cd} (0.02)	0.99^{c} (0.01)	0.42^{b} (0.02)	0.23^{d} (0.02)
Irradiated dry flour (10 kGy)	0.59^{cde} (0.02)	1.49^{c} (0.02)	0.42^{b} (0.02)	0.30^{a} (0.02)	0.14^{a} (0.02)	0.34^{ab} (0.02)	0.32^{a} (0.02)	0.68^{ab} (0.02)	0.62^{a} (0.02)	0.27^{c} (0.02)	0.37^{abcd} (0.02)	0.18^{bc} (0.02)	0.30^{abcd} (0.02)	0.94^{b} (0.02)	0.42^{b} (0.02)	0.18^{a} (0.02)
Irradiated dry flour (50 kGy)	0.56^{bc} (0.02)	1.45^{b} (0.02)	0.40^{ab} (0.02)	0.29^{a} (0.02)	0.14^{a} (0.02)	0.34^{ab} (0.02)	0.31^{a} (0.02)	0.69^{abcd} (0.02)	0.63^{ab} (0.02)	0.20^{a} (0.02)	0.37^{abcd} (0.02)	0.19^{bc} (0.02)	0.29^{abc} (0.02)	0.92^{b} (0.02)	0.37^{a} (0.02)	0.17^{a} (0.02)
Irradiated wet flour (10 kGy)	0.55^{b} (0.01)	1.43^{b} (0.02)	0.40^{ab} (0.02)	0.29^{a} (0.02)	0.13^{a} (0.02)	0.34^{ab} (0.02)	0.31^{a} (0.02)	0.69^{abc} (0.02)	0.63^{ab} (0.02)	0.29^{cd} (0.02)	0.37^{ab} (0.02)	0.19^{bc} (0.02)	0.28^{ab} (0.02)	0.94^{b} (0.02)	0.39^{ab} (0.02)	0.21^{bcd} (0.02)
Irradiated wet flour (50 kGy)	0.54^{b} (0.02)	1.45^{b} (0.02)	0.40^{ab} (0.02)	0.28^{a} (0.02)	0.13^{a} (0.02)	0.35^{bc} (0.02)	0.30^{a} (0.02)	0.69^{abcd} (0.02)	0.64^{ab} (0.02)	0.24^{b} (0.02)	0.36^{a} (0.02)	0.18^{bc} (0.02)	0.27^{a} (0.02)	0.93^{b} (0.02)	0.38^{a} (0.02)	0.18^{abc} (0.02)
Porridge from unirradiated flour	0.56^{b} (0.02)	1.38^{a} (0.02)	0.38^{a} (0.02)	0.29^{a} (0.02)	0.13^{a} (0.02)	0.31^{a} (0.02)	0.30^{a} (0.02)	0.67^{a} (0.02)	0.62^{a} (0.02)	0.28^{c} (0.02)	0.34^{a} (0.02)	0.13^{a} (0.02)	0.27^{a} (0.02)	0.88^{a} (0.02)	0.37^{a} (0.02)	0.18^{ab} (0.02)
Porridge from irradiated dry flour (10 kGy)	0.57^{bcd} (0.02)	1.57^{e} (0.02)	0.40^{ab} (0.02)	0.29^{a} (0.02)	0.15^{a} (0.02)	0.38^{d} (0.02)	0.32^{a} (0.02)	0.70^{bcd} (0.02)	0.66^{b} (0.02)	0.29^{cd} (0.02)	0.37^{abc} (0.02)	0.17^{b} (0.02)	0.29^{abc} (0.02)	0.96^{bc} (0.02)	0.39^{ab} (0.02)	0.21^{cd} (0.02)
Porridge from irradiated dry flour (50 kGy)	0.60^{de} (0.01)	1.53^{d} (0.01)	0.41^{ab} (0.01)	0.31^{a} (0.01)	0.15^{a} (0.01)	0.34^{ab} (0.01)	0.33^{ab} (0.01)	0.72^{cd} (0.01)	0.65^{ab} (0.01)	0.29^{cd} (0.01)	0.45^{e} (0.01)	0.21^{c} (0.01)	0.38^{e} (0.01)	1.15^{f} (0.01)	0.48^{c} (0.01)	0.24^{d} (0.01)
Porridge from irradiated wet flour (10 kGy)	0.49^{a} (0.01)	1.54^{de} (0.01)	0.49^{c} (0.01)	0.35^{b} (0.01)	0.22^{b} (0.01)	0.38^{cd} (0.01)	0.36^{b} (0.01)	0.69^{abc} (0.01)	0.84^{c} (0.01)	0.32^{d} (0.01)	0.40^{cd} (0.01)	0.12^{a} (0.01)	0.31^{bcd} (0.01)	1.10^{e} (0.01)	0.46^{c} (0.01)	0.22^{cd} (0.01)
Porridge from irradiated wet flour (50 kGy)	0.60^{de} (0.01)	1.54^{de} (0.01)	0.41^{b} (0.01)	0.30^{a} (0.01)	0.13^{a} (0.01)	0.36^{bcd} (0.01)	0.31^{a} (0.01)	0.72^{d} (0.01)	0.64^{ab} (0.01)	0.24^{b} (0.01)	0.39^{bcd} (0.01)	0.12^{a} (0.01)	0.33^{d} (0.01)	1.05^{d} (0.01)	0.45^{c} (0.01)	0.20^{abc} (0.01)

[1]Values in the same column with different letters are significantly ($p<0.05$) different from each other; [2]Values in parentheses are standard deviations for duplicate analyses (n = 2)

Table 2.1.2c. Effect of irradiating wet and dry maize PAN 6043 flours, followed by cooking to make porridges on their amino acid composition (g/100g flour db)

Sample	Asp	Glu	Ser	Gly	His	Arg	Thr	Ala	Pro	Tyr	Val	Met	Iso	Leu	Phe	Lys
Unirradiated Flour	[1]0.54^{b} [2](0.02)	1.71^{def} (0.03)	0.51^{bc} (0.02)	0.36^{b} (0.02)	0.27^{d} (00)	0.40^{ab} (0.02)	0.36^{ab} (0.03)	0.69^{bcd} (0.02)	0.89^{de} (00)	0.35^{bcd} (0.02)	0.41^{abc} (0.02)	0.16^{bc} (0.02)	0.29^{ab} (00)	1.13^{bc} (0.03)	0.46^{ab} (0.03)	0.28^{de} (00)
Irradiated dry flour (10 kGy)	0.56^{b} (0.02)	1.75^{f} (0.02)	0.50^{bc} (0.02)	0.36^{b} (0.02)	0.25^{bcd} (0.02)	0.42^{bcd} (0.02)	0.38^{bc} (0.02)	0.73^{ef} (0.02)	0.91^{ef} (0.02)	0.36^{cd} (0.02)	0.45^{c} (0.02)	0.21^{e} (0.02)	0.36^{c} (0.02)	1.25^{d} (0.02)	0.56^{d} (0.02)	0.24^{bc} (0.02)
Irradiated dry flour (50 kGy)	0.57^{bc} (0.02)	1.72^{def} (0.02)	0.48^{ab} (0.02)	0.34^{ab} (0.02)	0.22^{a} (0.02)	0.44^{cde} (0.02)	0.38^{bc} (0.02)	0.70^{cde} (0.02)	0.91^{ef} (0.02)	0.36^{cd} (0.02)	0.42^{abc} (0.02)	0.13^{a} (0.02)	0.31^{ab} (0.02)	1.12^{b} (0.02)	0.45^{ab} (0.02)	0.24^{bc} (0.02)
Irradiated wet flour (10 kGy)	0.59^{c} (0.02)	1.74^{ef} (0.02)	0.52^{c} (0.02)	0.37^{b} (0.02)	0.24^{abc} (0.02)	0.47^{f} (0.02)	0.40^{c} (0.02)	0.74^{f} (0.02)	0.94^{f} (0.02)	0.37^{d} (0.02)	0.43^{bc} (0.02)	0.19^{de} (0.02)	0.30^{ab} (0.02)	1.16^{c} (0.02)	0.48^{bc} (0.02)	0.29^{e} (0.02)
Irradiated wet flour (50 kGy)	0.55^{b} (0.02)	1.70^{ed} (0.02)	0.49^{abc} (0.02)	0.35^{ab} (0.02)	0.22^{ab} (0.02)	0.47^{ef} (0.02)	0.38^{bc} (0.02)	0.72^{ef} (0.02)	0.92^{ef} (0.02)	0.33^{bc} (0.02)	0.41^{abc} (0.02)	0.19^{cde} (0.02)	0.30^{ab} (0.02)	1.12^{b} (0.02)	0.45^{ab} (0.02)	0.22^{ab} (0.02)
Porridge from unirradiated flour	0.56^{bc} (0.02)	1.67^{bc} (0.02)	0.52^{c} (0.02)	0.35^{ab} (0.02)	0.27^{d} (0.02)	0.41^{bc} (0.02)	0.36^{abc} (0.02)	0.67^{abc} (0.02)	0.85^{bc} (0.02)	0.37^{d} (0.02)	0.41^{ab} (0.02)	0.20^{de} (0.02)	0.30^{ab} (0.02)	1.13^{bc} (0.02)	0.47^{bc} (0.02)	0.27^{de} (0.02)
Porridge from irradiated dry flour (10 kGy)	0.55^{b} (0.01)	1.65^{b} (0.01)	0.49^{abc} (0.01)	0.35^{ab} (0.01)	0.27^{d} (0.01)	0.44^{def} (0.01)	0.36^{ab} (0.01)	0.68^{bcd} (0.01)	0.87^{cd} (0.01)	0.28^{a} (0.01)	0.41^{ab} (0.01)	0.17^{bcd} (0.01)	0.30^{ab} (0.01)	1.12^{bc} (0.02)	0.46^{b} (0.01)	0.26^{cde} (00)
Porridge from irradiated dry flour (50 kGy)	0.56^{bc} (0.01)	1.71^{cde} (0.01)	0.52^{c} (0.01)	0.37^{b} (0.01)	0.26^{cd} (0.01)	0.42^{bcd} (0.01)	0.36^{ab} (0.01)	0.71^{de} (0.01)	0.90^{de} (0.01)	0.36^{cd} (0.01)	0.42^{bc} (0.01)	0.16^{ab} (0.01)	0.32^{b} (0.01)	1.16^{bc} (0.01)	0.45^{ab} (0.01)	0.25^{bcd} (0.01)
Porridge from irradiated wet flour (10 kGy)	0.49^{a} (0.01)	1.53^{a} (0.01)	0.46^{a} (0.01)	0.32^{a} (0.01)	0.24^{abc} (0.01)	0.37^{a} (0.01)	0.33^{a} (0.01)	0.64^{a} (0.01)	0.82^{ab} (0.01)	0.32^{b} (0.01)	0.38^{a} (0.02)	0.19^{bcde} (00)	0.28^{a} (0.01)	1.06^{a} (0.01)	0.42^{a} (0.01)	0.23^{ab} (0.01)
Porridge from irradiated wet flour (50 kGy)	0.50^{a} (0.01)	1.56^{a} (0.01)	0.47^{a} (0.01)	0.32^{a} (0.01)	0.21^{a} (0.01)	0.41^{bcd} (0.01)	0.35^{ab} (0.01)	0.66^{ab} (0.01)	0.81^{a} (0.01)	0.32^{b} (0.01)	0.40^{ab} (0.01)	0.19^{bcde} (0.01)	0.31^{b} (0.01)	1.14^{bc} (0.01)	0.51^{c} (0.01)	0.20^{a} (0.01)

[1]Values in the same column with different letters are significantly ($p<0.05$) different from each other; [2]Values in parentheses are standard deviations for duplicate analyses (n = 2)

In Madjeri sorghum (Table 2.1.2b) amino acid contents decreased with irradiation of flour except for methionine whose content increased. A reverse trend was observed in the porridges, where, with the exception of tyrosine, the amino acid content increased with irradiation. In maize (Table 2.1.2c) amino acid composition increased with irradiation of flour, with the exception of lysine and histidine. In maize porridges, on the other hand, amino acid composition generally decreased with irradiation.

2.1.5.3 Pepsin Protein Digestibility

Table 2.1.3 shows the effects of irradiation on the *in vitro* pepsin protein digestibility of sorghum and maize flours with and without cooking into porridge. Digestibility was significantly ($p<0.05$) affected by irradiation dose, wet cooking, and the type of cereal. Protein digestibility of BR7 sorghum flour was not significantly ($p>0.05$) affected by irradiation be it in dry or in wet medium. With Madjeri sorghum and PAN 6043 maize however, digestibility decreased somewhat with irradiation in the wet medium but not so much in the dry medium. Protein digestibility of the unirradiated sorghum samples decreased significantly ($p<0.05$) with cooking (17.5% for BR7 and 12.6% for Madjeri) compared to only 4.2% for unirradiated maize. However, when sorghum flour samples were irradiated before cooking, it alleviated the adverse effect of cooking on sorghum protein digestibility. Irradiation of dry sorghum flour at 10 kGy in particular maintained digestibility of sorghum porridges at levels similar with those in the uncooked samples. Digestibility of porridges from dry flour irradiated at 10 kGy was higher on average by 20.6% and 10.3% in BR7 and Madjeri sorghums, respectively, than those of porridges from unirradiated flour. With a higher dose of irradiation (50 kGy) and with irradiation of wet flour, digestibility of the sorghum porridges was lower compared to porridges from 10 kGy dry irradiated flour, but still higher than that of porridges from unirradiated flour. Maize porridges prepared from dry irradiated flour showed little difference in digestibility compared to that from unirradiated flour but decreased significantly ($p<0.05$) in porridges made from wet irradiated flour at both 10 and 50 kGy.

Table 2.1.3. Effects of irradiating wet and dry sorghum and maize flours, followed by cooking to make porridges, on their pepsin protein digestibility (%)

Sample[3]	Sorghum BR7	Sorghum Madjeri	Maize PAN 6043
Unirradiated flour	[1]70.5 ^{d} (0.7)[2]	73.3 ^{f} (0.4)	74.2 ^{e} (0.8)
Irradiated dry flour (10 kGy)	71.0 ^{d} (0.7)	73.5 ^{f} (1.9)	72.3 ^{cd} (0.6)
Irradiated dry flour (50 kGy)	70.3 ^{d} (0.7)	73.6 ^{f} (0.5)	72.8 ^{de} (2.1)
Irradiated wet flour (10 kGy)	71.4 ^{d} (2.9)	70.0 ^{cde} (1.0)	72.5 ^{cd} (0.9)
Irradiated wet flour (50 kGy)	71.4 ^{d} (0.7)	71.7 ^{ef} (2.9)	69.9 ^{b} (0.7)
Porridge from unirradiated flour	58.2 ^{a} (1.2)	64.1 ^{a} (1.0)	71.1 ^{bc} (0.6)
Porridge from irradiated dry flour (10 kGy)	70.2 ^{d} (1.0)	70.7 ^{de} (0.8)	71.2 ^{bcd} (0.8)
Porridge from irradiated dry flour (50 kGy)	63.2 ^{bc} (2.7)	68.0 ^{bc} (0.6)	72.6 ^{cde} (0.3)
Porridge from irradiated wet flour (10 kGy)	65.5 ^{c} (1.8)	68.7 ^{bcd} (1.9)	66.6 ^{a} (1.6)
Porridge from irradiated wet flour (50 kGy)	62.8 ^{b} (1.3)	66.7 ^{b} (1.3)	67.5 ^{a} (1.5)

[1]Values in the same column with different letters are significantly ($p<0.05$) different from each other

[2]Values in parentheses are standard deviations for duplicate experiments (n = 4)

[3]The wet irradiated and porridge samples were freeze-dried

2.1.5.4 Multi-enzyme Protein Digestibility

Multienzyme protein digestibility of uncooked flour decreased more with irradiation of wet flour than of dry flour (Table 2.1.4). Cooking the unirradiated flour significantly ($p<0.05$) decreased protein digestibility of the porridges. Protein digestibility of porridges from 10 kGy dry irradiated sorghum and maize flours were not significantly different from those of porridges from unirradiated flour. Except for sorghum BR7 dry flour irradiated at 50 kGy, irradiation of dry sorghum and maize flours at 50 kGy, and of wet flours reduced their protein digestibility significantly ($p<0.05$).

2.1.5.5 Nitrogen Solubility Index

No significant ($p>0.05$) difference was observed in NSI of sorghum BR7 with irradiation and cooking into porridge (Table 2.1.5). For sorghum Madjeri, NSI was unaffected by irradiation of dry flour at 10 kGy, but decreased significantly at 50 kGy and with irradiation of the wet flour. A decrease in NSI occurred in the Madjeri porridges at high irradiation dose and with wet irradiation. NSI of maize generally decreased with irradiation in both dry and wet medium for the uncooked and cooked samples.

2.1.5.6 Albumins and Globulins

Albumin and globulin (AG) content of uncooked sorghum BR7 flour decreased significantly ($p<0.05$) with irradiation in both dry and wet medium, more so in the latter (Table 2.1.6). In uncooked sorghum Madjeri and maize flours, AG content was basically unaffected by irradiation in dry medium but decreased significantly ($p<0.05$) with irradiation in the wet medium. When the flour samples were cooked into porridges AG contents of all three cereals decreased. AG contents of porridges from 10 kGy dry irradiated flours were similar to those of porridges from unirradiated flours, but decreased in porridges made from 50 kGy dry and from wet irradiated flours.

Table 2.1.4. Effects of irradiating wet and dry sorghum and maize flours, followed by cooking to make porridges, on their multienzyme protein digestibility (%)

Sample[3]	Sorghum BR7	Sorghum Madjeri	Maize PAN 6043
Unirradiated flour	[1]97.2 ^{e} (1.9)[2]	98.0 ^{f} (1.8)	97.3 ^{f} (2.3)
Irradiated dry flour (10 kGy)	92.0 ^{d} (2.4)	94.9 ^{e} (1.1)	96.3 ^{ef} (0.2)
Irradiated dry flour (50 kGy)	92.8 ^{d} (2.2)	93.3 ^{e} (2.5)	91.5 ^{d} (1.4)
Irradiated wet flour (10 kGy)	84.6 ^{ab} (2.1)	86.3 ^{bc} (0.9)	86.1 ^{b} (2.4)
Irradiated wet flour (50 kGy)	88.2 ^{c} (1.9)	89.5 ^{d} (1.6)	88.9 ^{c} (1.2)
Porridge from unirradiated flour	91.0 ^{d} (1.4)	94.0 ^{e} (0.7)	94.5 ^{e} (1.8)
Porridge from irradiated dry flour (10 kGy)	92.6 ^{d} (1.0)	93.5 ^{e} (1.0)	95.2 ^{e} (0.6)
Porridge from irradiated dry flour (50 kGy)	92.0 ^{d} (1.1)	87.8 ^{cd} (1.5)	90. 8 ^{cd} (0.4)
Porridge from irradiated wet flour (10 kGy)	82.2 ^{a} (1.0)	83.4 ^{a} (0.9)	83.7 ^{a} (0.7)
Porridge from irradiated wet flour (50 kGy)	85.5 ^{b} (1.8)	85.3 ^{ab} (1.2)	86.3 ^{b} (1.6)

[1]Values in the same column with different letters are significantly ($p<0.05$) different from each other

[2]Values in parentheses are standard deviations of duplicate experiments (n = 4)

[3]The wet irradiated and porridge samples were freeze-dried

Table 2.1.5. Effects of irradiating wet and dry sorghum and maize flours, followed by cooking to make porridges, on their Nitrogen Solubility Indices (% of total protein)

Sample[3]	Sorghum BR7	Sorghum Madjeri	Maize PAN 6043
Unirradiated Flour	[1]19.2 ^{ab} (1.6)[2]	21.8 ^{e} (1.7)	21.2 ^{gh} (0.3)
Irradiated dry flour (10 kGy)	19.4 ^{ab} (1.3)	21.7 ^{e} (1.2)	19.8 ^{def} (1.2)
Irradiated dry flour (50 kGy)	19.9 ^{b} (0.9)	18.1 ^{abc} (0.5)	18.9 ^{cd} (1.0)
Irradiated wet flour (10 kGy)	18.5 ^{ab} (1.7)	19.1 ^{bcd} (0.6)	18.6 ^{bc} (0.7)
Irradiated wet flour (50 kGy)	18.7 ^{ab} (1.2)	19.8 ^{cd} (1.2)	16.7 ^{a} (0.6)
Porridge from unirradiated flour	18.9 ^{ab} (0.5)	18.0 ^{ab} (1.1)	22.3 ^{i} (1.0)
Porridge from irradiated dry flour (10 kGy)	18.5 ^{ab} (2.0)	20.4 ^{de} (0.8)	20.1 ^{ef} (0.6)
Porridge from irradiated dry flour (50 kGy)	18.2 ^{ab} (1.6)	17.6 ^{ab} (0.1)	20.2 ^{fg} (0.5)
Porridge from irradiated wet flour (10 kGy)	19.0 ^{ab} (1.5)	17.1 ^{a} (2.1)	17.6 ^{ab} (0.6)
Porridge from irradiated wet flour (50 kGy)	17.4 ^{a} (1.0)	17.1 ^{ab} (1.1)	19.0 ^{cde} (0.5)

[1]Values in the same column with different letters are significantly ($p<0.05$) different from each other

[2]Values in parentheses are standard deviations of duplicate experiments (n = 4)

[3]The wet irradiated and porridge samples were freeze-dried

Table 2.1.6. Effects of irradiating wet and dry sorghum and maize flours, followed by cooking to make porridges, on their albumin and globulin content (% of total protein)

Sample[3]	Sorghum BR7	Sorghum Madjeri	Maize PAN 6043
Unirradiated Flour	[1]12.3 ^{g} (1.1)[2]	14.4 ^{e} (0.8)	12.1 ^{f} (0.9)
Irradiated dry flour (10 kGy)	6.3 ^{e} (0.4)	15.1 ^{e} (1.3)	12.1 ^{f} (0.8)
Irradiated dry flour (50 kGy)	7.9 ^{f} (0.4)	16.0 ^{e} (1.8)	10.7 ^{e} (0.2)
Irradiated wet flour (10 kGy)	3.1 ^{d} (0.2)	7.0 ^{d} (0.7)	5.6 ^{d} (0.9)
Irradiated wet flour (50 kGy)	3.0 ^{d} (0.1)	6.5 ^{cd} (1.2)	3.2 ^{c} (0.3)
Porridge from unirradiated flour	3.5 ^{d} (0.4)	4.7 ^{b} (0.2)	3.8 ^{c} (0.6)
Porridge from irradiated dry flour (10 kGy)	3.4 ^{d} (0.6)	5.2 ^{bc} (0.7)	3.9 ^{c} (0.2)
Porridge from irradiated dry flour (50 kGy)	1.2 ^{a} (0.2)	3.0 ^{a} (0.1)	2.1 ^{b} (0.2)
Porridge from irradiated wet flour (10 kGy)	2.4 ^{bc} (0.1)	3.8 ^{ab} (1.2)	2.1 ^{b} (0.2)
Porridge from irradiated wet flour (50 kGy)	1.8 ^{ab} (0.2)	2.2 ^{a} (0.3)	1.1 ^{a} (0.1)

[1]Values in the same column with different letters are significantly ($p<0.05$) different from each other

[2]Values in parentheses are standard deviations of duplicate experiments (n = 4)

[3]The wet irradiated and porridge samples were freeze-dried

2.1.5.7 Colour

In general, there was a reduction in L-value (whiteness) and an increase in b-value (yellowness) of the flour samples with dry and wet irradiation (Table 2.1.7). The same pattern occurred in the freeze-dried porridge samples but with lower L and higher b-values, indicating more browning in the porridges. However, porridges from unirradiated samples were lighter in colour than those from irradiated samples, indicating little or no browning in these samples. L colour was significantly correlated with albumin and globulin content in both BR7 ($r = 0.75$; $p<0.05$) and Madjeri ($r = 0.74$; $p<0.05$) sorghums but not in the maize.

2.1.5.8 Polyphenols

Polyphenol content was highest in sorghum BR7, followed by Madjeri, whereas no polyphenols could be detected in maize (Table 2.1.8). The polyphenols in the sorghums were significantly reduced by irradiation and were essentially eliminated in the wet irradiated flours and their porridges. Polyphenols were reduced more when irradiation was combined with cooking, than by irradiation or cooking alone.

2.1.5.9 Antioxidant Activity

All three cereals showed antioxidant activity (Table 2.1.9). Sorghum BR7 had the highest antioxidant activity. The antioxidant activity of sorghum Madjeri and maize were similar. Irradiation of dry flour at 10 kGy had no significant effect ($p<0.05$) on antioxidant activity, but it increased slightly in dry flour samples irradiated at 50 kGy. Antioxidant activity however, decreased with wet irradiation and with cooking. The decrease was greatest in sorghum BR7. There was a significant positive correlation ($r = 0.67$; $p<0.05$) between antioxidant activity and polyphenols for sorghum BR7 but not for sorghum Madjeri or maize.

Table 2.1.7. Effects of irradiating wet and dry sorghum and maize flours, followed by cooking to make porridges, on their L and b colour

Sample[3]	Sorghum BR7		Sorghum Madjeri		Maize PAN 6043	
	L[4]	b[5]	L	b	L	b
Unirradiated Flour	[1]71.4 [i] (0.2)[2]	9.8 [b] (0.1)	81.8 [g] (0.1)	8.5 ([b] 0.1)	86.6 [f] (0.3)	10.2 [b] (0.2)
Irradiated dry flour (10 kGy)	70.7 [h] (0.3)	10.1 [cd] (0.2)	81.0 [f] (0.4)	9.2 [d] (0.1)	85.2 [e] (0.3)	10.9 [c] (0.1)
Irradiated dry flour (50 kGy)	70.6 [h] (0.1)	11.3 [e] (0.1)	80.6 [f] (0.5)	11.3 [g] (0.1)	84.3 [d] (0.1)	13.2 [f] (0.1)
Irradiated wet flour (10 kGy)	67.3 [f] (0.1)	9.9 [bc] (0.1)	79.3 [e] (0.1)	8.7 [c] (0.1)	86.4 [f] (0.1)	11.1 [d] (0.2)
Irradiated wet flour (50 kGy)	68.6 [g] (0.2)	11.4 [e] (0.1)	80.8 [f] (0.2)	11.2 [g] (0.2	84.3 [d] (0.1)	14.8 [g] (0.1)
Porridge from unirradiated flour	66.7 [e] (0.2)	9.5 [a] (0.1)	78.4 [d] (0.3)	7.9 [a] (0.1)	87.4 [g] (0.2)	9.7 [a] (0.1)
Porridge from irradiated dry flour (10 kGy)	64.8 [d] (0.4)	10.2 [d] (0.1)	69.3 [a] (0.4)	10.6 [f] (0.1)	82.6 [c] (0.2)	13.1 [f] (0.1)
Porridge from irradiated dry flour (50 kGy)	60.2 [b] (0.1)	12.2 [f] (0.1)	73.2 [c] (0.4)	12.9 [h] (0.1)	78.5 [a] (0.1)	15.9 [h] (0.2)
Porridge from irradiated wet flour (10 kGy)	58.3 [a] (0.2)	9.8 [b] (0.2)	69.8 [a] (0.4)	10.1 [e] (0.1)	84.9 [e] (0.2)	11.6 [e] (0.1)
Porridge from irradiated wet flour (50 kGy)	62.9 [c] (0.2)	12.1 [f] (0.1)	72.3 [b] (0.4)	14.0 [i] (0.1)	80.8 [b] (0.1)	14.9 [g] (0.1)

[1]Values in the same column with different letters are significantly ($p<0.05$) different from each other

[2]Values in parentheses are standard deviations of duplicate experiments (n = 4); [3]The wet irradiated and porridge samples were freeze-dried

L-value = degree of whiteness (White 100 ↔ 0 Black); b-value = degree of yellowness (Yellow + b ↔ - b Blue)

Table 2.1.8. Effects of irradiating wet and dry sorghum and maize flours, followed by cooking to make porridges, on their total polyphenol content (g/100g tannic acid equivalent db)

Sample[3]	Sorghum BR7	Sorghum Madjeri	Maize PAN 6043
Unirradiated Flour	[1]0.17 [f] (0.01)[2]	0.04 [e] (0.01)	0.00 [b] (0.02)
Irradiated dry flour (10 kGy)	0.07 [e] (0.03)	0.02 [b] (0.02)	-0.02[ab] (0.04)
Irradiated dry flour (50 kGy)	0.04 [d] (0.01)	0.01[bcd] (0.02)	-0.02[ab] (0.06)
Irradiated wet flour (10 kGy)	0.03 [cd] (0.01)	0.00 [bc] (0.01)	-0.06 [a] (0.03)
Irradiated wet flour (50 kGy)	0.00 [ab] (0.01)	-0.03 [a] (0.02)	-0.06 [a] (0.02)
Porridge from unirradiated flour	0.08 [e] (0.02)	0.03 [cd] (0.01)	-0.02[ab] (0.06)
Porridge from irradiated dry flour (10 kGy)	0.03 [cd] (0.01)	0.02 [cde] (0.04)	-0.01[ab] (0.02)
Porridge from irradiated dry flour (50 kGy)	0.02 [bc] (0.01)	0.01[bcd] (0.01)	-0.03[ab] (0.02)
Porridge from irradiated wet flour (10 kGy)	0.01 [ab] (0.01)	-0.01[abc](0.01)	-0.06[ab] (0.04)
Porridge from irradiated wet flour (50 kGy)	-0.01 [a] (0.01)	-0.01[ab] (0.01)	-0.03[ab] (0.05)

[1]Values in the same column with different letters are significantly ($p<0.05$) different from each other

[2]Values in parentheses are standard deviations of duplicate experiments (n = 4)

[3]The wet irradiated and porridge samples were freeze-dried

Table 2.1.9. Effects of irradiating wet and dry sorghum and maize flours, followed by cooking to make porridges, on their antioxidant activity (mMTE/g db)

Sample[3]	Sorghum BR7	Sorghum Madjeri	Maize PAN 6043
Unirradiated Flour	[1]56.1 ^{g} (0.1)[2]	42.8 ^{f} (0.2)	40.2 ^{d} (0.5)
Irradiated dry flour (10 kGy)	56.0 ^{g} (0.2)	42.7 ^{f} (0.2)	40.3 ^{d} (0.3)
Irradiated dry flour (50 kGy)	56.5 ^{h} (0.2)	44.6 ^{g} (0.1)	42.9 ^{h} (0.4)
Irradiated wet flour (10 kGy)	52.7 ^{f} (0.2)	40.1 ^{c} (0.2)	41.6 ^{g} (0.1)
Irradiated wet flour (50 kGy)	50.6 ^{e} (0.1)	39.2 ^{a} (0.2)	40.7 ^{e} (0.1)
Porridge from unirradiated flour	50.3 ^{d} (0.1)	41.3 ^{d} (0.1)	39.0 ^{b} (0.1)
Porridge from irradiated dry flour (10 kGy)	48.7 ^{b} (0.2)	39.5 ^{ab} (0.2)	36.8 ^{a} (0.1)
Porridge from irradiated dry flour (50 kGy)	49.6 ^{c} (0.2)	42.6 ^{f} (0.2)	39.2 ^{b} (0.1)
Porridge from irradiated wet flour (10 kGy)	48.8 ^{b} (0.1)	42.3 ^{e} (0.1)	41.2 ^{f} (0.2)
Porridge from irradiated wet flour (50 kGy)	47.7 ^{a} (0.2)	39.7 ^{b} (0.3)	39.7 ^{c} (0.1)

[1]Values in the same column with different letters are significantly ($p<0.05$) different from each other

[2]Values in parentheses are standard deviations of duplicate experiments (n = 4)

[3]The wet irradiated and porridge samples were freeze-dried

2.1.6 Discussion

For the purpose of this discussion high irradiation dose will refer to 50 kGy dry and 10 and 50 kGy wet irradiated flour and porridge samples.

That protein content did not change with irradiation alone was not unexpected, as irradiation does not affect nitrogen amount. Previous studies with barley irradiated at doses upto 200 kGy also showed no significant changes in protein content (MacArthur & D'Appolonia, 1983; Bhatty & MacGregor, 1988; Al-Kaisey, Mohammed, Alwan & Mohammed, 2002). The reason for the apparent increase in protein content of unirradiated and irradiated sorghum and maize samples with cooking is not certain, and may be due to experimental error.

Amino acid profiles of sorghum and maize proteins in this study showed high concentrations of glutamic acid, alanine, proline and leucine, but low levels of lysine. These results are similar to those reported in the literature for sorghum (Chibber *et al.*, 1978) and maize (Landry, Paulis & Fey, 1983) proteins. Changes in amino acids with irradiation may result from free radicals splitting peptide bonds in proteins, and the subsequent deamination-decarboxylation of some of the amino acids or the crosslinking of two or more amino acids (Diehl, 1990). The reduction in amino acid contents with irradiation could be due to some of these reactions. The reduction in tyrosine levels in particular could suggest the formation of bityrosine complexes. The decrease in lysine could also be attributed to the formation of complexes involving lysine. However, the reason for the general increase observed in amino acid values in sorghum porridges and in maize flours with irradiation is not certain. The increases observed in methionine content with irradiation is not clear as this amino acid is susceptible to oxidation during acid hydrolysis. However, where disulphide bonds involving methionine are broken by irradiation, it could lead to an increase in the concentration of detectable methionine.

In vitro protein digestibility of sorghum and maize decreased with cooking as determined by the pepsin and multienzyme methods. However, the extent of reduction in protein digestibility of sorghum in particular, with cooking alone, using the multienzyme assay is

not consistent with what has been reported in literature using pepsin (Hamaker *et al*., 1987; Oria *et al.,* 1995b; Duodu *et al*., 2002, Duodu *et al.,* 2003), and with the results obtained in this study. Given that pepsin preferentially hydrolyses peptide bonds containing hydrophobic residues (Huang & Tang, 1968), and that sorghum and maize prolamins which make up the majority of proteins in these cereals are largely hydrophobic (Wall & Paulis, 1978), it suggests that pepsin may give a better indication of protein digestibility in these cereals. In addition, pepsin protein digestibility of sorghum has been shown to be similar to *in vivo* protein digestibility (Axtell *et al.,* 1981; MacLean *et al.,* 1981). This discussion will therefore focus on protein digestibility as determined by the pepsin method.

The fact that *in vitro* pepsin protein digestibility of sorghum decreased substantially on wet cooking, compared to maize is in agreement with previous work (Mertz *et al.,* 1984; Hamaker *et al*., 1987; Duodu *et al*., 2002). However, irradiation (10 kGy) of dry sorghum flour before wet cooking prevented this decrease and maintained protein digestibility of their porridges at levels comparable with the unirradiated flour. The improvement (10-20%) brought about in pepsin protein digestibility of sorghum porridges by irradiation of dry flour at 10 kGy over porridge from unirradiated flour was similar to that reported with extrusion cooking (Hamaker *et al.* 1994) and by cooking with reducing agents (Rom *et al*., 1992).

It is hypothesized that irradiation cleaved disulphide bonds in the sorghum prolamin proteins, as observed by Köksel *et al.* (1998) for wheat, resulting in unfolding of protein structure with possible fragmentation that could also prevent formation of disulphide crosslinks during cooking. This would result in a more open protein network that would expose more protein sites to proteolytic enzymes, and hence improve digestibility. Sorghum prolamin proteins have a high content of disulphide bonds (Oria *et al*., 1995a) and these bonds can be cleaved by irradiation (Di Simplicio *et al.,* 1991; Köksel *et al*., 1998). Splitting of the disulphide bonds by irradiation will no doubt modify protein structure. Porridge pepsin digestibility of sorghum, however, decreased significantly with high irradiation dose, although it remained higher than that of porridge from unirradiated flour. It is possible that under these conditions the unfolded proteins formed crosslinks

(aggregates) that were less susceptible to enzyme hydrolysis. Cho *et al.* (1999) reported crosslinking in BSA and β-lactoglobulin protein solutions irradiated at 10 kGy to form high molecular weight polymers. The decreases in solubility of albumin and globulin (AG) proteins of both sorghums and in NSI of sorghum Madjeri with high irradiation dose are indications of the formation of insoluble complexes that could impair digestibility.

Maillard reactions have been associated with irradiation of protein containing foods (Wootton *et al*., 1988; Krumhar & Berry, 1990; Cunha *et al.,* 1993)**.** Maillard reactions are accompanied by the formation of brown or yellow pigments (Whistler & Daniel, 1985). Thus the L and b-values of the flour and porridge samples was determined. The decrease in whiteness, and increase in yellowness of flour colour with irradiation and with cooking may be indicative of the occurrence of Maillard reactions. Some Maillard products inhibit proteolytic activity (Öste *et al.,* 1986, 1987). Maillard browning could therefore be in part responsible for the reduction in digestibility observed with porridges from flour irradiated at high dose. It is, however, not certain to what extent the colour changes are related to the formation of Maillard products. Another possible indication of the occurrence of Maillard reactions was the slight increase in antioxidant activity in 50 kGy dry irradiated sorghum and maize flours. Baltes (1982) reported that the antioxidant effects of melanoidins are greatest at the beginning of the browning reactions, which was attributed to Maillard intermediates such as the reductones. These Maillard intermediates give yellow coloured products (Whistler & Daniel, 1985). The yellow colouration (b-value) was highest in 50 kGy dry irradiated flours and could therefore represent the onset of Maillard browning. The lower porridge digestibility observed with wet irradiated samples at 10 kGy compared to the dry irradiated samples supports the accepted tenet that the effects of irradiation are enhanced in wet medium, because of indirect effects from free radicals generated from the radiolysis of water (Cieśla *et al*., 2000).

Protein digestibility of maize porridge was affected differently by irradiation compared to the two sorghum cultivars. Digestibility decreased significantly in maize porridge made from wet irradiated flour in comparison to porridge from unirradiated flour. Part of the

reason could be the lower concentration of radiation susceptible disulphide bonds in maize prolamin proteins (Esen, 1986; Duodu *et al*., 2002), which could be related to the lack of effect of irradiation of the dry flour. Irradiation of wet flour may have enhanced the effects of irradiation through reactions of water radiolysis products with protein molecules (Cieśla *et al*., 2000), resulting in crosslinking or aggregation of proteins. The decrease in NSI and in AG content of maize flours irradiated in wet medium indicates some crosslinking or aggregation of proteins that could negatively affect digestibility. Nitrogen solubility is thought to be related to protein digestibility as an increase in soluble nitrogen is in most cases accompanied by an increase in protein digestibility (Cheftel *et al.,* 1985). This was, however, not always the case in sorghum. No significant correlation was found between NSI and protein digestibility in this study, suggesting that protein digestibility in irradiated sorghum was improved through modification of protein structure rather than degradation of proteins to smaller peptides. Taylor & Taylor (2002) had also proposed that fermentation improved digestibility of sorghum protein by modifying protein structure.

The AG proteins exhibited a pattern of change that was not consistent with that of digestibility. This could mean that the changes in AG content do not have a direct bearing on overall protein digestibility of sorghum and maize. The AG proteins are high in lysine (Taylor & Schüssler, 1986), an amino acid implicated in Maillard reactions (Whistler & Daniel, 1985). The decline in solubility of AG suggests the formation of some insoluble complexes. Irradiation at 10 kGy induced aggregate formation in bovine serum albumin that reduced its solubility (Krumhar & Berry, 1990). The greater reduction in AG content in BR7 compared to Madjeri could be related to its higher polyphenol content. Condensed tannin-free sorghum contains polyphenols such as phenolic acids and flavonoids (Hahn, Rooney & Earp, 1984). BR7 is a red sorghum and this colour appears to be due to the presence of flavonoids (Hahn *et al*., 1984), hence, its higher content of polyphenols compared to the white sorghum, Madjeri. Sorghum polyphenols are more likely to bind to large proteins, rich in proline and having a loose open structure (Butler *et al.,* 1984). AG proteins from sorghum have molecular weight ranging from 14-70 kDa with the majority of the proteins in the high molecular weight range, and they do contain proline (Taylor & Schüssler, 1986). They thus may complex with polyphenols. Polyphenols may be

oxidised to *o*-quinones by oxygen (Haslam, 1989). Irradiation produces free radicals with oxidising ability (Thakur & Singh, 1994) that could oxidise polyphenols. These *o*-quinones may then react with amino acid residues in AG through covalent interactions to polymerise proteins (Haslam, 1989). A positive correlation ($r = 0.88$; $p<0.05$) between polyphenols and AG content in BR7 sorghum supports this suggestion. Thus polyphenols could in part account for the reduction in solubility of AG in sorghum BR7 following irradiation. Duodu *et al*. (2002) using SDS-PAGE showed that the indigestible residues from wet cooked sorghums were mainly prolamin proteins. There is, however, a possibility that at high doses of irradiation combined with cooking crosslinks may be formed with the proteins in the AG fraction that could negatively affect protein digestibility. This is inferred from the significant ($p<0.05$) reduction in AG content of porridges from flour samples irradiated at high doses.

The BR7 polyphenol content was similar to values reported by Glennie (1983) for Barnard Red (0.1%) and NK 283 (0.08%); both red condensed tannin free sorghums. Polyphenols decreased with irradiation possibly through oxidation by free radicals and reaction of the oxidised polyphenols with AG proteins. They decreased more in wet irradiated samples, which is consistent with the fact that free radicals generated during irradiation have a direct bearing on reduction of polyphenols, as more free radicals are generated in wet than in dry medium (Thakur & Singh, 1994).

Antioxidant activity was measured to determine whether or not polyphenols had an effect on the outcome of irradiation. Polyphenols can react with free radicals and in so doing act as antioxidants (Velioglu, Mazza, Gao & Oomah, 1998). In sorghum BR7 polyphenol content was positively correlated with antioxidant activity ($r = 0.67$; $p<0.05$). However, the polyphenols were not the only components responsible for antioxidant activity. This was apparent, as there was high antioxidant activity in Madjeri sorghum and maize, which had negligible polyphenol contents. Cereal grains contain vitamin E (tocopherols) and tocotrienols that are present in the lipid fraction of the germ and these possess antioxidant activity (MacEvilly, 2003). As suggested, irradiation may have induced Maillard reactions, and products from these reactions are reported to possess antioxidant activity (Baltes, 1982; Eiserich & Shibamoto, 1994). Aromatic amines and sulphur

containing compounds present in these cereals also possess antioxidant activity (Yu, Haley, Perret, Harris, Wilson & Qian, 2002). All of these may contribute to the antioxidant potential of these samples. Polyphenols can act as antioxidants by scavenging free radicals (Velioglu *et al*., 1998) and could thus offer some protection against the effects of irradiation (Cho *et al*., 1999). This protection could be responsible for the lack of change in NSI of BR7 flour and porridge samples with irradiation. However, the oxidized polyphenols may have complexed with AG proteins in BR7 to reduce solubility and cause the lower porridge digestibility of sorghum BR7 samples irradiated at high dose (50 kGy) and in wet medium, compared to sorghum Madjeri and maize. These results, however, indicate that the polyphenols were the most potent of all the antioxidants in these samples.

2.1.7 Conclusions

These findings indicate that irradiation (especially of dry flour at 10 kGy) has the potential to alleviate the adverse effects of wet cooking on pepsin protein digestibility of sorghum porridge. This seems to occur through a modification in protein structure with the result that more peptide bonds are exposed to hydrolysis. However, at higher doses of irradiation, Maillard reactions, crosslinking or aggregation of proteins may be triggered, leading to a reduction in digestibility. Polyphenols appear to influence the observed effects of irradiation on protein digestibility.

2.1.8 References

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2.2 Effects of Irradiation and Cooking of Sorghum and Maize Flours on the Structure of their Proteins

2.2.1 Abstract

Sorghum proteins become less digestible when cooked into porridge compared to maize. In section 2.1, it was shown that irradiation of sorghum flour prior to cooking can alleviate somewhat the reduction in protein digestibility that occurs on cooking. The prolamins of sorghum became very much less extractable upon cooking, but the effect was much less in maize. SDS-PAGE showed that cooked sorghum proteins contained more disulphide-linked dimers than maize proteins. Irradiation of dry sorghum flours at 10 kGy before cooking somewhat relieved the reduction in prolamin extractability, but at high doses the effects were inconsistent. Maize prolamins became more in extractable with irradiation alone at high doses, possibly due to crosslinking of the proteins. Free sulphydryl groups increased, while disulphide bonds decreased in sorghum porridges from irradiated flours, compared to porridges from unirradiated flour, indicating breakdown of disulphide bonds to free sulphydryls. SDS-PAGE under non-reducing conditions showed less dimers in porridges from irradiated flour, suggesting cleavage of disulphide bonds. FTIR showed no consistent changes in protein secondary structure, with irradiation and cooking. It appears that irradiation, especially of sorghum, and to a lesser extent maize, flours followed by cooking altered protein structure, as evidenced from the lower concentration of disulphide bonds and disulphide-linked dimers in porridges from irradiated compared to unirradiated flour.

Key words: Irradiation, sorghum, maize, porridge, prolamins, sulphydryl groups, disulphide bonds, SDS-PAGE, FTIR

2.2.2. Introduction

During wet cooking of sorghum flour, as in porridge making, sorghum prolamin proteins form disulphide crosslinks with themselves and probably with other proteins (Hamaker *et al*., 1987; Oria *et al*., 1995b). These crosslinked proteins are less digestible and cause a reduction in protein digestibility when sorghum is, for example, cooked into porridge (Hamaker *et al*., 1987; Oria *et al*., 1995b; Duodu *et al*., 2003). When maize is treated similarly, fewer disulphide crosslinks are formed and protein digestibility of maize is only slightly reduced by wet cooking (Hamaker *et al*., 1987; Duodu *et al*., 2003).

It has been shown in section 2.1 that irradiation prior to cooking can to some extent alleviate the reduction in protein digestibility that normally occurs upon wet cooking of sorghum. This was attributed to the fact that irradiation can split disulphide bonds (Di Simplicio *et al*., 1991; Köksel *et al*., 1998) which occur in the proteins of mature sorghum and maize (Landry *et al*., 1983; Oria *et al*., 1995a; El Nour *et al*., 1998). This could modify protein structure to allow proteolytic enzymes better access to the proteins, hence the better digestibility.

It has also been suggested that the extractability of prolamin proteins in sorghum and maize may have a bearing on their protein digestibility, with digestibility increasing with increased prolamin extractability (Hamaker *et al*., 1994; Duodu *et al*., 2003). Cooking of sorghum generally renders the prolamin proteins less extractable, in aqueous alcohol both with and without a reducing agent, because of the formation of disulphide and other crosslinks (Hamaker *et al*., 1986; Oria *et al*., 1995a; Duodu *et al*., 2003; Nunes *et al*., 2004).

To understand the changes taking place in sorghum and maize proteins following irradiation and cooking, it is important to investigate certain molecular characteristics of these proteins. The extractability of the prolamin proteins of sorghum and maize were determined, since prolamin extractability is believed to be related to protein digestibility in sorghum and maize (Hamaker *et al.,* 1994). Protein-rich flours were prepared from the irradiated and cooked samples, and used to determine the amount of free sulphydryl

groups and disulphide bonds, and also for sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and Fourier transform infrared (FTIR) spectroscopy analyses.

2.2.3. Materials

Irradiated and unirradiated, uncooked and cooked sorghum and maize flour samples were used for analyses. The grains, flour preparation, and irradiation and cooking procedures were as described in section 2.1.

2.2.4. Methods

2.2.4.1. Prolamin Extractability

Prolamins were extracted from unirradiated, irradiated, uncooked and cooked sorghum and maize flours, according to the method of Taylor *et al.* (1984a). Albumins and globulins were extracted thrice in 1.25 M NaCl and the residue rinsed with distilled water. The residue was stirred for two 1 h periods and then overnight at room temperature with aqueous alcohol alone and then the same procedure repeated with aqueous alcohol with reducing agent (0.05% w/v dithiothreithol, (DTT)) to obtain the uncrosslinked and crosslinked prolamins, respectively. Sorghum kafirins were extracted in 60% (v/v) *tert*-butanol, while maize zeins were extracted in 55% (v/v) isopropanol. The combined prolamin supernatants were frozen, freeze-dried and their protein content (N x 6.25) determined by the Dumas combustion method (AACC, 2000) method 46-30.

2.2.4.2. Preparation of Protein-rich Flour

Porridge samples that showed the most significant changes in protein digestibility with irradiation and cooking (porridges from dry flour samples irradiated at 10 kGy and from wet flour samples irradiated at 50 kGy) (section 2.1) together with control porridges from unirradiated flours were used to prepare protein-rich flour. The wet milling procedure of Taylor *et al.* (1984b) was used with modification. Using approximately 30 g of sample,

the salt soluble fraction was extracted with 1.25 M sodium chloride (1:5 w/v). The residue was then digested with α-amylase (Sigma, A6211) using 1300 units/g of sample in sodium acetate buffer (0.2 M), pH 5.5, at 37 ºC for 12 h to hydrolyse the gelatinised starch and thus facilitate wet milling and sieving. The digested samples were centrifuged at 5000 *g* for 10 min. The residue was mixed with cold water (150 ml) at about 4ºC and then wet milled using a Retsch Ultra Centrifugal Mill (Haan, Germany) to pass first through a 750 µm opening screen and again milled through a 250 µm opening screen. The milled samples were passed through sieves of decreasing aperture (180, 106 and 75 µm) to remove fibre. The filtrate was centrifuged as above and the supernatant discarded. The protein fraction at the top of the pellet was removed, diluted with water and re-centrifuged. The process was repeated until most of the starch had been removed as observed by the colour of the sample. The protein-rich flour was then freeze-dried, and ground into powder using a mortar and pestle. The protein-rich samples had protein contents (N x 6.25) ranging from 35 to 50%, as determined by the Dumas method.

2.2.4.3. Free Sulphydryl (SH) Groups and Disulphide (SS) Bonds

Free sulphydryl groups were determined in the solid-state using Ellman's reagent (5,5'-dithiobis 2-nitrobenzoic acid, DTNB, Sigma) and the direct colorimetric assay of Chan & Wasserman (1993). Protein-rich flour (30 mg) was incubated at room temperature with 1 ml of reaction buffer containing 8 M urea, 0.2 M Tris-HCl, 3 mM EDTA, 1% SDS (w/v) and 0.4 mM Ellman's reagent at pH 8.0 for 30 min. Samples were then centrifuged at 7500 *g* for 20 min in a microcentrifuge (Labnet, Edison, NJ, USA). A 0.2 ml aliquot of the supernatant was diluted 10 times in a solution of the reaction buffer without added DTNB and the absorbance read at 412 nm. Sample blanks were prepared with buffer without added DTNB.

Disulphide bond content was determined as sulphydryls after reduction of the disulphide bonds with sodium sulphite. The procedure was essentially as described for free sulphydryls with a reaction buffer consisting of 8 M urea, 0.1 M sodium sulphite, 3 mM EDTA, 0.2 M Tris-HCl at pH 9.5 and 10 mM $NTSB^{2-}$ (disodium-2-nitro-5-thiosulphobenzoate) synthesised from DTNB in the presence of sodium sulphite and

oxygen as described by Thannhauser, Konishi & Scheraga (1987). After reacting for 30 min, samples were centrifuged and diluted as for free sulphydryl groups and the absorbance read at 412 nm. Sample blanks were prepared with buffer without added $NTSB^{2-}$.

The SH content was calculated using the equation given by Li-Chan (1983).

$$\mu M\ SH/g = (73.53\ A_{412}\ D)/C$$

Where A_{412} is the net absorbance at 412 nm after correcting for reagent and sample blanks; C is the sample concentration (mg/ml); D is the dilution factor; and 73.53 is $10^6/1.36 \times 10^4$ (1.36×10^4 being the molar absorptivity of DTNB) and 10^6 the conversion factor from mole/mg to µM/g.

Disulphide group content was calculated as the difference in sulphydryl content before and after reduction of disulphide bonds.

Total cysteine content was calculated as (SH) + 2(SS).

2.2.4.4. SDS-PAGE

SDS-PAGE was conducted using the discontinuous Tris-HCl/glycine buffer system (Laemmli, 1970) with a 7-14% (w/v) linear gradient gel using 40% acrylamide-bis (19:1) stock solution, under reducing and non-reducing conditions. Molecular weight standards (Combithek calibration proteins for SDS-PAGE, Boehringer Mannheim, Mannheim, Germany) comprising α_2-macroglobulin (M_r 170000), β-galactosidase (M_r 116353), fructose-6-P-kinase (M_r 85204), glutamate dehydrogenase (M_r 55562), aldolase (M_r 39212), triose phosphate isomerase (M_r 26626), trypsin inhibitor (M_r 20100) and lysozyme (M_r 14307) were mixed with reducing sample buffer to give 1 µg protein per expected band of standard. Samples were loaded at approximately 37.5 µg protein per well.

SDS-PAGE was conducted with a Protean II xi vertical cell system (Bio-Rad laboratories, Hercules, CA, USA) at a constant current of 13 mA per gel for 1 h at 120 V and then at

25 mA per gel at 250 V for a further 8 h with cooling at 12 ºC. Gels were stained with 0.03% (w/v) Coomassie Brillant Blue R250 in 7% (v/v) acetic acid and 20% (v/v) methanol and 3.2% (w/v) trichloroacetic acid (TCA) and destained with 4% (v/v) acetic acid, 29% (v/v) methanol and 3% (w/v) TCA. The gels were then scanned on a flat bed scanner. M_r of the protein bands was estimated from the log-linear plot of molecular weight versus relative mobility of the protein standards.

2.2.4.5. FTIR

FTIR absorbance spectra were recorded on a Perkin Elmer, Spectrum GX 2000 FTIR system (Beaconsfield, UK) adapted to a Perkin Elmer auto-image microscope system using high pressure diamond optics, between 4000 and 700 cm^{-1}. For each spectrum, a total of 500 scans were collected at a resolution of 8 cm^{-1}. All samples were analysed in duplicate. Fourier self deconvolution (band narrowing) was achieved with a full width at half height (FFHH) of 13 cm^{-1} and a resolution enhancement factor of 2.0, using Opus software (Bruker Instruments, Billerica, MA, USA).

2.2.4.6. Statistical Analysis

Data on prolamin extraction, sulphydryl and disulphide groups were subjected to analysis of variance (ANOVA) using Statistica (Version 6.0, Statsoft Inc., Tulsa, OK, USA) and the means separated using the least significance difference test at the 5% level. Prolamins were extracted once and analysed for protein content in triplicate, whereas sulphydryl groups were determined in triplicate.

2.2.5. Results

2.2.5.1 Prolamin Extractability

Based on reported values of about 50% or more prolamins in sorghum and maize whole grain flour (Taylor *et al*., 1984a; Hamaker *et al*., 1995), it is apparent from the data on

Table 2.2.1 that not all of the prolamins in the unirradiated flours had been extracted. Possible reasons for this will be discussed in the discussion section (section 3.1). Consequently, only general trends will be considered.

Irradiation alone did not bring about any major changes in prolamin extractability of the sorghum samples. In maize, irradiation of dry flour at 50 kGy and of wet flour at 10 and 50 kGy (high doses) caused a marked reduction in prolamin extractability. Cooking the unirradiated flour rendered the prolamins significantly ($p<0.05$) less extractable, more so in sorghum than in maize. The extractability of sorghum prolamins was reduced by over 50%, whereas that of maize was reduced by about half this amount upon cooking. However, irradiation of dry flour at 10 kGy prior to cooking relieved the reduction somewhat in prolamin extractability, more so in sorghum than in maize. But, when the irradiation dose was increased to 50 kGy (dry) and when samples were irradiated in wet medium (10 and 50 kGy) before cooking, no clear consistent trends were observed in sorghum prolamin extractability. In maize on the other hand, prolamin extractability was only significantly reduced in porridges from 50 kGy wet irradiated flours.

2.2.5.2. Free Sulphydryl (SH) Groups and Disulphide (SS) Bonds

Determination of disulphide bonds showed that maize and sorghum porridges from unirradiated flour contained similar amounts of disulphide bonds (Table 2.2.2). Free sulphydryl groups in both sorghum and maize increased with increasing dose of irradiation. This increase was accompanied by a significant ($p<0.05$) reduction in disulphide bond and total cysteine content in sorghum, but not in maize.

Table 2.2.1. Effects of irradiating wet and dry sorghum and maize flours followed by cooking to make porridges on the extractability of their prolamin proteins (% total protein)

Sample and treatment [3]	Sorghum BR7	Sorghum Madjeri	Maize PAN 6043
Unirradiated Flour	[1]38.5 ^{de} (0.9)[2]	33.0 ^{f} (1.1)	37.5 ^{f} (2.3)
Irradiated dry flour (10 kGy)	37.4 ^{d} (0.2)	32.9 ^{f} (0.4)	34.5 ^{e} (1.1)
Irradiated dry flour (50 kGy)	43.7 ^{g} (0.2)	35.2 ^{g} (0.5)	22. 6 ^{a} (0.8)
Irradiated wet flour (10 kGy)	39.8 ^{ef} (1.0)	33.0 ^{f} (0.8)	25.5 ^{b} (1.3)
Irradiated wet flour (50 kGy)	40.4 ^{f} (1.2)	30.7 ^{e} (0.8)	30.2 ^{d} (0.2)
Porridge from unirradiated flour	17.0 ^{b} (0.3)	14.4 ^{a} (0.3)	28.1 ^{c} (0.6)
Porridge from irradiated dry flour (10 kGy)	24.6 ^{c} (1.1)	21.5 ^{bc} (1.0)	31.2 ^{d} (1.1)
Porridge from irradiated dry flour (50 kGy)	17.8 ^{b} (1.2)	22.5 ^{c} (0.7)	31.8 ^{d} (1.0)
Porridge from irradiated wet flour (10 kGy)	16.3 ^{ab} (0.6)	20.5 ^{b} (1.8)	31.0 ^{d} (1.0)
Porridge from irradiated wet flour (50 kGy)	15.1 ^{a} (1.5)	29.1 ^{d} (0.5)	21.6 ^{a} (0.5)

[1]Values in the same column with different letters are significantly ($p<0.05$) different from each other

[2]Values in parentheses are standard deviations of triplicate analyses

[3]The wet irradiated and porridge samples were freeze dried

Table 2.2.2. Effects of irradiating wet and dry sorghum and maize flours followed by cooking to make porridges on the free sulphydryl (SH), total cysteine and disulphide (SS) contents of their protein-rich flours (μM/g protein)

Porridge sample	Sorghum BR7			Sorghum Madjeri			Maize PAN 6043		
	Free SH	Total Cysteine	SS Bonds	Free SH	Total Cysteine	SS Bonds	Free SH	Total Cysteine	SS Bonds
From unirradiated flour	0.7[a1] (0.1)[2]	97.9[b] (2.4)	48.6[c] (1.2)	1.0[a] (0.1)	90.8[b] (2.0)	44.9[b] (1.0)	1.7[a] (0.1)	102.1[ab] (3.2)	50.2[ab] (1.7)
From 10 kGy dry irradiated flour	2.4[b] (0.5)	86.0[a] (1.9)	41.8[b] (1.2)	2.2[b] (0.3)	84.2[ab] (4.2)	41.0[a] (2.1)	2.3[a] (0.6)	108.5[b] (5.4)	53.1[b] (3.0)
From 50 kGy wet irradiated flour	3.4[c] (0.3)	82.0[a] (2.2)	39.3[a] (1.2)	3.1[c] (0.1)	78.3[a] (4.9)	37.6[a] (2.4)	3.4[b] (0.4)	97.8[a] (3.1)	47.2[a] (1.7)

[1]Values in the same column with different letters are significantly ($p<0.05$) different from each other
[2]Values in parentheses are standard deviations of triplicate analyses

2.2.5.3. SDS-PAGE

SDS-PAGE under non-reducing conditions (Fig 2.2.1A) shows bands < 26 k, bands at 47 k and bands at the top of the gel, at about 170 k. The 47 k bands were essentially absent with SDS-PAGE under reducing conditions (Fig 2.2.1B), whereas some of the 170 k bands remained after reduction. Under non-reducing conditions, the 47 k band in the unirradiated samples (lane 1), is more intense in sorghum than in maize. This band together with the bands of M_r < 26 k decreased in intensity with irradiation (lanes 2 and 3), more so in sorghum than in maize. The monomer bands of M_r < 26 k are attributed to γ-, α- and β-prolamins, based on the reported M_rs for these polypeptides (Esen, 1987; Shull *et al*., 1991; Mazhar, Chandrashekar & Shetty, 1993), although the M_rs for γ-prolamins in this study were lower than reported values. Based on comparisons with the work of Duodu *et al.* (2002), the 47 k and 170 k bands could be attributed to kafirin dimers and polymers, respectively

2.2.5.4. FTIR

The spectra of the proteins from unirradiated and irradiated sorghum and maize porridges were normalized using the highest absorbance peak in the α-helical band of the Amide I region (Fig 2.2.2). Generally, the spectra showed essentially the same bands, with small shifts in band positions and changes in band intensities when flour samples were irradiated before cooking, but the changes were not consistent between the samples and there was not a dose-response relationship.

In the Amide I region (1620-1700 cm^{-1}), bands between 1650 and 1658 cm^{-1} are attributed to α-helical segments and those between 1620 and 1640 cm^{-1}, and between 1670 and 1695 cm^{-1} to β-sheets (Surewicz & Mantsch, 1988). In the Amide II region (1510-1580 cm^{-1}) α-helix bands are found between 1545-1547 cm^{-1} and β-sheets at about 1524 cm^{-1} (Surewicz & Mantsch, 1988; Bandekar, 1992).

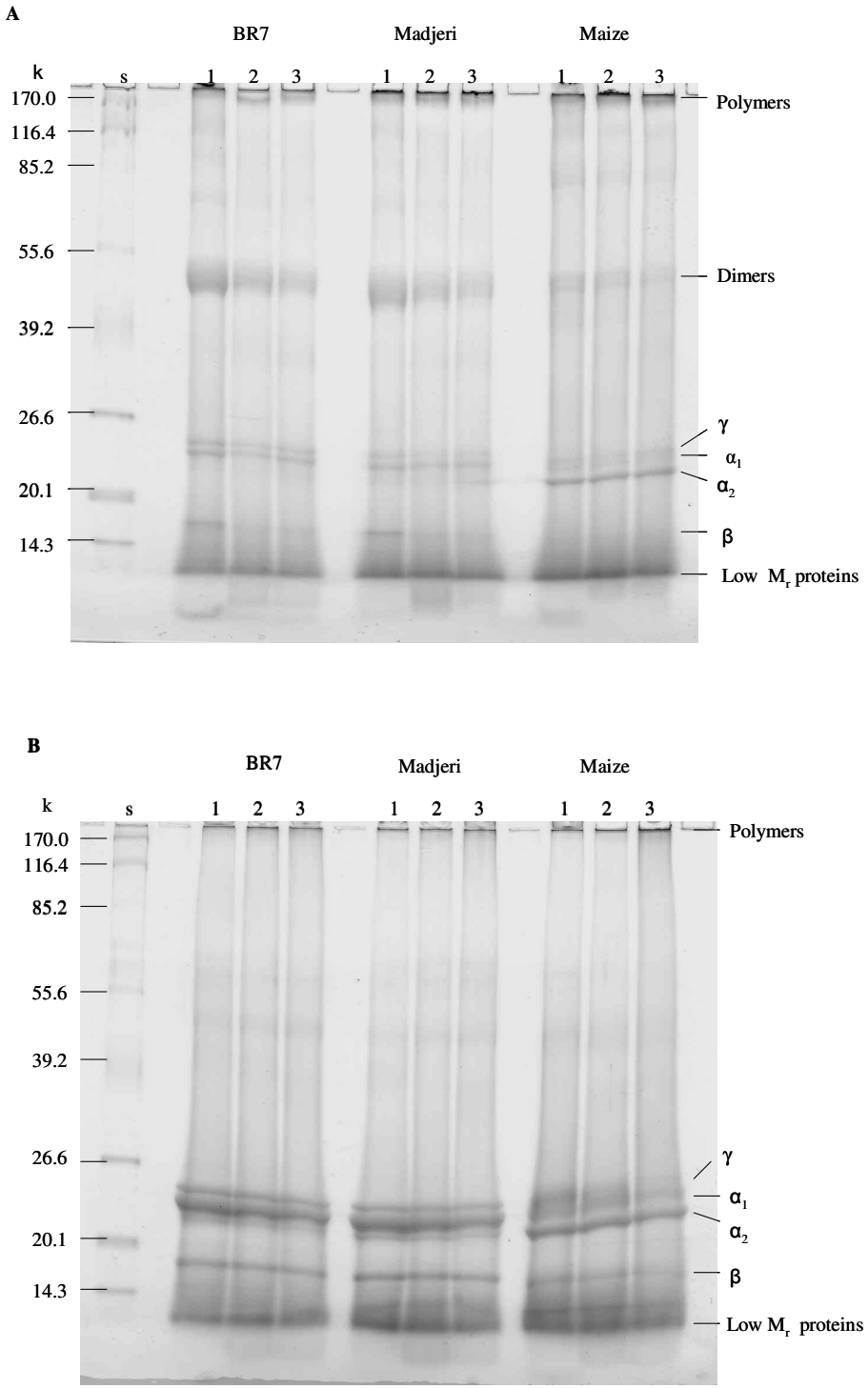

Figure 2.2.1. Gradient SDS-PAGE (7-14% acrylamide) of sorghum BR7 and Madjeri and maize PAN 6043 protein-rich flours from porridges of unirradiated (lane 1), 10 kGy dry irradiated (lane 2) and 50 kGy wet irradiated (lane 3) flours, performed under non-reducing (A) and reducing (B) conditions. Lane s is molecular weight standards.

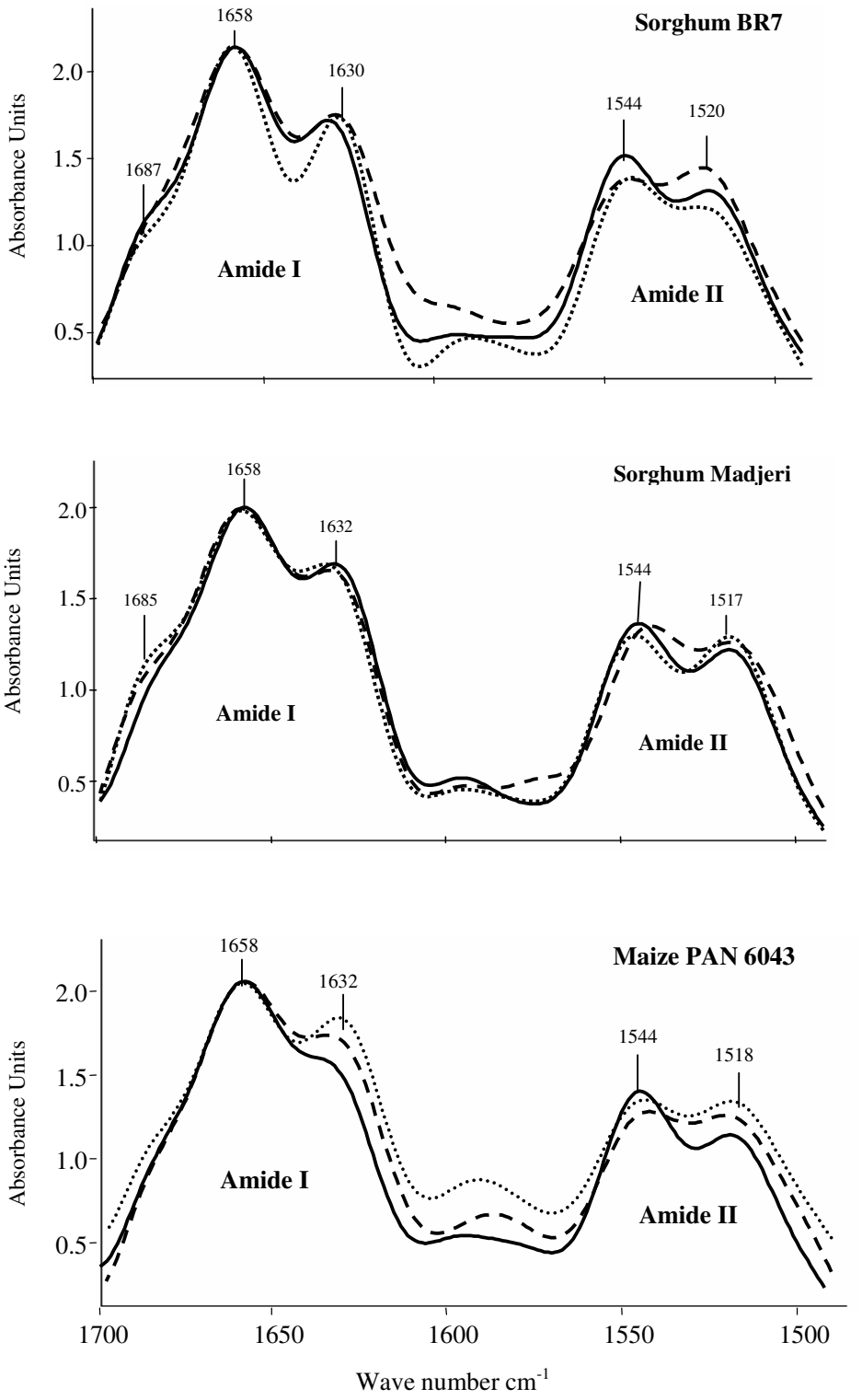

Fig 2.2.2. FTIR spectra of sorghum BR7 and Madjeri and maize PAN 6043 protein-rich flours from porridges of unirradiated (smooth lines), 10 kGy dry irradiated (dotted lines) and 50 kGy wet irradiated (dashed lines) flours, normalized to the highest absorbance peak of the alpha-helix band (1658) in the Amide I region.

In sorghum BR7, in the Amide I region, the β-sheet band at 1633 cm^{-1} was slightly shifted to lower wavelengths (1630 cm^{-1}) with irradiation. In the Amide II region, the absorbance of the α-helix (1544 cm^{-1}) band decreased with irradiation, while that of β-sheet (1520 cm^{-1}) increased at 50 kGy, but decreased at 10 kGy.

With irradiation of sorghum Madjeri, the β-sheet band (1632cm^{-1}) was basically unchanged in the Amide I region, but the β-sheet band (1517 cm^{-1}) in the Amide II region increased slightly in intensity following irradiation. The α-helix band (1544 cm^{-1}) in the Amide II region was slightly shifted to higher wavelengths with irradiation at 10 kGy and to lower wavelengths with irradiation at 50 kGy.

Irradiation of maize caused an increase in the absorbance of β-sheet bands in both the Amide I (1632 cm^{-1}) and II (1518 cm^{-1}) regions, with the increase being greater at 10 kGy. The absorbance of the α-helix band (1544 cm^{-1}) in the Amide II region was slightly reduced by irradiation.

2.2.6. Discussion

With reference to prolamin extractability (Table 2.2.1), it is possible that the effects of irradiation, such as breaking of disulphide bonds (Di Simplicio *et al*., 1991; Köksel *et al*., 1998) that could have led to increased extractability of the prolamins were masked by the use of the reducing agent (DTT) in the prolamin extraction solvent. Hence, this explains why prolamin extractability was generally only slightly affected by irradiation alone. Reducing agents will cleave disulphide bonds and improve prolamin extractability (Taylor *et al.,* 1984a). It is, however, not certain why irradiation alone at high doses decreased prolamin extractability in maize, but this could possibly have been due to non-disulphide crosslinking of the prolamins.

As stated, sorghum and maize prolamins become less extractable after cooking as a result of disulphide crosslinking (Hamaker *et al.,* 1986). It is proposed that during cooking, less soluble disulphide-linked polymers are formed between α- and γ-prolamins with

themselves and with other proteins (Hamaker *et al*., 1987; Oria *et al*., 1995b). This phenomenon occurs to a greater extent in sorghum, and is considered a major cause for the greater reduction in protein digestibility when sorghum is cooked into porridge (reviewed by Duodu *et al*., 2003). It could also be the reason prolamin extractability was reduced more with cooking in sorghum compared to maize. Therefore, the higher levels of 47 k disulphide-linked dimers observed in sorghum (Fig 2.2.1A) compared to maize porridges from unirradiated flour may reflect the higher levels of disulphide bonding in sorghum porridges that may have contributed to the lower extractability of sorghum prolamins on cooking.

The concentration of disulphide bonds in proteins from porridges of both sorghum and maize from unirradiated flour were however, similar (Table 2.2.2), which contradicts the proposition that sorghum forms more disulphide crosslinks on cooking than maize (Hamaker *et al.,* 1987), as well as the SDS-PAGE data (Fig 2.2.1). It has been suggested that the formation of a stable tertiary structure, as can occur during heating may cause disulphide bonds to be locked in the protein core, thus restraining their accessibility, reactivity and sensitivity to reducing agents, and consequently, to determination (Chan & Wasserman, 1993; Narayan, Welker, Wedemeyer & Scheraga, 2000). It is possible that such locking in of disulphide bonds may have occurred in the sorghum porridges, since they crosslink more during cooking, and thus caused the observed anomaly. This could also explain in part why the total cysteine content in sorghum porridges decreased with irradiation of flour (Table 2.2.2).

It was expected that total cysteine content would remain unchanged with irradiation if all the cleaved disulphide bonds were converted to sulphydryl groups, but this was clearly not the case. When disulphide bonds are cleaved by irradiation they yield sulphydryl groups. These sulphydryl groups can be oxidized back to disulphide bonds, or they may react further with free radicals to form new products such as sulphinic and sulphonic acids, neither of which can be converted back to sulphydryl groups or disulphide bonds (Garrison, 1987; Swallow, 1991). Some loss of disulphide bonds and sulphydryl groups may have occurred through the latter reactions, leading to a reduction in total cysteine content with irradiation.

The partial relief obtained in the reduction of prolamin extractability in sorghum porridges from 10 kGy dry irradiated flour may therefore suggest that disulphide bonds have been cleaved following irradiation and cooking. Free sulphydryl groups in sorghum porridges increased, with irradiation of dry flour at 10 kGy, while disulphide bonds decreased (Table 2.2.2), indicating cleavage of disulphide bonds by irradiation to yield free sulphydryl groups. In addition, the reduction in the amount of kafirin dimers (Fig 2.2.1A) in porridges from 10 kGy dry irradiated flour compared to unirradiated flour is also consistent with the breaking of disulphide bonds by irradiation. As stated, it is believed that the formation of disulphide bonds contributes to the reduction in prolamin extractability when sorghum is cooked into porridge (Hamaker *et al.,* 1986). A reduction in their concentration therefore, should lead to more extractable prolamins. It was interesting to observe that this was the case, as the reduction in sorghum prolamin extractability was alleviated in sorghum porridges from 10 kGy dry irradiated flour.

On the contrary, free sulphydryl and disulphide bonds in maize porridge were not significantly ($p>0.05$) affected by irradiation of dry flour at 10 kGy (Table 2.2.2) and the amount of zein dimers appeared unchanged (Fig 2.2.1A). Likewise, the reduction in zein extractability was only slightly alleviated, with irradiation of dry flour at 10 kGy. This apparent lack of effect of irradiation of dry flour at 10 kGy on maize porridge may be related to the fact, that, maize porridges had more free sulphydryl groups and a lower concentration of dimers than sorghum porridges, which is consistent with less disulphide bonds in maize porridges. Disruption of disulphide bonds can alter protein structure (Byun, Kang, Hayashi, Matsumura & Mori, 1994), and because there are fewer of these in maize compared to sorghum, splitting them by irradiation would probably have very little effect on maize protein structure, accounting for the observed absence of irradiation effect on these proteins.

At high doses of irradiation (50 kGy dry, and 10 and 50 kGy wet irradiation), followed by cooking, the effects on prolamin extractability in sorghum are obviously complex, as clear trends could not be discerned. The fact that there was no consistent further improvement in prolamin extractability of sorghum at high doses suggests that some crosslinking may have been taking place counteracting the positive effects of breaking

disulphide bonds. Although, free sulphydryl groups in sorghum increased further in porridges from 50 kGy wet irradiated flour, the amount of kafirin dimers, as observed by SDS-PAGE under non-reducing conditions (Fig 2.2.1A), was not different from those of porridges from 10 kGy dry irradiated flour, once more highlighting the complexity of the reactions at high doses.

In maize, free sulphydryl groups increased significantly ($p<0.05$) in porridge from 50 kGy wet irradiated flour (high dose) compared to porridge from unirradiated flour. The fact that the amount of dimers also decreased at high dose suggests that disulphide bonds may have been broken. However, prolamin extractability decreased in porridges from 50 kGy wet irradiated flour compared to porridges from unirradiated flour. It was mentioned earlier that at high doses of irradiation alone, non-disulphide crosslinking of maize prolamins could be taking place. It is possible therefore, that this type of crosslinking may have continued during cooking, thus, contributing to the reduction in prolamin extractability of porridge from wet maize flour irradiated at 50 kGy.

FTIR was carried out to determine if there were any changes in protein structure at the secondary level. The spectra (Fig 2.2.2) showed small inconsistent changes between samples, in bands corresponding to different secondary structures, indicating that the changes that took place with irradiation (such as cleaving of disulphide bonds) did not affect protein secondary structure considerably. The Argos *et al*. (1982) model for the α-zein polypeptide (which represent about 80% of total zein; Esen, 1987) does not show the presence of intra-molecular disulphide crosslinks. It is probable that most of the disulphide crosslinks in zeins and kafirins are formed between α- and γ-prolamins and are inter-molecular. Splitting these crosslinks therefore, may not significantly alter protein secondary structure, which would explain the results observed here. Wu, Paulis, Sexson & Wall (1983) observed that the α-helical content of zein proteins remained essentially constant when disulphide bonds in zein were broken by 2-mercaptoethanol, suggesting that disulphide bonds in zein are not important in maintaining α-helical structure. Similarly, work carried out by Smeller, Meersman, Fidy & Heremans (2003) on horse-radish peroxidase protein and using FTIR spectroscopy, suggested that cleavage of

disulphide bonds may lead to a state that has a higher flexibility rather than a changed secondary structure, which may also have been the case here.

2.2.7. Conclusions

As suggested by sulphydryl, disulphide and SDS-PAGE data, porridges from irradiated sorghum flours appear to have fewer disulphide bonds, and less disulphide linked kafirin dimers than porridges from unirradiated flour. Such changes may modify protein structure to give a more open protein network that would expose the proteins to solvents and relieve the reduction in prolamin extractability, as seen with sorghum porridges from 10 kGy dry irradiated flour. At high doses however, the situation is more complex, with possible formation of non-disulphide crosslinks that could cause refolding of the protein structure and reduce solvent access to proteins, hence the inconsistent results in prolamin extractability at high doses. Maize prolamin extractability on the other hand is markedly reduced by irradiation alone at high doses, and in porridge from 50 kGy wet irradiated flour, probably due to non-disulphide crosslinking of maize proteins under these conditions.

2.2.8 References

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3 DISCUSSION

This section will first discuss some of the methodologies used in this study. Subsequently, it will go on to examine the main finding of this research, which is that irradiation of sorghum flour before cooking into porridge partially alleviated the reduction usually observed in pepsin protein digestibility when sorghum is cooked into porridge. A mechanism by which this occurs is proposed, and reasons are advanced as to why the relief in reduction of digestibility is higher at low dose (10 kGy dry) of irradiation, but decreases when the dose is increased to 50 kGy and when the flour samples are irradiated in wet medium.

3.1 Methodological Considerations

Various methods were used in this study. The key methods will be discussed here; their principles, strengths and limitations, as well as modifications made to original methods, and why. A discussion of how good the methods were in measuring the desired effects and what could have been done to improve their performance where necessary will also be provided.

This study set out to investigate the effects of irradiation and cooking on the protein digestibility of sorghum in comparison with maize. It has been established that the effects of irradiation on proteins, as well as on any food component, are influenced by the moisture content of the sample (Cieśla *et al*., 2000; Lee *et al*., 2003). This is so because radiolysis of water generates free radicals with high oxidizing and reducing potentials. In a liquid environment these free radicals are free to move about and react with food components, thus causing extensive modification to the proteins compared to when irradiation is carried out on dry solids or flour samples with low moisture content (Cieśla *et al*., 2000). Irradiation was therefore carried out additionally in wet medium, as this was expected to heighten the effects of irradiation on sorghum and maize proteins for clearer understanding. High dose (50 kGy) of irradiation was also employed for similar reasons. The use of 30% solids content for the wet irradiated and porridge samples was prompted by the fact that it approximates the concentration at which stiff porridges are prepared

from sorghum and maize (Murty & Kumar, 1995). From the results obtained, it was apparent that the effects of irradiation were more intense at high dose and also in the wet irradiated samples, and this was particularly evident in the cooked samples, where the problem of sorghum protein digestibility resides.

Whole grain sorghum and maize flour are commonly used for preparing stiff porridges in the northern and western parts of Cameroon respectively (Fombang, personal observation). Hence, the choice of whole grain flours. It was an added advantage that the sorghum grains used were condensed tannin-free cultivars, which avoids the complication of tannin-protein interactions interfering with digestibility (Chibber *et al,* 1980).

In order to be able to determine the effects of irradiation combined with cooking on proteins at the molecular level, it was essential to concentrate the proteins in the samples. Given that the problem of reduced protein digestibility in sorghum occurs when it is cooked into porridge, it was decided to consider only the cooked samples for molecular studies, with the unirradiated cooked samples serving as the control. This decision may not have been the best as it was later discovered that it would have been helpful to have the unirradiated uncooked control samples to be better able to judge if the observed effects were due to irradiation alone or a combination of irradiation and cooking.

Protein concentration was carried out according to the wet milling procedure of Taylor *et al*. (1984b). Given that the prolamin proteins are the ones that influence protein digestibility of sorghum and maize significantly (Hamaker *et al*., 1987; Oria *et al*., 1995a; Duodu *et al*., 2003), a decision was made to first extract the salt soluble proteins before wet milling. Wet milling mechanically disrupts the protein and starch complex and separates cell wall fibres from the starch and proteins (Eckhoff *et al*., 2003). The fibres are then removed by sieving. In the normal wet milling procedure uncooked samples are used. Because the samples used in this study were cooked porridges, the gelatinized starch made sieving the wet milled products impossible. To overcome this problem, the porridges were treated with α-amylase to break down gelatinized starch before wet milling. This treatment thinned the slurry and it could then be sieved.

The wet milling did not, however, produce pure proteins as would have been desired. The low protein concentration (35-50%) of the protein preparations suggests that the proteins were not completely separated from the starch and fibres. Starch-protein interactions that occur during cooking (Zhang & Hamaker, 1998) may hinder the effective separation of starch from proteins and thus contribute to the low purity of the protein preparations. Nonetheless, the samples were sufficiently protein rich that they could be used for the proposed analyses.

Concerning assaying for protein digestibility, the pepsin assay is a single enzyme assay that has been widely used in estimating protein digestibility of sorghum and maize proteins and especially in determining the effects of wet cooking on sorghum and maize protein digestibility (Mertz *et al*., 1984; Hamaker *et al*., 1987; Duodu *et al*., 2002). It involves incubating the sample with pepsin at acidic pH and measuring the amount of insoluble protein. One of the drawbacks of this method is that it could underestimate protein digestibility for proteins that are resistant to pepsin because of their primary structure or acid stability of their tertiary structure (Swaisgood & Catignani, 1991). However, in the case of sorghum and maize it could be expected to give a good indication of protein digestibility given that pepsin preferably hydrolyses bonds containing hydrophobic amino acid residues (Huang & Tang, 1968). In sorghum and maize, the predominant proteins are the prolamins (Guiragossian *et al*., 1978; Taylor *et al*., 1984b), which are high in the hydrophobic amino acids proline, alanine and leucine (Esen *et al*., 1985, Esen, 1987; Taylor & Belton, 2002). Added to this is the fact that results obtained in human studies with sorghum (MacLean *et al*., 1981, 1983) paralleled those obtained with pepsin (Axtell *et al*., 1981; Mertz *et al*., 1984).

The multienzyme pH stat assay on the other hand, determines protein digestibility using a combination of three enzymes; trypsin, chymotrypsin and amino-peptidase (Pederson & Eggum, 1983). It is based on the principle that during proteolysis protons are released from the cleaved peptide bonds resulting in a decrease in pH of the suspension (Boisen & Eggum, 1991). Assuming a correlation between the initial rate of peptide release and protein digestibility, the later can be predicted by measuring the amount of base needed to maintain the pH of the reaction mixture constant for a period of 10 min. It has been used

to estimate protein digestibility of uncooked sorghum and maize (Weaver *et al*., 1998; Aboubacar *et al*., 2001) and found to correlate (r = 0.86) well with pepsin digestibility (Aboubacar *et al*., 2001) under those conditions. With extruded sorghum (Gomez, Waniska, Rooney & Lusas, 1988), however, the pH-stat multienzyme method indicated that digestibility of protein was less affected by extrusion than was indicated by the pepsin assay. It is, however, difficult to draw any conclusion from these two studies about the performance of the multienzyme assay, as the problem of protein digestibility in sorghum is with the wet cooked sorghum, and not with the uncooked or extruded samples (reviewed by Duodu *et al*., 2003).

Given that the pH-stat assay correlated well with pepsin in determining protein digestibility of uncooked sorghum and maize, and has the advantage over pepsin of being relatively more rapid, its performance was investigated with wet cooked sorghum and maize. The results obtained with the pH-stat method in this study do not show a reduction in protein digestibility of sorghum with cooking as has repeatedly been reported in literature (reviewed by Duodu *et al*., 2003), and suggests therefore, that, this assay may not be suitable for determining the effects of wet cooking on sorghum protein digestibility.

As stated, in section 2.2, the amount of extractable prolamins in sorghum and maize is believed to be somehow related to their protein digestibility (Hamaker *et al*., 1986; Hamaker *et al*., 1994). The prolamin proteins of the sorghum and maize flour samples were extracted first without and then with reducing agent to obtain the uncrosslinked (prolamin I) and crosslinked prolamins (prolamin II) (Guiragossian *et al*., 1978; Shewry, 2002), and the extracts freeze-dried. The lower kafirin and zein content obtained in this study for the unirradiated uncooked samples in comparison with reported values (Taylor *et al*., 1984a; Hamaker *et al.,* 1995) indicates that not all of the kafirins and zeins were extracted. The low extractability of the prolamins was due to some problems encountered while carrying out the extraction. It was impossible to determine the protein content of the liquid extracts using the Dumas method because their protein concentration was too dilute (~0.09% N) to be detected using the Leco protein/nitrogen analyzer. It was then resolved to freeze-dry the extracts before determining their protein content. The extracts

were poured into weighed aluminium plates for freeze-drying. After drying, the plates together with the samples were weighed before transferring the powder samples to bottles for storage at 4ºC. The dry samples were stored for about three weeks before determining their protein content, and during this period they probably picked up moisture that would have diluted their nitrogen content. Because of the small quantity of the samples (< 1 g) their moisture content could not be determined before analysis, and the protein content was calculated relative to the weight of the samples obtained immediately after freeze-drying, thus giving a low protein content for these proteins. Losses encountered during handling may have additionally contributed to the low recoveries. Determining the protein content in the liquid extracts would most certainly have improved accuracy. This shortcoming, notwithstanding, the results obtained could be expected to reflect general trends in prolamin extractability with irradiation and cooking.

Polyphenols were determined according to the International Organization for Standardization (ISO, 1988) method for the determination of tannins in sorghum grain. It is based on the interaction of ferric ions with phenols in the presence of ethanolamine to give a coloured complex (Mole & Waterman, 1987). This method is not specific for one single type of polyphenol, which makes it appropriate for our study as the sorghum samples used were condensed tannin-free sorghums. However, such sorghums can contain phenolics such as phenolic acids and flavonoids (Hahn *et al*., 1984; Serna-Saldivar & Rooney, 1995), which would give a positive reaction with this assay.

To establish whether or not the effects of irradiation were influenced by the presence of polyphenols, which are known to possess antioxidant activity (AOA) (Rice-Evans, Miller & Paganga, 1997), AOA was measured using the ABTS radical cation decolorization assay (Re *et al*., 1999). The $ABTS^{\cdot+}$ radical is generated by oxidation of ABTS with potassium persulphate and the radical is then reduced in the presence of hydrogen-donating antioxidants. The percent inhibition of absorbance at 734 nm is a measure of the AOA. The ABTS method is suitable for use with samples where pigmentation is present (Awika, 2002) as in the red sorghum BR7, since the pigmentation would not interfere with the results. The method also has the advantage of being applicable to antioxidants

including flavonoids and carotenoids (Re *et al*., 1999), the former being present in sorghum (Hahn *et al*., 1984; Serna-Saldivar & Rooney, 1995).

Concerning the determination of sulphydryl groups and disulphide bonds, the solid-state direct colorimetric assay for disulphide bond determination is said to be a rapid and convenient method for determining cysteine and cystine content of suspensions that contain a mixture of soluble and insoluble proteins (Thannhauser *et al*., 1987). This assay was developed to avoid the tedious extraction procedure that could not solubilise all the proteins and required removal of the reducing agent before assay by colorimetric means (Thannhauser *et al*., 1987). Ellman's reagent, 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) reacts specifically with thiol groups and $NTSB^{2-}$ (disodium-2-nitro-5-thiosulphobenzoate) reacts with cysteine and thiol groups formed after reduction of disulphide bonds with sodium sulphite (Thannhauser *et al*., 1987; Chan & Wasserman, 1993; Pöyri, Mikola, Sontag-Strohm, Kaukovirta-Norja & Home, 2002). To maximize reaction of the protein with DTNB and $NTSB^{2-}$, a combination of urea and SDS are added to the reaction mixture to expose any thiol or disulphide bonds that may be buried within the hydrophobic protein matrix (Chan & Wasserman, 1993). This was particularly important for the porridge samples used in this study, as it is known that the kafirins and zeins are hydrophobic (Wall & Paulis, 1978; Wilson, 1983) and crosslink through disulphide bonds during cooking (Hamaker *et al*., 1987). Some of these disulphide bonds may become buried in the protein matrix, hence the need for SDS and urea to expose them. However, it appears from my results that not all of the disulphide bonds, particularly in the case of sorghum, were exposed for assay possibly because the proteins were extensively crosslinked and folded after cooking. Studies by Chan & Wasserman (1993) and Narayan *et al.,* 2002 had shown that formation of stable tertiary structure in proteins may result in disulphides bonds being 'locked in' and therefore not detectable. It is possible that crosslinking of sorghum during cooking may have caused folding of the protein that may in turn have prevented some of the disulphide bonds from being detected. This poses a limitation to the use of this assay, especially with highly crosslinked products, since it appears that not all of the disulphide bonds may be accessed for determination. In view of this limitation, therefore, the results of this assay only represent apparent values and should therefore be treated with caution.

One major advantage of this assay though, is that regardless of whether DTNB and $NTSB^{2-}$ reacts with soluble or insoluble proteins, the product of this reaction, the chromophore NTB^{2-} (2-nitro-5-thiobenzoate) is soluble in aqueous solution (Chan & Wasserman, 1993). The solids can then be separated by centrifugation and the absorbance of the supernatant read. In addition, the reaction of NTSB with thiols and disulphides can be carried out in the presence of dissolved oxygen and the reducing agent, sodium sulphite, which eliminates the inaccuracy and inconvenience associated with the necessity to work under an oxygen free atmosphere as well as the need to remove the reducing agent (Thannhauser *et al*., 1987). The reliability of this assay was tested with a reference protein, bovine serum albumin (BSA), and the measured cysteine content was 35.6 mol %, which agrees with that calculated from the amino acid sequence of BSA (35 mol %). Different concentrations of sulphite were also tested to ensure sufficient sulphite was available to reduce all the disulphide bonds, and the 0.1M sulphite reported in this method was found to be optimal.

Electrophoresis describes the migration of a charged particle under an electric field (Wilson & Walker, 1995). SDS-PAGE separates proteins according to subunit size (Smith, 1994) and has been used to determine changes in the molecular weight of proteins following irradiation (Bhatty & MacGregor, 1988; Puchala & Schuessler, 1993; Nićiforović, Radojčić & Milosavljević, 1999).

In SDS-PAGE, the protein is saturated with SDS, which masks the natural charge of the protein. SDS also denatures the protein, degrading its secondary, tertiary and quaternary structure and aids solubilisation (Otter, 2003). Reducing agent is added to break disulphide bonds when electrophoresis is carried out under reducing conditions. Polyacrylamide gel is used because it has pore sizes similar to that of protein molecules and so contributes a molecular sieving effect (See & Jackowski, 1989; Smith, 1994). The proteins bind SDS, become negatively charged and are separated based on size alone (Smith, 1994). It is, however, important that the protein standards and unknown proteins bind equal amounts of SDS per gram of protein, which will ensure identical mobility on SDS-PAGE gels. If this does not happen, it will result in differential mobility on the gel and give an unreliable estimation of molecular weight (Bunce, White & Shewry, 1985).

The lower molecular weight estimations obtained for the γ-prolamins in this study (22-26 k) as opposed to 27-28 k (Shewry, 2002) is believed to be a problem associated with the Laemmli method used. It has been observed that this method underestimates the molecular weight of γ-prolamins in sorghum and maize (Prof JRN Taylor, Department of Food Science, University of Pretoria, personal communication).

The molecular weight of the proteins in this study was initially determined using the uniform gel system, but because band separation was not clear on this system, gradient gels were subsequently used. Gradient gels because of their decreasing pore sizes give increased protein sieving effect at high polyacylamide concentrations, leading to the formation of sharper bands at lower molecular weights, since the proteins are about the same size as the sieve. Gradient gels also allow determination of polypeptides with a wider range of molecular weights (Bollag, Rozycki & Edelstein, 1996).

Infrared spectra are increasingly utilized for the analysis of peptides and proteins because they detect the amide (peptide) bond, which display distinct IR signals for differently folded peptides and proteins (Singh, 2000). Proteins are composed of a mixture of different structural elements, including α-helical, β-sheets, turns and unordered strands. Each of these has a characteristic hydrogen bonding pattern involving amide C=O and N-H groups, and therefore the amide C=O groups associated with each structural element may be expected to have a characteristic electron density. This in turn will provide a characteristic absorption frequency (Jackson & Mantsch, 1993). It is this separation of the amide absorptions that underlies the determination of protein secondary structure by IR spectroscopy

In the infrared spectra, the secondary structure of proteins is most clearly reflected by the Amide I and II regions, particularly the former which absorbs around 1620-1690 cm^{-1} (Byler & Susi, 1986, Bandekar, 1992). Despite the clarity of protein secondary structure in the Amide I region, it has the disadvantage of absorbing water bands at 1640 cm^{-1} interfering with protein bands at 1650 cm^{-1} and the difficulty of assigning peaks correctly due to serious overlapping of the random coil and the α-helix bands (Singh, 2000). Water interference was not expected to be a problem in this study because the samples used

were freeze-dried. Despite its disadvantages, the Amide I region is, however, considered useful for monitoring structural changes caused by protein denaturation (Christy Yukihiro & Gregoriou, 2001). The Amide II region, on the other hand, is not as sensitive as the Amide I and III regions to variations in secondary structure content and is not by itself used for secondary structure estimation (Singh, 2000). The Amide I and II bands were used in this study to determine secondary structure components of the FTIR spectra because they gave high signal bands.

The Amide III region (1200-1350 cm^{-1}) does not have interfering OH vibrations from water and no overlapping bands arising from different secondary structures (Singh, 2000). It is, however, limited for secondary structure analysis because of a lower signal band that requires higher signal-to-noise ratio spectra for accurate analysis, and interference from CH vibrations of amino acid side chains and other non-protein groups (Singh, 2000). The Amide III band was not used in this study because of low signal bands.

FTIR was carried out in this study using high pressure diamond optics and a microscope, ATR (Attenuated Total Reflectance) Zinc selenide cell and KBr pellets. High pressure diamond optics had the advantage of needing smaller samples (about 10 mg) and gave better resolution spectra compared to ATR Zinc selenide cell and KBr pellets. ATR requires perfect contact between the ATR crystal and the sample for good intensity spectra (Spectrum, 2.0 Users Guide, 1998), and uses large quantities of sample (about 0.3 g), which was a handicap in this study due to limited protein samples. In addition, ATR did not give good intensity spectra compared to the diamond cell possibly due to poor contact between the sample and the ATR cell. With KBr pellets, it is possible that the samples were unevenly distributed within the pellet, and this may have caused the lower resolution of the spectra obtained with this method.

Compared to other methods of protein secondary structure analysis such as nuclear magnetic resonance (NMR), circular dichroism (CD) and Raman spectroscopy, FTIR spectroscopy has the advantage that it can be used with protein preparations of low protein concentration (Jackson & Mantsch, 1995). High molecular weight proteins can also be studied using FTIR (Singh, 2000). CD analysis requires an accurate protein

concentration and is limited to clear protein solutions. NMR requires protein preparations of high protein concentration and is limited to small proteins of about 200 amino acids (Jackson & Mantsch, 1993; Singh, 2000). However, FTIR alone is not sufficient to unequivocally establish protein secondary structure without verification by other methods such as NMR and X-ray diffraction (Wilder, Friedrich, Potts, Daumy & Francoeur, 1992; Li-Chan, 1998).

Raman spectroscopy has the advantage of having a high signal-to-noise ratio, but its use is impaired by the fact that it requires samples with high protein concentrations; otherwise there is excessive fluorescence (Jackson & Mantsch, 1993; Singh, 2000; Thygesen, Lokke, Micklander & Engelsen, 2003). Raman spectroscopy was attempted in this study, but, because of the low purity of the samples, there was too much fluorescence and no reasonable spectra could be obtained.

3.2 Sorghum and Maize Proteins and the Effects of Cooking on their Protein Digestibility

The aim of this study was to determine if irradiation prior to cooking could alleviate the reduction observed in protein digestibility when sorghum flour is cooked into porridge. To assess the effects of these treatments on protein digestibility, the *in vitro* pepsin and multienzyme assays were used. Results of sorghum protein digestibility obtained using the pepsin assay has previously been shown to correlate with *in vivo* protein digestibility (Axtell *et al.,* 1981; MacLean *et al.,* 1981). In this study, the results of sorghum protein digestibility as determined by the multienzyme assay did not correlate with the pepsin assay (Table 3.1). Because pepsin digestibility has been shown to correlate with *in vivo* digestibility, this discussion will focus on the effects of irradiation and cooking on protein digestibility as determined by the pepsin assay.

Table 3.1. Correlation matrix showing the relationship between pepsin and multienzyme digestibilities with prolamin extractability for sorghum and maize flours

Sorghum BR7	Prolamins	Pepsin	pH stat
Prolamins	1		
Pepsin	0.83**	1	
pH stat	0.20	-0.05	1
Sorghum Madjeri	Prolamins	Pepsin	pH stat
Prolamins	1		
Pepsin	0.75*	1	
pH stat	0.17	0.42	1
Maize PAN 6043	Prolamins	Pepsin	pH stat
Prolamins	1		
Pepsin	0.32	1	
pH stat	0.49	0.62	1

* $p<0.05$; ** $p<0.01$

Sorghum and maize prolamin proteins in the uncooked state contain disulphide bonds (Esen, 1987; Oria *et al*., 1995a). Argos *et al*. (1982) proposed a model for α-zeins that shows the presence of intra- and inter-molecular hydrogen bonds within and between α-zein polypeptides. According to Koppelman, Nieuwenhuizen, Gaspari, Knippels, Penninks, Knol, Hefle & De Jongh (2005), disulphide bonds contribute to the stability of protein structure on the secondary and tertiary folding levels. Likewise, in soybean proteins hydrogen and disulphide bonds are considered to be important in maintaining network structure (Marsman, Gruppen, de Groot & Voragen, 1998). One can therefore presume that the disulphide bonds (Esen, 1987; Oria *et al*., 1995a) and hydrogen bonds (Argos *et al*., 1982), present in sorghum and maize prolamins are involved in stabilising the protein structure.

During wet cooking of sorghum, the formation of disulphide bonds between prolamin proteins and possibly other proteins may cause the protein structure to become more compact and folded (Fig 3.1b), considering that disulphide bonds contribute to the folded structure of proteins (Koppelman *et al*., 2005).

Because disulphide bonds can crosslink proteins polypeptides to form macropolymers, they could cause folding of the protein, with the result that some peptide bonds susceptible to proteolysis could become inaccessible to proteolytic enzymes, thus rendering these macropolymers less digestible (Carbonaro, Bonomi, Iametti, Cappelloni & Carnovale, 1998). This, is so, because, the digestibility of a protein is dependent on the accessibility of susceptible peptide bonds to specific proteolytic enzymes (Carbonaro *et al*., 1998). Crosslinking of proteins, as suggested by Hurrell & Finot (1982), reduces their digestibility by preventing enzyme access to peptide bonds or by masking sites of enzyme attack. Thus in wet cooked sorghum, crosslinking of proteins through the formation of disulphide bonds may result in folding of the proteins, preventing enzyme access to them, hence, the reduction in digestibility.

As in the case of wet cooked sorghum, reduced proteolysis as a result of the formation of disulphide-bonded macropolymers during processing has also been reported in whey proteins (Carbonaro *et al*., 1998) and in soy bean proteins (Hager, 1984). In maize, on the

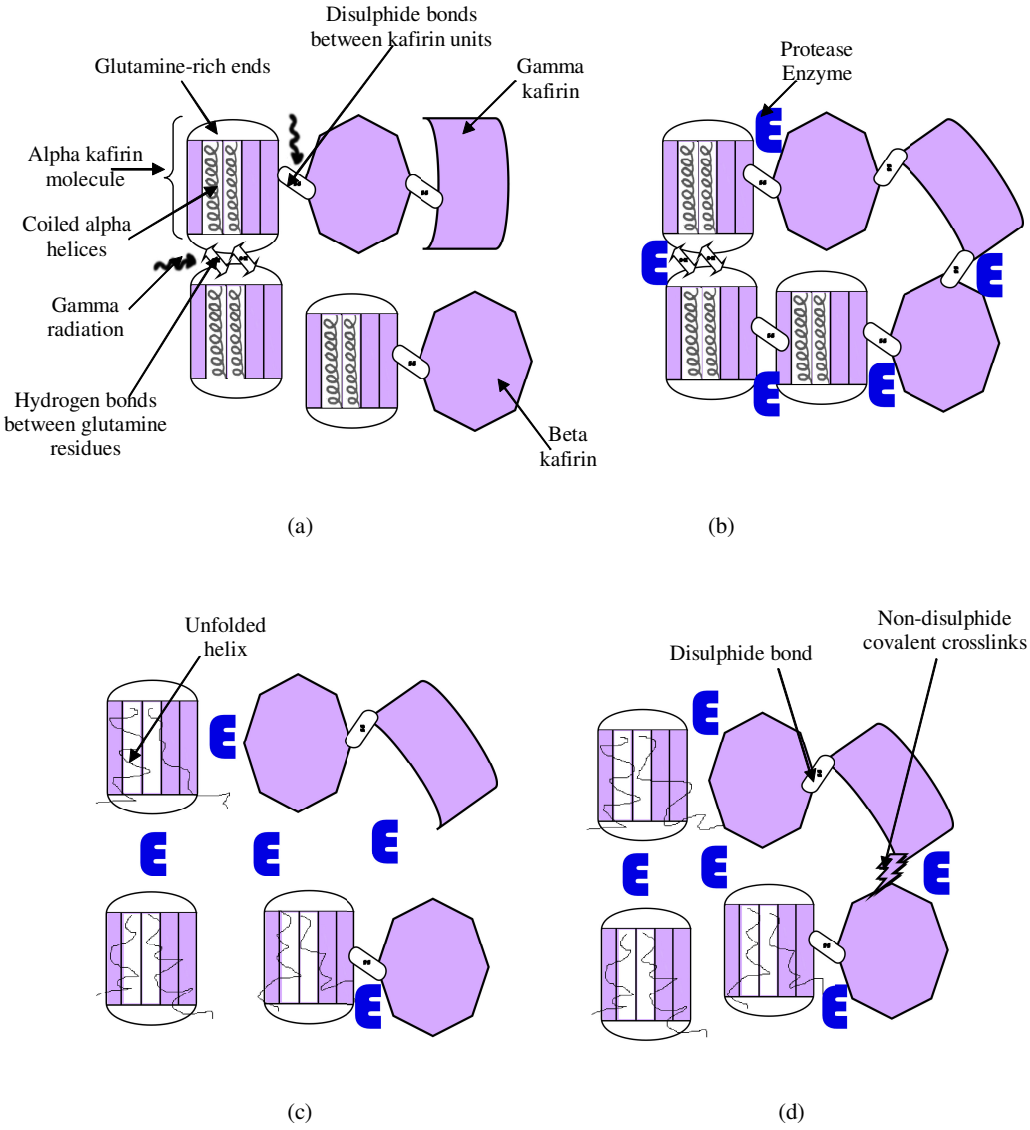

Fig 3.1 Model showing sorghum kafirin proteins, and proposed changes in sorghum kafirin crosslinking patterns with cooking alone and irradiation combined with cooking, and the effects of these crosslinks on protease enzyme access to the proteins; (a) kafirins in uncooked flour showing possible sites of attack by irradiation, (b) porridge from unirradiated flour, (c) porridge from 10 kGy dry irradiated flour, (d) porridge from 50 kGy wet irradiated flour.

other hand, disulphide bond formation during wet cooking is less extensive compared to sorghum, and has been advanced as the reason for the lower reduction in maize protein digestibility on cooking (Duodu *et al*., 2002). The finding that porridges from unirradiated sorghum flour had more dimers compared to their maize counterparts (section 2.2) supports this proposition. However, the results of disulphide bonds did not agree with this proposition. The results, showed similar levels of disulphide bonds in sorghum and maize porridges. Possible reasons for this discrepancy have been discussed in section 3.1.

Pepsin hydrolyses peptide bonds between hydrophobic amino acids such as phe-val and phe-ala residues, which frequently occupy the interior of the proteins (Brody, 1994). Such amino acids and peptide bonds occur in kafirin (DeRose *et al*., 1989) and zein (Viotti, Cairo, Vitale & Sala, 1985). It is possible therefore that during cooking, with the formation of disulphide bonds, the proteins become more folded enclosing the hydrophobic residues further inside the protein core, thus preventing them from coming into contact with the enzyme. This may contribute to the reduced protein digestibility of sorghum when cooked into porridge.

3.3 Effects of Irradiation and Cooking on Protein Digestibility of Sorghum and Maize Flours

Irradiation generates free radicals that can attack protein molecules. Radiation energy can disrupt non-covalent bonds (hydrogen bonds and hydrophobic interactions) and covalent (disulphide) bonds (Garrison, 1987; Swallow, 1991; Kempner, 1993) that stabilise protein structure. Hydrogen bonds, such as those in the Argos model between glutamine residues, can be disrupted when the glutamine residues are deamidated following irradiation (Venkatachalam & Sathe, 2003). Cleaving of disulphide bonds by irradiation can occur either, through the direct action of ionising radiation on disulphide bonds or through the secondary effects of water radiolytic products on disulphide bonds (Di Simplicio *et al*., 1991). Direct action will most likely have dominated during irradiation of the dry flours, where radiation energy reacts directly with the protein with the ejection of electrons to

form free radicals (Swallow, 1991). On the other hand, during wet irradiation, secondary effects will be more prevalent. In wet systems, radiation energy is absorbed by water, which then dissociates to give hydroxyl radical, hydrated electrons and other radical species, which are very reactive, and will in turn react with proteins producing free radicals. The reaction below shows the possible radiolytic products that can be obtained from the radiolysis of water:

$$H_2O \longrightarrow \cdot OH + \acute{e}_{aq} + H\cdot + H_2 + H_2O_2 \qquad \text{(Thakur \& Singh, 1994)}$$

Energy not taken up by water is taken up directly by the protein and other molecules present with the ejection of electrons to form radicals. Irradiation in wet medium will therefore achieve greater radiolytic damage because of the combination of both direct and indirect effects. The finding that the protein digestibility of porridges from dry sorghum flours irradiated at 50 kGy was similar to digestibility of porridges from wet flours irradiated at 10 kGy, and that protein digestibility of maize porridges was only significantly affected in wet irradiated flour samples, demonstrates the enhanced effects of radiation damage in wet medium. Similar reports of the enhanced effects of radiation damage in wet compared to dry medium appear in the literature (Cieśla *et al*., 2000; Lee *et al*., 2003).

Since disulphide and hydrogen bonds are involved in stabilising protein structure, their breaking can result in loss of conformational or structural integrity that could expose additional peptide bonds and enhance proteolysis (Kananen, Savolainen, Mäkinen, Perttilä, Myllykoski & Pihlanto-Leppälä, 2000; Venkatachalam & Sathe, 2003; Koppelman *et al*., 2005). Working with dry beans, Reddy, Pubois & McGinnis (1979) concluded that irradiation of beans at 210 kGy prior to autoclaving could cause changes in their protein structure that enhanced denaturation of the protein during subsequent heating, and improved its digestibility. It is possible that in sorghum, following irradiation, the protein structure may have been changed, such that it impaired the formation of disulphide bonds during cooking. The observation of fewer disulphide bonds in proteins of porridges from irradiated flour compared to unirradiated flour lends support to this argument.

In an attempt to explain the mechanism by which irradiation alleviated the reduction in cooked sorghum protein digestibility, this discussion will focus on the prolamin proteins (kafirins), which are the majority of the proteins in sorghum, and are the proteins directly implicated in reduced protein digestibility in wet cooked sorghum (reviewed by Duodu *et al*., 2003). Figure 3.1 shows a graphical representation of the proposed mechanism for the alleviation of the reduction in cooked sorghum protein digestibility, when cooking was preceded by irradiation of flour.

As stated, kafirin, like zein, can be divided into three species α-, β- and γ-kafirins, based on similarities in their solubilities, molecular weight and cross reactivity of kafirins with zein antisera (Shull *et al*., 1991). Amongst these, the α-kafirins are the major group, representing about 80% of the total kafirins, while β- and γ-kafirins account respectively for about 8 and 12% of total kafirins (Shull *et al*., 1991). Argos *et al*. (1982) proposed a structural model for the zein 19 and 22 k protein (the α-zein polypeptide species) based on CD data and their amino acid sequences. Given the sequence homology between zein and kafirin (DeRose *et al*., 1989), the model can also be applied to α-kafirin. The model shows α-zein as consisting of a collection of nine helical rods packed in a cylinder, with the top and bottom of the cylinder populated by glutamine residues. Polar and hydrophobic residues distributed along the helical surfaces will allow intra- and intermolecular hydrogen bonds and van der Waals interaction among neighbouring helices such that the rod-shaped zein molecule could aggregate in molecular planes which can then stack through glutamine interactions (hydrogen bonds) at the cylinder caps (Fig 3.1a). This model does not show any intra- or intermolecular disulphide bonds, possibly because of the low cysteine content of the α-zein, about 1 or 2 cysteine residues (Shewry, 1995). The structure of β- and γ-zeins are, however not known.

It has been proposed that during wet cooking of sorghum, disulphide crosslinks are formed between the different kafirin species with themselves and possibly with matrix proteins, to form protein polymers that are less digestible, thus, accounting for the reduction in protein digestibility when sorghum is cooked into porridge (Oria *et al.,* 1995b). Fig 3.1b shows proposed disulphide bonding between kafirin species when sorghum is cooked into porridge, and how this affects enzyme access to the proteins.

However, sorghum kafirins in the uncooked flour contain disulphide bonds (Oria *et al*., 1995a) as well as hydrogen bond and van der Waals interaction, as seen in the Argos model. When sorghum flours are subjected to irradiation therefore, these disulphide and hydrogen bonds may become cleaved by radiation energy, as discussed.

Considering the kafirin proteins, it is conceivable therefore, that, breaking of the disulphide bonds between the kafirin species and between these and other proteins, and breaking of hydrogen bonds within and between the α-kafirin helices, would lead to unfolding of the helices within the α-kafirin molecule, and dissociation of the helices and kafirins species from one another. With the individual kafirins species now more free to move, this would increase the intermolecular distances between them and create an open protein network. Unfolded and free polypeptides, together with a more open protein network would reduce the chances of the cysteine residues coming together during subsequent cooking to form disulphide bonds or other crosslinks. Consequently fewer disulphide bonds would be expected in porridges from irradiated flours compared to porridges from unirradiated flours (Fig 3.1c). This is in agreement with the data for disulphide bonds and SDS-PAGE summarized in Table 3.2. With an open protein network following irradiation and cooking, it is probable that additional peptide bonds were exposed to proteolysis and the enzymes had better access to the proteins, hence, the alleviation in the reduction in protein digestibility.

As stated earlier, pepsin hydrolyses peptide bonds between hydrophobic amino acids. Although protein hydrophobicity was not measured in this study, work done by Dogbevi, Vachon & Lacroix (2000) with red kidney beans, whose proteins are also globular, indicated that irradiation at doses up to 8 kGy increased hydrophobicity through exposure of hydrophobic amino acids following unfolding of the protein structure. With a more open protein network in the irradiated cooked porridges, it is possible that the pepsin susceptible hydrophobic amino acid peptide bonds buried within the protein core were exposed, resulting in an increase in protein digestibility.

Table 3.2. Summary of results showing changes in pepsin protein digestibility of sorghum and maize flours with some protein attributes when unirradiated and irradiated (mild and severe) flour samples are cooked in to porridge

Sorghum BR7	Pepsin digestibility	Prolamin extractability	NSI	AG solubility	Free SH groups	SS bonds	SDS PAGE dimer	L-colour	b-colour
Unirradiated flour	Control	Control	Control	Control	ND	ND	ND	Control	Control
Porridge from unirradiated flour	↓↓	↓↓	-	↓	Control	Control	Control	↓	↓
Porridge from 10 kGy dry irradiated flour	-	↓	-	↓	↑	↓	↓	↓	↑
Porridge from 50 kGy wet irradiated flour	↓	↓↓	-	↓↓	↑↑	↓↓	↓	↓	↑

Sorghum Madjeri	Pepsin digestibility	Prolamin extractability	NSI	AG solubility	SH groups	SS bonds	SDS PAGE dimer	L-colour	b-colour
Unirradiated flour	Control	Control	Control	Control	ND	ND	ND	Control	Control
Porridge from unirradiated flour	↓↓	↓↓	↓	↓	Control	Control	Control	↓	↓
Porridge from 10 kGy dry irradiated flour	-	↓	-	↓	↑	↓	↓	↓	↑
Porridge from 50 kGy wet irradiated flour	↓	↓	↓	↓↓	↑↑	↓↓	↓	↓	↑

Table 3.2 Cont'd

Maize PAN 6043	Pepsin digestibility	Prolamin extractability	NSI	AG solubility	SH groups	SS bonds	SDS PAGE dimer	L-colour	b-colour
Unirradiated flour	Control	Control	Control	Control	ND	ND	ND	Control	Control
Porridge from unirradiated flour	↓	↓	-	↓	Control	Control	Control	-	↓
Porridge from 10 kGy dry irradiated flour	↓	↓	↓	↓	-	-	-	↓	↑
Porridge from 50 kGy wet irradiated flour	↓↓	↓↓	↓	↓↓	↑	-	↓	↓	↑

- Same as control
Arrows indicate degree of increase (↑) or decrease (↓) relative to control
ND Not determined
NSI Nitrogen solubility index
AG Albumin and globulin proteins
SH Sulphydryl groups
SS Disulphide bonds

As the prolamins crosslink through disulphide and other bonds during cooking, their extractability also decreases in parallel with protein digestibility (Hamaker *et al.*, 1986). Thus, as stated, Hamaker *et al* (1994) proposed that the extractability of prolamins could somehow be related to their protein digestibility. It was therefore interesting to observe that the alleviation in the reduction in sorghum protein digestibility with cooking of dry flour irradiated at 10 kGy paralleled a relief in the reduction of the extractability of its prolamin proteins, compared to prolamins of porridges from unirradiated flour. Furthermore, there were significant positive correlations between pepsin protein digestibility and prolamin extractability for sorghum BR7 ($r = 0.83$; $p<0.01$) and Madjeri ($r = 0.75$; $p<0.05$) (Table 3.1). Maize prolamin extractability was, however, not significantly correlated with pepsin protein digestibility. This finding is not surprising considering that the extractability of maize prolamins, like its protein digestibility, was less affected by cooking and the combination of irradiation and cooking, compared to sorghum (Table 3.2).

The flour samples prepared for irradiation were not packaged under vacuum, implying that some oxygen would have been present in the system at the onset of irradiation. It has been demonstrated that irradiation in the presence of oxygen leads to breakdown of proteins to smaller peptides, whereas in the absence of oxygen, polymerisation of proteins occurs (Davies, 1987; Puchala & Schuessler, 1993; Kume & Matsuda, 1995; Tuce *et al*., 2001). As discussed in the literature review (section 1.2.5.1), Schuessler & Schilling (1984) proposed a mechanism by which protein breakdown occurs during irradiation in an oxygenated system. It begins with an attack on the peptide bond of the protein by the hydroxyl radical to form a protein radical, which then reacts with oxygen to form a peroxy radical. The two peroxy radicals can combine to form an oxyradical which can later decompose into peptides in the presence of oxygen. If, on the other hand, oxygen is absent, the protein radicals can combine to form polymers.

During irradiation, the oxygen present in the system is being used up and may become depleted with time (Swallow, 1991). The flour samples for irradiation were packaged in polyethylene bags that were placed in polystyrene boxes. Both types of packaging material have poor gas barrier properties (Mauer, 2003). Thus the samples had access to

oxygen during irradiation. It is, however, not certain whether the rate of oxygen usage during irradiation equalled the rate of oxygen diffusion into the polyethylene bags. Nonetheless, it appears that under severe conditions of irradiation, protein polymers were formed as suggested by the reduction in the solubility of the albumin and globulin (AG) proteins, as well as the reduction in the alleviation in protein digestibility. It is possible therefore, that oxygen could have become limiting under severe conditions of irradiation and favoured protein polymerisation. This would, however, need to be investigated.

Protein polymers, often referred to as aggregates, formed during irradiation are proposed to be linked by disulphide (Garrison, 1987), bityrosine (Davies, 1987; Davies *et al*., 1987a; Mezgheni *et al*., 1998; Ressouany, Vachon & Lacroix, 1998), and other crosslinks involving protein radicals (Tuce *et al*., 2001; Lee *et al*., 2003). The absence of an increase in disulphide bonds with irradiation and cooking (Table 3.2) may suggest that disulphide bonds did not contribute significantly to polymer formation in porridges from irradiated flours. Presumably, other crosslinks may have been involved.

The reduction in the solubility of AG proteins under severe conditions of irradiation, and the reductions observed in lysine content in porridges from 50 kGy wet irradiated samples could suggest the formation of protein complexes involving lysine, such as lysinoalanine and Maillard products. Lysinoalanine is formed when a racemised amino acid (D-amino acid) undergoes β-elimination to form a dehydroalanine derivative, which can later crosslink with the ∈-amino group of a lysine side chain to form lysinoalanine (Cheftel *et al*., 1985; Friedman, 1999a). Irradiation of proteins can lead to racemization of amino acids and subsequently to lysinoalanine formation (Friedman, 1999b). Lysinoalanine formation results in reduced protein digestibility and in the destruction of amino acids involved in the crosslinking (Liardon & Hurrell, 1983). The AG proteins of sorghum, like those of maize, are rich in lysine (Van Scoyoc *et al*., 1988; Yau *et al*., 1999), and thus can participate in the formation of lysinoalanine. Proteins containing lysine can also undergo Maillard reactions with reducing sugars, resulting in the formation of insoluble polymers and a decrease in nitrogen solubility (Yen, Lee & Chichester, 1989). The occurrence of Maillard reactions was assessed by measuring the lightness and yellowness of the flour colour, since Maillard reactions produce products with brown and yellow coloured

pigments (Whistler & Daniel, 1985; Yen *et al*., 1989). The results summarised in Table 3.2 indicate that Maillard reactions may have occurred in porridges from irradiated flour.

Browning in irradiated foods has been associated with Maillard reactions (Wootton *et al*., 1988), and/or the oxidation of phenolics, and aromatic amino acids (phenylalanine and tyrosine) to produce melanin-type pigments (Wootton *et al*., 1988; Giroux & Lacroix, 1998). Oxidation of phenylalanine produces tyrosine and hydroxyl derivatives. Oxidation of tyrosine produces 3,4-dihydroxyphenylalanine (DOPA), which can be oxidised further to produce crosslinking reactions which provoke melanin-type pigment formation (Diehl, 1990; Giroux & Lacroix, 1998). Flour colour became darker and yellowish in porridges from irradiated flour (Table 3.2). The darkening of porridges with irradiation of flour, together with the decrease in solubility of AG proteins, suggests that Maillard browning and other crosslinks such as bityrosine may be involved in protein polymerisation under severe conditions of irradiation.

Another possible indication of Maillard browning was the slight increase in antioxidant activity in 50 kGy dry irradiated sorghum and maize flours, as described in section 2.1. Products from Maillard reactions have been shown to possess antioxidant activity (Baltes, 1982; Eiserich & Shibamoto, 1994), with the antioxidant effects being greatest at the beginning of the browning reactions, due to Maillard intermediates such as the reductones (Baltes, 1982). These Maillard intermediates give rise to yellow coloured products (Whistler & Daniel, 1985), and the yellow colouration was highest in uncooked dry sorghum and maize flours irradiated at 50 kGy, which could represent the onset of Maillard browning. With Maillard reaction initiated in the irradiated flour, cooking will most likely accentuate the process. Products from Maillard reactions can inhibit proteolytic enzymes (Öste *et al*., 1986, 1987), and cause a reduction in protein digestibility (Hafez, Mohamed, Hewedy & Singh, 1985). Maillard reactions may therefore have contributed to the reduced alleviation in protein digestibility of sorghum porridges from severely irradiated flours, through the formation of crosslinks that prevent enzyme access to peptide bonds and/or by inhibiting the enzyme (Hurrell & Finot, 1982).

As mentioned earlier, bityrosine crosslinks could also be involved in protein polymerisation during irradiation, and this could adversely affect its digestibility (Davies, 1987; Davies & Delsignore, 1987). Davies *et al* (1987a) reported increased formation of bityrosine in bovine serum albumin (BSA) with increased exposure to hydroxyl radical produced by gamma irradiation that decreased its susceptibility to proteolysis. Ressouany *et al*., (1998) also found that the amount of bityrosine crosslinks increased with increasing irradiation dose when calcium caseinate solution was irradiated with doses of up to 64 kGy. Bityrosine is a covalently bonded biphenol produced by reaction of two tyrosyl radicals or a tyrosyl radical plus a tyrosine molecule. The tyrosyl radicals are produced as a result of hydrogen abstraction by hydroxyl radicals (Davies *et al*., 1987a). Bityrosine crosslinks are more likely to form between two protein molecules (intermolecular) than within the same protein molecule (intramolecular), thus leading to polymerisation (Davies, 1987). Although bityrosine levels were not measured in this study, the possibility of their formation under severe conditions of irradiation could be indicated by the decreases observed in tyrosine content with irradiation. The formation of bityrosine crosslinks may therefore have contributed to the lower protein digestibility of these porridges, and needs to be investigated further.

Speculating further using the model proposed in Fig 3.1, it is possible that under severe conditions of irradiation of sorghum flour, the increased formation of covalent non-disulphide crosslinks (Maillard, bityrosine, lysinoalanine), as discussed above, may have caused some of the unfolded and free polypeptides to become crosslinked once again, with the result that the protein network closed up (Fig 3.1d). A tighter protein network would restrict enzyme access to peptide bonds and cause the subsequent reduction in the alleviation in protein digestibility of porridges from flour samples irradiated under severe conditions (Table 3.2).

With reference to the solubility and extractability of the sorghum proteins (NSI, AG solubility and prolamin extractability), the red sorghum BR7 behaved differently from the white sorghum Madjeri (Table 3.2). The reduction in prolamin extractability of porridges of sorghum Madjeri was alleviated by irradiation of flour, both under mild and severe conditions, before cooking, whereas, in sorghum BR7 the alleviation only occurred under

mild irradiation conditions. NSI was not significantly affected by irradiation and cooking in sorghum BR7, but it decreased in sorghum Madjeri with cooking alone. This decrease was relieved by irradiation of flour under mild conditions, but not under severe condition. Although, the behaviour of the AG was similar for sorghum BR7 and Madjeri with cooking alone or with irradiation and cooking, when flour samples were irradiated without cooking, the solubility of sorghum BR7 AG proteins decreased more with irradiation compared to Madjeri (Table 2.1.5). These differences in the solubility properties of sorghum BR7 and Madjeri were attributed to the higher levels of polyphenols in sorghum BR7, which could act as antioxidants (Awika & Rooney, 2004) and offer some protection against the effects of irradiation. Because the sorghums used were condensed tannin free cultivars, the values obtained for phenols could therefore have been due in part to phenolic acids, which are present in all sorghums (Hahn *et al*., 1984). The higher levels of phenols in sorghum BR7 may have been due to flavonoids (Hahn *et al*., 1984), which caused its red pigmentation. The flavonoids found in sorghum are the anthocyanidins, with the most common being the 3-deoxyanthocyanidins, which include apigeninidin and luteolinidin. Of these two, apigeninidin was found to be most prevalent in red sorghum bran (reviewed by Awika & Rooney, 2004).

The effect of irradiation on proteins depends on free radicals being freely available to react with the proteins. Polyphenols such as the flavonoids can act as antioxidants by directly scavenging free radicals such as superoxide, and peroxyl radicals (Rice-Evans *et al*., 1997; Velioglu *et al*., 1998), generated during irradiation. In so doing, they act as hydrogen or electron donors and in the process become oxidised themselves to *o*-quinones, which can react with available nucleophilic groups (XH) on proteins such as the ϵ-amino groups of lysine, thiol groups of cysteine and N-terminal amino groups, to bring about crosslinking and ultimately polymerisation (Haslam, 1989). Figure 3.2 shows a schematic representation of the mechanisms by which flavonoids can act as antioxidants and become oxidised to *o*-quinone radicals that can react with proteins to polymerise them, using apigeninidin, the flavonoid found in red sorghum bran.

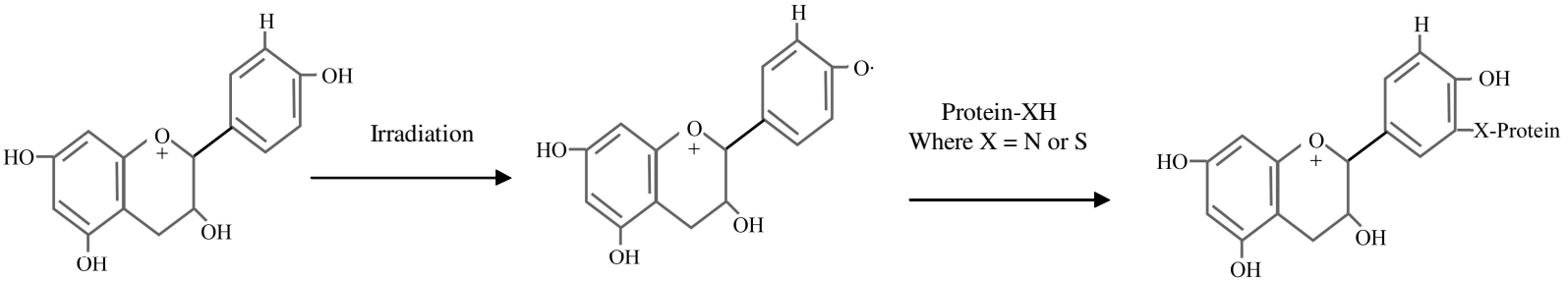

Fig 3.2. Model showing the proposed mechanism of crosslinking between oxidized polyphenols (*o*-quinone radical) with proteins through the amino group of lysine or the sulphur group of cysteine

The absence of a significant change in nitrogen solubility of sorghum BR7 with irradiation, without and with cooking (Table 3.2), may be a consequence of the protective antioxidant effects of BR7 polyphenols, which could have scavenged free radicals and thus reduced the effects of irradiation. However, in the case of AG solubility, which decreased more with irradiation alone in sorghum BR7 compared to Madjeri, the protective effects of the polyphenols may have been counterbalanced by the reaction of the *o*-quinones radical with the ϵ-amino groups of lysine (Fig 3.2) that is abundant in this fraction (Van Scoyoc *et al*., 1988; Yau *et al*., 1999), to polymerise the AG proteins and reduce their solubility. The possibility of the *o*-quinones complexing with cysteine sulphydryl groups (Haslam, 1989) may also be implicated in the reduced alleviation in the reduction of prolamin extractability in porridges from sorghum BR7 flours irradiated under severe conditions, compared to sorghum Madjeri.

In maize, cooked protein digestibility was only significantly reduced in porridges from wet irradiated flour (Table 3.1). In the case of sorghum, it was suggested that cleaving of disulphide and hydrogen bonds by irradiation may have effected a change in protein structure that limited the formation of disulphide crosslinks during cooking, and hence relieved the reduction in protein digestibility. As stated, maize zein proteins are very similar to sorghum kafirins, with the difference that zeins apparently have fewer disulphide bonds in the uncooked flour than kafirins (Hamaker *et al*., 1986). The amounts of disulphide bonds in uncooked sorghum and maize flours were, however, not measured in this study.

According to Swallow (1991), and based on rate constants, the sulphur-containing and aromatic amino acids are more susceptible to radiation damage. Therefore a protein that has more disulphide bonds would be expected to undergo greater conformational changes following irradiation. This has indeed been observed with soybean proteins (Byun *et al.,* 1994). These authors noted that soybean 11S proteins with 21 disulphide bonds and 2 free sulphydryl groups underwent greater conformational changes during irradiation at 20 kGy, compared to the 7S proteins that had neither disulphide bonds nor sulphydryl groups. By comparison therefore, one would expect less conformational changes in zeins compared to kafirins. The fact that the amount of disulphide bonds in zein were not

significantly affected by irradiation and cooking, whereas in sorghum disulphide groups decreased significantly with irradiation and cooking, suggests that protein structure of zein may not have been significantly affected by irradiation. The absence of a significant change in protein digestibility of maize porridges from dry irradiated flour compared to the unirradiated flour seems to agree with the above suggestion.

However, under wet conditions of irradiation, where non-disulphide crosslinks may have been formed, as in the case with sorghum, this may have caused the reduction in the protein digestibility of maize porridges from wet irradiated flours. It would therefore, appear that, because of the presence of fewer disulphide bonds in zeins, irradiation had no beneficial effect on the protein digestibility of their porridges, but rather under wet conditions of irradiation there was a direct negative effect, owing to the enhanced effects of irradiation in wet medium.

4 CONCLUSIONS AND RECOMMENDATIONS

The objective of this study was to determine whether irradiation of flour prior to cooking could alleviate the reduction in protein digestibility that occurs when sorghum flour is cooked into porridge. From the results obtained in this study, it emerges that this is possible.

Irradiation of flour before cooking can alleviate the negative effects of cooking on sorghum protein digestibility. This alleviation appears to be favoured by mild irradiation (10 kGy dry), and appears to occur through a change in protein structure brought about by irradiation breaking hydrogen and disulphide bonds in sorghum proteins, that in turn can reduce disulphide crosslinking during cooking. This apparently results in a more unfolded and open protein network, exposing additional peptide bonds to pepsin hydrolysis. Because 10 kGy was the lowest irradiation dose used, and it alleviated the reduction in protein digestibility most, it is recommended that the effects of lower doses (< 10 kGy) of irradiation on sorghum cooked protein digestibility be investigated. Sorghums, especially condensed-tannin free cultivars are prone to insect damage. If low dose irradiation such as used in insect disinfestations (0.5-5 kGy) as recommended by the International Consultative Group on Food Irradiation, ICGFI (1991), can give better cooked protein digestibility in sorghum, it may combine prolonged shelf life with better nutritional quality that in turn can contribute towards food security. In addition, lower doses of irradiation may cause less damage to proteins and other food components such as starch and vitamins.

Although, irradiation of sorghum flours under severe conditions (50 kGy dry and 10 and 50 kGy wet) also relieves the reduction in protein digestibility of their cooked porridges, the beneficial effects are however diminished compared to low dose irradiation (10 kGy dry). It appears that covalent non-disulphide crosslinks, such as Maillard crosslinks, lysinoalanine and bityrosine may be formed under severe conditions of irradiation. This may have caused refolding and closing up of the protein structure, resulting in the masking of some previously exposed digestible peptide bonds. It is recommended that the participation of bityrosine crosslinks and lysinoalanine formation in protein

polymerisation of sorghum and maize be determined. In addition, the extent of protein breakdown and polymerisation should be assessed in order to gain more understanding into the mechanism by which irradiation prior to cooking alleviates the reduction in cooked sorghum protein digestibility.

In the case of maize, whose proteins are similar to those of sorghum, irradiation of dry flour before cooking does not have a significant effect on the protein digestibility of their porridges, but when flour samples are irradiated in wet medium before cooking the digestibility of their porridges decreases compared to porridges from unirradiated flour. It seems that the presence of fewer disulphide bonds in uncooked maize flour compared to sorghum does not allow for significant changes in its protein structure when irradiated in dry form, whereas under wet (severe) conditions of irradiation, the formation of covalent non-disulphide bonds as in the case of sorghum may result in a direct negative effect on maize protein digestibility.

It appears that polyphenols in sorghum BR7 can act as antioxidants, scavenging free radicals during irradiation, thus influencing the extent of radiation damage in these proteins. NSI in BR7 did not change significantly with irradiation possibly due to polyphenols scavenging free radicals and reducing the extent of radiation damage. However, under severe conditions of irradiation, the oxidised polyphenols may have been converted to free radicals that reacted with the AG proteins, causing the greater reduction in solubility of AG proteins in sorghum BR7 with irradiation alone.

Although irradiation can somewhat alleviate the reduction in cooked sorghum protein digestibility, the practical application of this technique to improve the nutritional quality of sorghum porridge may not be immediately appealing considering the high initial capital investments. However, in areas where irradiation facilities already exist, this may not be so. It is therefore, important to take into account all the benefits that irradiation could offer in terms of nutritional quality and safety that could offset the cost of irradiation.

Irradiation does affect other components within the food such as carbohydrates and lipids. Previous studies in our laboratory had shown that irradiation of maize flour at low dose (2.5 kGy) can cause depolymerisation of starch leading to a reduction in viscosity and an improvement *in vitro* starch digestibility. Because of the reduced viscosity nutrient dense porridges could be made from irradiated maize flours. If such can occur in sorghum, then combining these benefits with better protein digestibility will make irradiation even more desirable. In addition, nutrient dense high digestible porridges may assist to some extent towards alleviating malnutrition in sorghum consuming areas.

Since the reduction in protein digestibility of sorghum occurs during cooking, and is due to disulphide crosslinking of their proteins, it is recommended that irradiation be investigated on the cooked porridges. If effective at improving protein digestibility, it could also provide a means of producing safer pre-cooked sorghum porridges, given that irradiation can have pasteurising and sterilizing effects, depending on the dose used.

In conclusion, irradiation is a potentially useful technique of processing that can improve nutritional quality and safety of sorghum foods, given that it can improve protein digestibility and nutrient density, and at the same time destroy pathogenic and spoilage micro organisms.

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LIST OF PUBLICATIONS FROM THIS RESEARCH

Journal paper

EN Fombang, JRN Taylor, CMF Mbofung and A Minnaar, (2005). Use of γ-irradiation to alleviate the poor protein digestibility of sorghum porridge. Food Chemistry, 91, 695-703.

Oral presentation

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Poster Presentation

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