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.

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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).

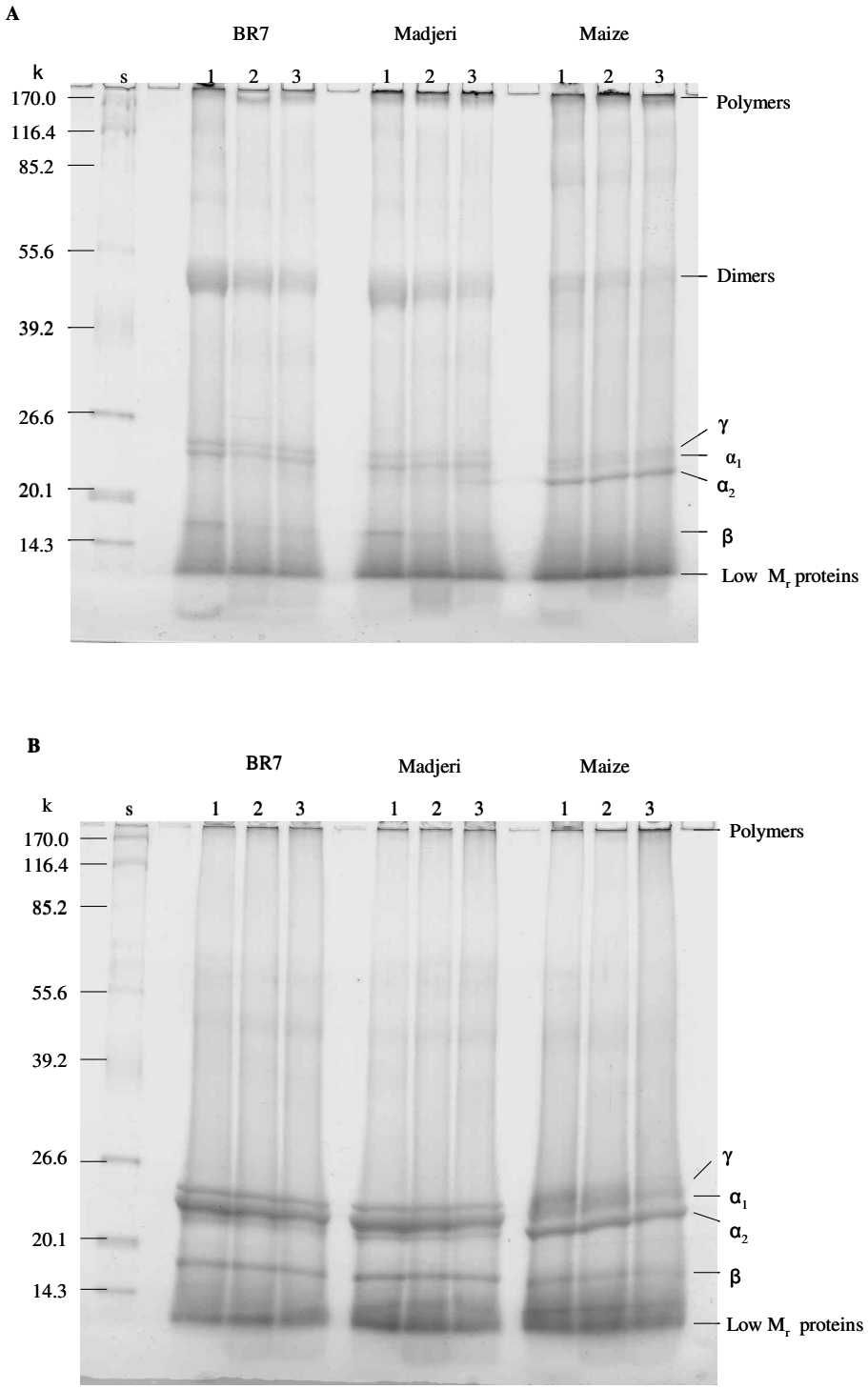

Figure 2.2.1. Gradient SDS-PAGE (7-14% acrylamide) of sorghum BR7 and Madjeri and maize PAN 6043 protein-rich flours from porridges of unirradiated (lane 1), 10 kGy dry irradiated (lane 2) and 50 kGy wet irradiated (lane 3) flours, performed under non-reducing (A) and reducing (B) conditions. Lane s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

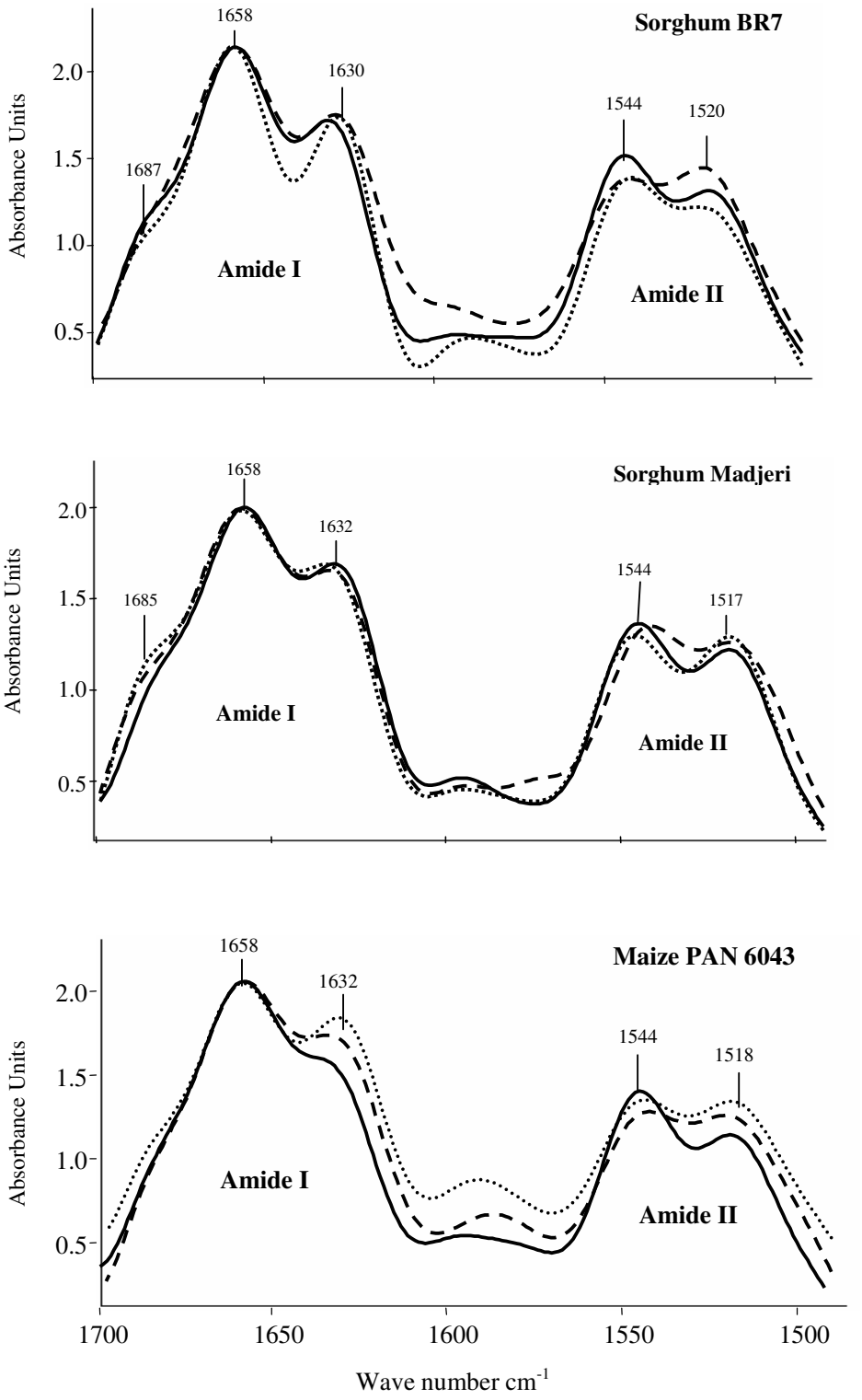

Fig 2.2.2. FTIR spectra of sorghum BR7 and Madjeri and maize PAN 6043 protein-rich flours from porridges of unirradiated (smooth lines), 10 kGy dry irradiated (dotted lines) and 50 kGy wet irradiated (dashed lines) flours, normalized to the highest absorbance peak of the alpha-helix band (1658) in the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