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Use of γ-irradiation to alleviate the poor protein digestibility of sorghum porridge

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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 effects 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, were 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 accompanied by an increase in protein solubility, suggesting that it was probably related to modification of protein structure, allowing better access to proteolytic enzymes. Maillard reactions and protein aggregation, at high doses, negatively affected digestibility. Polyphenols influenced the effects of irradiation.

Keywords: Protein digestibility; Sorghum; Maize; Porridge; Irradiation; Maillard reactions; Protein aggregation; Polyphenols

1. 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 (Duodu et al., 2002; Hamaker, Kirleis, Butler, Axtell, & Mertz, 1987; Mertz et al., 1984) and in vivo (Maclean, Lopez de Romana, Gastanaduy, & Graham, 1983; Maclean, Lopez de Romana, Placko, & Graham, 1981) studies have shown that wet-cooking of sorghum, as in porridge making, significantly decreases its protein digestibility. This, however, is not the case with maize, where digestibility of its proteins is only minimally affected by wet-cooking (Duodu et al., 2002; Hamaker et al., 1987). 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 (Hamaker et al., 1987; Oria, Hamaker, & Shull, 1995). 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., 1995); this may hence cause the reduction in digestibility (Hamaker et al., 1987; Oria et al., 1995).

Protein digestibility of wet-cooked sorghum has been improved using processes such as fermentation (El Khalifa & El Tinay, 1995; Taylor & Taylor, 2002), extrusion cooking (Hamaker, Mertz, & Axtell, 1994; Mertz et al., 1984) and cooking with reducing agents (Hamaker et al., 1987; Rom, Shull, Chandrashekar, & Kirleis, 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

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prevents the formation of disulphide bonds during cooking, with the result that proteins are more accessible to proteolytic enzymes. It would thus appear that the conformation of sorghum β and γ kafirin proteins and the consequent access of enzymes to the alpha kafirin proteins are important factors 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, Sapirstein, Celik, & Bushuk, 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, Lin, & Pacifici, 1987; Garrison, 1987; Kempner, 1993). However, high doses of irradiation may result in crosslinking and aggregation of proteins (Cieśla, Roos, & Głuszewski, 2000; Garrison, 1987; Kempner, 1993). In some cases, increased susceptibility of irradiated proteins to enzyme hydrolysis has been observed (Davies, 1987). Disulphide bonds occur in mature sorghum (Shull, Watterson, & Kirleis, 1991) and maize (Larkins, Pedersen, David Marks, & Wilson, 1984) prolamin proteins and, as indicated previously, their formation during cooking is associated with the lower digestibility of wet-cooked sorghum (Hamaker et al., 1987; Rom et al., 1992).

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

2. Materials and methods

2.1. Materials

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

2.2. 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 0, 10 and 50 kGy using a ^{60}Co γ irradiation source at the Isotron irradiation plant (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 gas-tight glass bottles and stored at 4 °C until analysed.

2.3. Methods of analyses

2.3.1. Protein digestibility

In vitro 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, and 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.

2.3.2. Nitrogen solubility index

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

2.3.3. Albumin and globulins

Flour samples were extracted in 1.25 M NaCl (1:5 w/v) (Taylor, Schüssler, & van der Walt, 1984) 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.

2.3.4. Colour

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

2.3.5. Polyphenols

Polyphenols in the flour samples were determined using a modified International Standardization Organisation (ISO) ferric ammonium citrate method (ISO, 1988). 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.

2.3.6. Antioxidant activity

Antioxidant activity was determined using the TEAC (trolox equivalents antioxidant activity) assay, as described by Re et al. (1999) with modification. Flour samples (0.3 g) were extracted into a 10-ml solution of 1% HCl in methanol 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, 2900 μl of $ABTS^{\cdot +}$ (2,2′-azinobis 3-ethylbenzothiazoline 6-sulfonic acid) solution were added 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.

2.4. 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 relationships between all of the parameters tested. Experiments were replicated twice and the samples analysed in duplicate.

3. Results

3.1. Protein digestibility

Table 1 shows the effects of irradiation on the in vitro protein digestibility of sorghum and maize flours with and without cooking into porridge. Digestibility was significantly ($p \leqslant 0.05$) 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 the dry or 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 ($p \leqslant 0.05$) 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, the process 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 comparable with those in the uncooked samples. Digestibility of porridge 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 than that of porridge from 10 kGy dry-irradiated flour, but still higher than that of porridge from unirradiated flour. Maize porridges prepared from dry-irradiated flour showed little difference in digestibility compared to that from unirradiated flour but digestibility decreased

Table 1
Effects of irradiating wet and dry sorghum and maize flours, followed by cooking to make porridges, on their protein digestibility (%)

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

[A] Values in the same column with different letters are significantly ($p \leqslant 0.05$) different from each other.
[B] Values in parentheses are standard deviations for duplicate experiments ($n = 4$).
[C] The wet-irradiated and porridge samples were freeze-dried.

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

[A] Values in the same column with different letters are significantly ($p \leqslant 0.05$) different from each other.
[B] Values in parentheses are standard deviations of duplicate experiments ($n = 4$).
[C] The wet-irradiated and porridge samples were freeze-dried.

significantly ($p \leqslant 0.05$) in porridges made from wet-irradiated flour at both 10 and 50 kGy.

3.2. Nitrogen solubility index

No significant ($p > 0.05$) difference was observed in NSI of BR7 sorghum with irradiation and cooking into porridge (Table 2). For Madjeri sorghum, 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 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.

3.3. Albumins and globulins

Albumin and globulin (AG) contents of uncooked BR7 sorghum flour decreased significantly ($p \leqslant 0.05$) with irradiation in both dry and wet medium, and more so in the latter (Table 3). In uncooked Madjeri sorghum and maize flours, AG content was basically unaffected by irradiation in dry medium but decreased significantly ($p \leqslant 0.05$) 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 wet-irradiated flours.

3.4. 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 4). 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

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

[A] Values in the same column with different letters are significantly ($p \leqslant 0.05$) different from each other.
[B] Values in parentheses are standard deviations of duplicate experiments ($n = 4$).
[C] The wet-irradiated and porridge samples were freeze-dried.

Table 4
Effects of irradiating wet and dry sorghum and maize flours, followed by cooking to make porridges, on their L and b colour

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

[A] Values in the same column with different letters are significantly ($p \leqslant 0.05$) different from each other.
[B] Values in parentheses are standard deviations of duplicate experiments ($n = 4$).
[C] The wet-irradiated and porridge samples were freeze-dried.
[D] L-value = whiteness.
[E] b-value = yellowness.

browning in these samples. L colour was significantly correlated with albumin and globulin contents in both BR7 ($r = 0.75$; $p \leqslant 0.05$) and Madjeri ($r = 0.74$; $p \leqslant 0.05$) sorghums but not in the maize.

3.5. Polyphenols

Polyphenol content was highest in BR7 sorghum, followed by Madjeri, whereas no polyphenols could be detected in maize (Table 5). 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.

3.6. Antioxidant activity

All three cereals showed antioxidant activity (Table 6). BR7 sorghum had the highest antioxidant activity. The antioxidant activities of Madjeri sorghum and maize were similar. Irradiation of dry flour at 10 kGy had no significant effect ($p \leqslant 0.05$) 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 greater in BR7 sorghum. A significant positive correlation ($r = 0.67$; $p \leqslant 0.05$) was obtained between antioxidant activity and polyphenols for BR7 sorghum but not for Madjeri sorghum or maize.

4. Discussion

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

The fact that in vitro protein digestibility of sorghum decreased substantially on wet-cooking, compared to maize is in agreement with previous work (Duodu

Table 5
Effects of irradiating wet and dry sorghum and maize flours, followed by cooking to make porridges, on their total polyphenol content (%)

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

[A] Values in the same column with different letters are significantly ($p \leqslant 0.05$) different from each other.
[B] Values in parentheses are standard deviations of duplicate experiments ($n = 4$).
[C] The wet-irradiated and porridge samples were freeze-dried.

Table 6
Effects of irradiating wet and dry sorghum and maize flours, followed by cooking to make porridges, on their antioxidant activity (mMTE/g)

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

[A] Values in the same column with different letters are significantly ($p \leqslant 0.05$) different from each other.
[B] Values in parentheses are standard deviations of duplicate experiments ($n = 4$).
[C] The wet-irradiated and porridge samples were freeze-dried.

et al., 2002; Hamaker et al., 1987; Mertz et al., 1984). However, irradiation (10 kGy) of dry sorghum flour before wet-cooking prevented this decrease and maintained protein digestibility of its porridge at levels comparable with the unirradiated flour. The improvement (12–18%), brought about in protein digestibility of sorghum porridge 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). Irradiation (30–90 kGy) of barley and oats has been reported to improve feed conversion and weight gain in chicks (Campbell, Classen, & Ballance, 1986).

It is hypothesized that irradiation cleaved disulphide bonds in 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 (Shull et al., 1991) and these can be cleaved by irradiation (Doguchi, 1969; Köksel et al., 1998). Splitting of these bonds by irradiation will no doubt modify protein structure. Porridge 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 and/or aggregates that were less susceptible to enzyme hydrolysis (Cho, Yang, & Song, 1999). Decreases in solubility of albumin and globulin (AG) proteins of both sorghums and in NSI of Madjeri sorghum with high irradiation dose are indications of the formation of insoluble complexes that could impair digestibility.

Maillard reactions have been associated with irradiation of proteins (Cunha, Sgabieri, & Damasio, 1993; Krumhar & Berry, 1990). 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 were 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, 1991). Maillard browning could therefore in part be 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 varieties. 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 (Duodu et al., 2002; Esen, 1986), 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 and 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, Cuq, & Lorient, 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 and Taylor (2002) have 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 crosslinks (Whistler & Daniel, 1985). The decline in solubility of AG suggests the formation of some insoluble complexes. Irradiation at 10 kGy induces aggregate formation in bovine serum albumin that reduces its solubility (Krumhar & Berry, 1990). The greater reduction obtained with 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); this explains its higher content of polyphenols compared to the white Madjeri sorghum. Sorghum polyphenols are more likely to bind to large proteins, rich in proline and having a loose open structure (Butler, Riedl, Lebryk, & Blutt, 1984). AG proteins from sorghum have molecular weights 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 may thus 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 ($r = 0.88$; $p \leqslant 0.05$) 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 BR7 sorghum following irradiation. Duodu et al. (2002), using electrophoresis, 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 ($p \leqslant 0.05$) reduction in AG content of porridges in flour samples irradiated at high doses.

The BR7 polyphenol content was similar to those 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 BR7 sorghum, polyphenol content was positively correlated with antioxidant activity ($r = 0.67$; $p \leqslant 0.05$). 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 et al., 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 BR7 sorghum samples irradiated at high dose (50 kGy) and in wet medium, compared to Madjeri and maize. These results, however, led us to propose that the polyphenols were the most potent of all the antioxidants in these samples.

5. Conclusion

Our findings indicate that irradiation (especially of dry flour at 10 kGy) has the potential to alleviate the adverse effects of wet-cooking on 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, cross-slinking and aggregation of proteins may have been triggered, leading to a reduction in digestibility. Polyphenols appeared to have influenced the observed effects of irradiation on protein digestibility.

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