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Gamma irradiation of cowpea (*Vigna unguiculata* L. Walp) flours and pastes: Effects on functional, thermal and molecular properties of isolated proteins

Joseph Oneh Abu [a,b], Klaus Müller [c], Kwaku Gyebi Duodu [a], Amanda Minnaar [a,*]

[a] *Department of Food Science, University of Pretoria, Pretoria 0002, South Africa*
[b] *Department of Food Science and Technology, University of Agriculture, P.M.B. 2373, Makurdi, Nigeria*
[c] *Department of Food Process Engineering, Fraunhofer Institut Verfahrenstechnik und Verpackung, Giggenhauser Street 35, D-85354, Freising, Germany*

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Abstract

In a previous study irradiation of cowpea flours and pastes at medium (10 kGy) and high (50 kGy) doses resulted in significant changes in protein-related functional properties. To understand some of the effects of gamma irradiation on cowpea proteins in particular, we isolated proteins from cowpea flours (FPC) and pastes (PPC) treated with gamma irradiation at 2, 10, and 50 kGy and analyzed their functional, thermal and molecular properties. Nitrogen solubility index of both FPC and PPC decreased, whereas oil absorption and emulsion capacities increased significantly with increasing irradiation dose. Differential scanning calorimetry showed decreases in transition temperatures (T_d) and enthalpies (ΔH), indicating a progressive denaturation of cowpea proteins with increasing irradiation. Size exclusion HPLC revealed increases in protein molecular weights, probably due to protein–protein cross-linking with irradiation in a dose-dependent manner. Reducing SDS-PAGE of FPC and PPC samples seems to suggest that the contribution of disulphide bonds to irradiation-induced cowpea protein–protein cross-linking is small.

Keywords: Cowpea; Protein; Functional properties; DSC; SE-HPLC; SDS-PAGE

1. Introduction

Cowpea flours, pastes and their respective protein concentrates, isolates and fractions have been studied extensively (McWatters, 1990; Ragab, Babiker, & Eltinay, 2004; Rangel, Domont, Pedrosa, & Ferreira, 2003; Sefa-Dedeh & Stanley, 1979; Sosulski, Kasirye-Alemu, & Sumner, 1987) and may potentially be employed to complement soy flours and proteins as functional ingredients in food systems. Cowpea proteins have also been characterized (Carasco, Croy, Derbyshire, & Boulter, 1978; Freitas, Teixeira, & Ferreira, 2004; Khan, Gatehouse, & Boulter, 1980; Rangel et al., 2003; Sefa-Dedeh & Stanley, 1979) although information on their application is inadequate.

The modification of functional properties of proteins using chemical methods, such as succcinylation, alkylation, disulphide bond reduction, phosphorylation, and physical methods, such as heat treatment as well as the use of transglutaminase to induce covalent cross-linking of lysyl residues have been reported (Damadoran, 1997; Gerrard, 2002; Kinsella & Whitehead, 1987). The possibility of modifying protein functional properties may enable targeted and more specific use of cowpea proteins in food systems.

* Corresponding author. Tel.: +27 12 4203239; fax: +27 12 4202839.
E-mail address: amanda.minnaar@up.ac.za (A. Minnaar).

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Gamma irradiation, usually applied to cowpeas for reasons such as insect and pest disinfestations (Diop, Marchioni, Ba, & Hasselmann, 1997) and to enhance cowpea flour protein digestibility (Dario & Salgado, 1994), may also affect cowpea protein functionality. Gamma irradiation has been employed to cross-link biodegradable films from whey, casein and soy proteins (Lacroix et al., 2002). Ionizing radiation, through the production of free radicals, can affect proteins by promoting reactions such as protein–protein association, deamination, cleavage of peptide and disulphide bonds and by association of aromatic and heterocyclic residues (Cho, Yang, & Song, 1999; Simic, 1978; Urbain, 1986). These changes depend on factors such as dose, pH, hydration state and temperature during irradiation (Simic, 1978) as well as the presence or absence of oxygen (Davies, 1987; Davies & Delsignore, 1987; Giroux & Lacroix, 1998).

Literature has shown gamma irradiation to be capable of modifying functional properties of some legumes. Emulsion, foam, water and oil absorption capacities were affected by gamma irradiation in peanut flour (Rahma & Mostafa, 1988), and protein solubility in soy (Byun & Kang, 1995; Hafez, Mohamed, Singh, & Hewedy, 1985) and red kidney bean (Dogbevi, Vachon, & Lacroix, 1999). In some of these studies, irradiation was applied to isolated proteins. The effects of irradiation on proteins in multi-component food systems such as cowpea flours and pastes may be expected to be less drastic given that large molecules such as starch may confer some protection against irradiation effects on smaller molecules such as proteins (Urbain, 1986). However, earlier findings in our laboratory (Abu, Muller, Duodu, & Minnaar, in press) showed significant changes in most protein-related functional properties of multi-component cowpea flours and pastes following their exposure to medium and high (but not low) dose irradiation, despite such potential protective effects.

To elucidate some of the effects of irradiation on cowpea proteins in irradiated cowpea flours and pastes, we isolated proteins from irradiated cowpea flours and pastes and studied them in terms of their functional, thermal and molecular properties.

2. Materials and methods

2.1. Irradiation of cowpea flours and pastes

Cowpea flour and paste (200 g) samples were sealed in polyethylene bags (ca. 80 μm thick) and kept chilled in ice-cooler boxes prior to and during irradiation. Samples were irradiated at Isotron S.A. in Isando, South Africa using a ^{60}Co source. Target doses were 2, 10 and 50 kGy, respectively. Irradiation was performed on three different occasions. Non-irradiated (control) and irradiated samples were stored at −18 °C. Before protein preparation, cowpea pastes were freeze-dried and milled through a 0.8 mm mesh.

2.2. Protein preparation

Three replicate samples of non-irradiated and irradiated cowpea flours and pastes were pooled per treatment prior to protein isolation. A three-step extraction process was used to prepare protein concentrates from irradiated cowpea flours and pastes. Samples dispersed in tap water (1:4) were adjusted to pH 4.5 (isoelectric point precipitation) with 3 M HCl at 15 °C for 45 min under constant stirring, followed by centrifuging for 10 min at 25 °C at 2500*g*. In the second step, the supernatant obtained from the previous step was discarded and the residue (mainly precipitated proteins) was extracted twice at 30 °C for 30 min under constant stirring at pH 7, followed by centrifuging for 10 min at 25 °C at 2500*g* (to remove insoluble suspensions). The supernatants containing the solubilised proteins were pooled, filtered through a 250 μm sieve and spray-dried (65–70 °C). The protein concentrates obtained from this method, on average, comprised approximately 80% protein, 10% moisture and 10% non-protein constituents.

2.3. Functional properties

2.3.1. Nitrogen solubility index (NSI)

Nitrogen solubility index was determined using the AACC method 46-23 (AACC, 2000). One gramme protein samples were analysed as described previously by Abu et al. (in press).

2.3.2. Emulsion capacity (EC)

Emulsion capacity was determined using a method described by Marshall, Dutson, and Carpenter (1975). The density of oil employed for EC and oil absorption capacity determinations was 0.92 g/ml.

2.3.3. Foam capacity (FC)

The method described by Akubor, Isolokwu, Ugbane, and Onimawo (2000) was used with modifications, as described previously by Abu et al. (in press).

2.3.4. Water and oil absorption capacities (WAC and OAC)

Water and oil absorption capacities were determined using the AACC method 56-20 (AACC, 2000). One gramme protein samples were analysed as described previously by Abu et al. (in press).

2.3.5. Gel strength (GS)

Gel strength was determined with 16% (w/v) protein sample dispersions in tap water as described previously by Abu et al. (in press).

2.3.6. Viscosity

The viscosity of protein concentrates was determined using a Bohlin CVO Rheometer (Bohlin Instruments Ltd., UK). The cup and bob (coaxial cylinder) method was used in actual sample determinations while a 4/40 cone (4° angle with 40 mm diameter) and plate were used in 'gap size' calibration. Samples were solubilized for at least 15 min prior to analyses at constant shear rate of 50 (s^{-1}). Protein solutions (10%) were heated from 30 to 90 °C. Viscosity at 90 °C was noted for all protein concentrates.

2.4. Available lysine

The furosine method of Bujard and Finot (1978) was employed to determine the available lysine in cowpea proteins.

2.5. Differential scanning calorimetry (DSC)

DSC was performed with a micro-DSC III system (Setaram, Caluire, France). Ten percent protein solutions were scanned from 24 to 115 °C at 1 °C/min. The reference pan was filled with distilled water. Denaturation temperatures and enthalpy values were noted.

2.6. Size exclusion high performance liquid chromatography (SE-HPLC)

Analyses were performed on an HPLC apparatus equipped with a C-10AD controller using a Superdex 200 column. The column was equilibrated with 50 mM Tris–HCl and 150 mM NaCl, pH 6.8. The molecular weight standards used were: thyroglobulin (5.0 mg: 670 kDa), bovine gamma globulin (5.0 mg: 158 kDa), chicken ovalbumin (5.0 mg: 44 kDa), equine myoglobin (2.5 mg: 17 kDa) and vitamin B_{12} (0.5 mg: 1.35 kDa). Both myoglobin and vitamin B_{12} served as colour markers. The eluant was comprised of 0.05 M Na_2HPO_4, 0.15 M NaCl and 0.01 M NaN_3. Chromatography was carried out at pH 6.8 at ambient temperature at 0.9 ml/min flow rate using a detector with UV absorbance at 220 nm.

2.7. SDS-Polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was conducted using a discontinuous Tris–HCl/glycine buffer system 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 gel electrophoresis, Boehringer Mannheim, Germany), comprising α_2-macroglobulin (170 kDa), β-galactosidase (116.3 kDa), fructose-6-P-kinase (85.2 kDa), glutamate (55.6 kDa), aldolase (39.2 kDa), triose phosphate isomerase (26.6 kDa), trypsin inhibitor (20.1 kDa) and lysozyme (14.3 kDa) were mixed with reducing sample buffer to give 1 μg protein per expected band of standard. Samples were loaded at 50 μg proteins per well.

SDS-PAGE was performed with a Protean II 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 °C. 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% (v/v) trichloroacetic acid (TCA) and destained with 4% (v/v) acetic acid, 29% (v/v) methanol and 3% (v/v) TCA. The gels were scanned on a flat bed scanner.

2.8. Statistical analysis

Most analyses were performed in triplicate ($n = 3$). Analysis of variance (ANOVA), followed by the least significant difference test (LSD-test), was applied to functional properties. Correlation coefficients (r) of functional properties were also determined. The level of significance used was 95%.

3. Results and discussion

3.1. Functional properties

Irradiation caused a progressive decrease in NSI in both FPC and PPC (Table 1). Protein-related functional properties, such as NSI, are, in general, influenced by various factors, such as protein denaturation, size, structure and conformation, charge, amino acid composition and amino acid sequence of the protein molecules (Zayas, 1997). The decreases in NSI may be due in part to protein denaturation following the production of free radicals during irradiation (Urbain, 1986). Denaturation of globular proteins tends to promote the exposure of previously buried non-polar protein sites, leading to increased hydrophobicity (Zayas, 1997) and, subsequently, decreased solubility. Data from differential scanning calorimetry (DSC) (Figs. 3 and 4) show decreases in peak denaturation temperatures (T_d) and/or enthalpies (ΔH). These may be indicative of progressive denaturation (Arntfield & Murray, 1981) of cowpea proteins with increasing irradiation dose. The decrease in NSI was more pronounced in FPC than in PPC, consistent with the higher protein denaturation in the former, as indicated by the DSC peak denaturation temperatures and enthalpies. Nitrogen solubility may therefore, be useful in predicting protein denaturation. Taha and Mohamed (2004) employed decreases in NSI as an index of protein denaturation in soy.

Table 1
Effect of irradiation on the functional properties of proteins isolated from cowpea flours and pastes irradiated at 2, 10 and 50 kGy

Sample	Nitrogen solubility index (%) at pH 7	Emulsion capacity (ml oil/g sample)	Foam capacity (%)	Oil absorption capacity (g oil/g sample)	Water absorption capacity (g water/g sample)	Gel strength (N/cm^2)	Viscosity at 90 °C ($Pa \times 10^3$)
Protein concentrates from cowpea flours (kGy)							
0	88.9^{e} (0.40)	590 (0.00)	811 (1.00)	1.26^{ab} (0.07)	ND	0.67^{a} (0.06)	31.17^{d} (0.29)
2	86.2^{bc} (1.10)	638 (2.89)	809 (1.15)	1.42^{c} (0.01)	ND	0.70^{a} (0.00)	31.50^{d} (0.50)
10	85.6^{b} (0.71)	702 (10.41)	807 (1.15)	1.52^{d} (0.04)	ND	0.77^{b} (0.66)	34.23^{e} (0.25)
50	83.5^{a} (0.74)	787 (5.77)	807 (1.15)	1.59^{d} (0.03)	ND	0.80^{b} (0.00)	94.90^{f} (0.85)
Protein concentrates from cowpea pastes (kGy)							
0	89.0^{e} (0.40)	630 (5.00)	807 (1.15)	1.19^{a} (0.04)	ND	0.70^{a} (0.00)	15.33^{a} (0.31)
2	88.6^{de} (0.00)	645 (10.00)	807 (1.15)	1.33^{b} (0.09)	ND	0.70^{a} (0.00)	17.30^{b} (0.30)
10	88.2^{de} (1.21)	660 (5.00)	807 (1.15)	1.44^{c} (0.02)	ND	0.70^{a} (0.00)	19.57^{c} (0.15)
50	87.5^{cd} (0.75)	698 (7.64)	803 (1.73)	1.55^{d} (0.02)	ND	0.80^{b} (0.00)	33.73^{e} (0.12)

ND, Not determinable owing to high solubility.
Values are means plus or minus standard deviation of three determinations ($n = 3$).
Values followed by different superscript letter in a column are significantly ($p < 0.05$) different from each other.

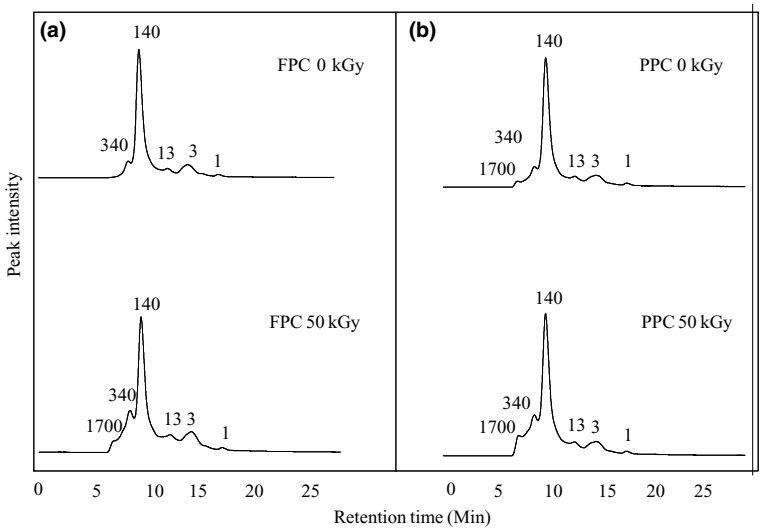

Fig. 1. Effect of irradiation on molecular weight distribution (kDa) of proteins isolated from cowpea flours (FPC) (a) and pastes (PPC) (b) as determined by size-exclusion HPLC.

Protein–protein cross-linking, leading to increases in molecular weight, may have also contributed somewhat to decreases in NSI of both FPC and PPC. Increases in protein molecular weight with irradiation (evident from SE-HPLC; Table 2 and Figs. 1(a) and (b)) may be due in part to bityrosine cross-linking of polypeptides (Giulivi, Traaseth, & Davies, 2003). Increased molecular weight may have led to increased hydrophobicity (Zayas, 1997) via, perhaps, the exposure of more non-polar protein sites, which may have in turn contributed to NSI decreases. However, given the higher protein cross-linking (Fig. 2 and Table 2), coupled with the lower reduction in NSI of proteins extracted from pastes compared to the proteins from flours, it seems that the contribution of protein molecular weight to the observed decreases in NSI was relatively small.

Irradiation, at all doses studied, caused progressive increases in emulsion capacities of FPC and PPC (Table 1), presenting a negative correlation ($r = -0.86$) with NSI (Table 4c). With decreasing NSI following irradiation treatment, decreases in EC would have been expected since these two functional properties are known, in general, to have high positive correlations (Zayas, 1997). It is possible that irradiation-induced increases in the ratio of exposed hydrophilic:hydrophobic amino residues may have contributed to improved emulsification. The increases in OAC with increasing irradiation (Table 1) seem to suggest increased protein hydrophobicity. Unfortunately, WAC (Table 1) which, together with OAC, would have enabled an estimation of the hydrophilic:hydrophobic ratio, was not determinable, owing

to reasons of high water solubility, as explained later in this paper. Increases in emulsion activity with increasing protein aggregation have been attributed to aggregated lecithin–soy protein complex having an amphipathic structure with increased hydrophobic surface (Hirotsuka, Taniguchi, Narita, & Kito, 1984). A statistically significant positive correlation ($r = 0.85$) between OAC and emulsifying properties of cowpea protein concentrates (Table 4c) was observed in this study. Similar correlations have been reported for proteins (Kato, Osako, Matsudomi, & Kobayashi, 1983). According to Hill (1996), estimating protein surface hydrophobicity (such as suggested by OAC) may be a useful indicator of emulsification properties.

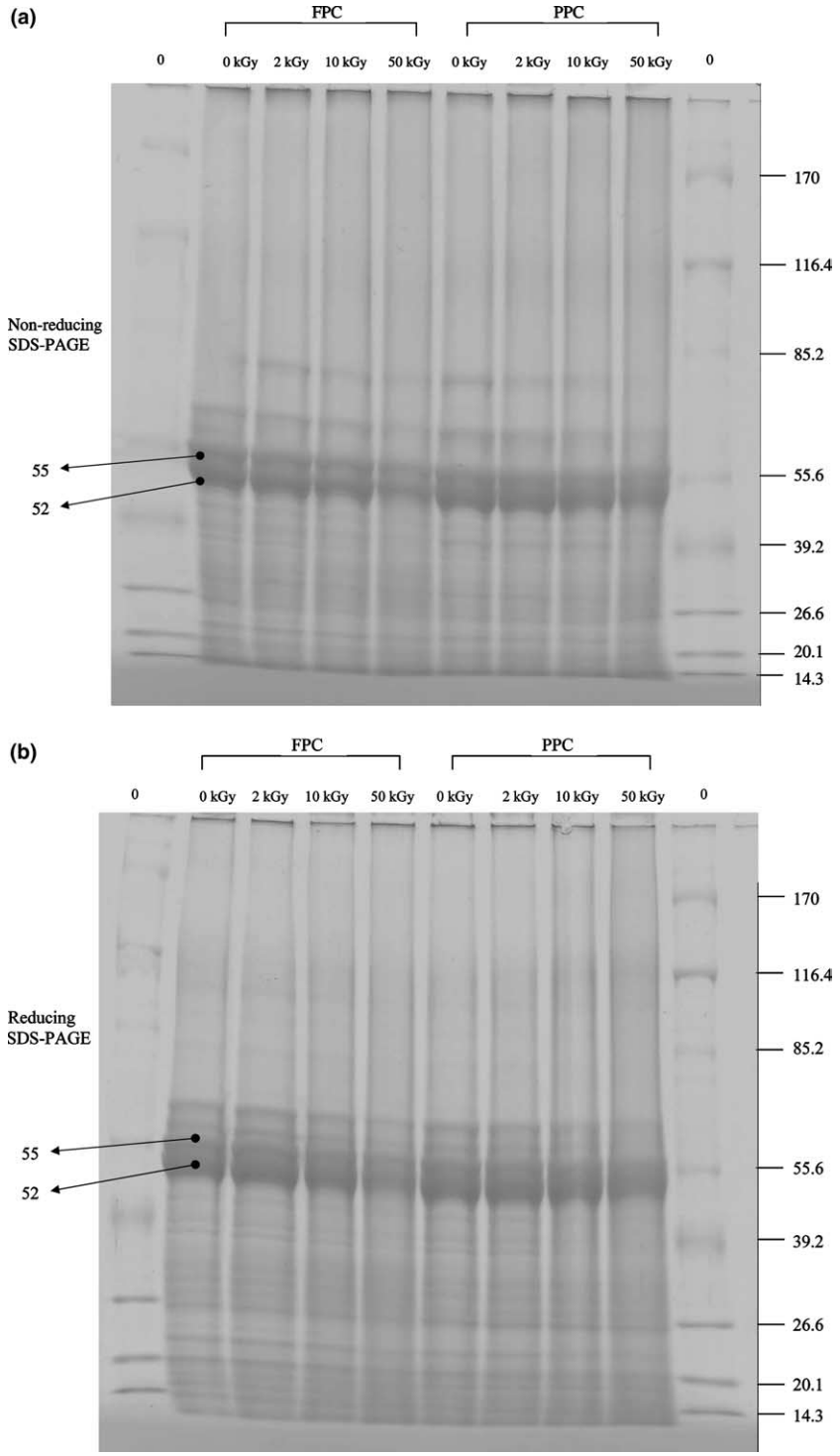

Fig. 2. SDS-polyacrylamide gel electrophoresis of proteins isolated from irradiated cowpea flours (FPC) and pastes (PPC) under non-reducing (a) and reducing (b) conditions. (Tracks 0 = Molecular weight markers (kDa)).

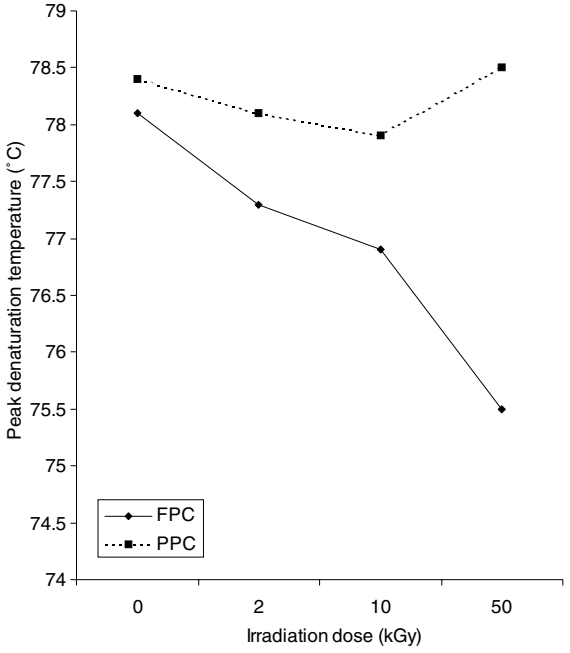

Fig. 3. Effect of irradiation on peak denaturation temperature of proteins isolated from irradiated cowpea flours (FPC) and pastes (PPC) determined using differential scanning calorimetry (DSC).

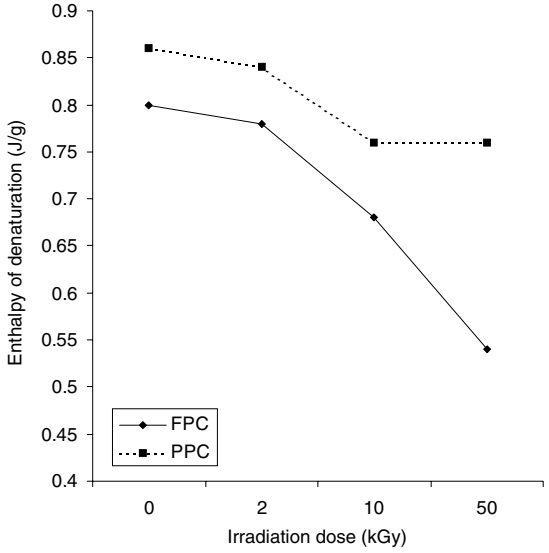

Fig. 4. Effect of irradiation on enthalpy of denaturation of proteins isolated from irradiated cowpea flours (FPC) and pastes (PPC) determined using differential scanning calorimetry (DSC).

Irradiation caused a significant decrease in FC of FPC at 2 kGy but not at higher doses, whereas FC of PPC was decreased only at 50 kGy (Table 1). The decreases in FC may be related to decreases in NSI given the high positive correlations ($r = 0.97$, and $r = 0.8$ in FPC and PPC, respectively) (Table 4). These decreases may be due, in part, to the earlier advanced reasons for NSI decreases, namely protein denaturation (for the changes in FC of FPC) and protein–protein cross-linking (for changes in FC of PPC). However, given the FC values recorded in the present study, cowpea flours and pastes may be irradiated at up to 50 kGy without totally compromising foaming properties.

The OAC of non-irradiated FPC and PPC (Table 1) were slightly higher than the value (1.10 ml oil/g sample or 1.01 g oil/g sample) reported for cowpea protein isolate (Ragab et al., 2004). As expected, oil absorption of cowpea protein samples (Table 1) increased significantly as irradiation dose increased. This may be due, in part, to the earlier advanced reason of possible increases in protein hydrophobicity with increasing irradiation dose. A significant negative correlation (minimum $r = -0.8$) was found between NSI and OAC (Table 4).

Owing to high solubility of FPC and PPC, water absorption capacity (WAC) was not determinable (Table 1) as the amounts of residue after centrifugation were far less than the starting protein material, probably because solubilised proteins were in close association with water. Similar reports have been presented for highly soluble plant proteins elsewhere (Zayas, 1997).

Irradiation at 10 and 50 kGy caused significant increases in GS of FPC and PPC, respectively (Table 1). Increases in gel strength with irradiation dose may be related, in part, to protein–protein cross-linking via bityrosine cross-links. Gels are made up of cross-linked

Table 2
Estimated peak areas (%) of protein fractions in proteins isolated from cowpea flours and pastes irradiated at 2, 10 and 50 kGy

Sample	Estimated peak areas (%)					
	1 (kDa)	3 (kDa)	13 (kDa)	140 (kDa)	340 (kDa)	1700 (kDa)
Protein concentrates from cowpea flours (kGy)						
0	1.1	13.3	5.9	72.7	7	ND
2	1.3	12.8	7.5	69.7	8.8	ND
10	1.3	12.5	7.8	64.8	12	1.6
50	1.2	14.3	10.4	52.7	18	3.5
Protein concentrates from cowpea pastes (kGy)						
0	1.2	11.6	7.7	67	10.6	2
2	1.2	11.2	6	68	10.7	2.9
10	1.2	11.1	5.9	66.2	12.6	3
50	1.1	10.7	7.4	57.3	18.1	5.4

ND, not detected.

protein polymers, via either covalent or non-covalent bonds, forming a network that is able to entrap water and other low-molecular-weight substances (Damadoran, 1997). Protein cross-linking may be expected to favour increases in gel strength by contributing to the pre-requisite network structure. Protein cross-linking is evident from SE-HPLC data (Fig. 1(a) and (b) and Table 2) as discussed later in this paper.

Although not always statistically significant, the viscosity of FPC and PPC increased with irradiation dose (Table 1) in a consistent manner with the results of GS. The increase in viscosity may be due to increases in molecular weight of proteins owing to protein cross-linking. An increase in viscosity may be suggestive of increased molecular weight of polymers (Damadoran, 1997; Mitchell & Areas, 1992). Gamma irradiation may, therefore, be potentially useful in positively modifying cowpea protein functional properties such as viscosity.

3.2. Total and available lysine of protein concentrates from irradiated cowpea flours

The total and available lysine of proteins extracted from irradiated cowpea flours are shown in Table 3. The total and available lysine values of FPC were slightly lower than those reported elsewhere for air-classified and wet-processed cowpea protein concentrates (Sosulski et al., 1987) probably owing to differences in cowpea variety.

Irradiation caused an insignificant ($p > 0.05$) but consistent decrease in available lysine with dose. This may indicate that reactive lysine contributed insignificantly to Maillard browning reactions with up to 50 kGy irradiation. Consequently, the contribution of Maillard-type reaction products to the protein cross-links formed with irradiation (as shown by SE-HPLC) may be insignificant. This finding seems to suggest that the colour darkening in cowpea flours and pastes with irradiation, reported earlier in our laboratory (Abu et al., in press), may be attributed only in part to Maillard-type reaction products. Perhaps, the formation of non-Maillard-type pigments was mostly responsible for the observed colour changes. Melanin-type pigments may form from tyrosine and tryptophan oxidation during irradiation treatment (Ley, Bleby, Coates, & Patterson, 1969).

3.3. Differential scanning calorimetry (DSC)

Data from the DSC thermograms are summarised in Figs. 3 and 4. The DSC thermograms (not shown)

Table 3
Total and available lysine contents of proteins isolated from cowpea flours irradiated at 2, 10 and 50 kGy

Protein concentrates from cowpea flours (kGy)	Total lysine (g/100 g)	Available lysine (g/100 g)
0	5.00[a] (0.29)	4.83[a] (0.28)
2	4.94[a] (0.13)	4.72[a] (0.12)
10	4.81[a] (0.16)	4.60[a] (0.15)
50	4.71[a] (0.02)	4.54[a] (0.02)

Values are means plus standard deviations (in parentheses) of duplicate determinations ($n = 2$).
Values followed by the same superscript letter in the same column are not significantly different ($p > 0.05$) from each other.

Table 4
Correlation coefficients (r) of functional properties of proteins isolated from irradiated cowpea flours (a), pastes (b) and their combination (c)

Functional property	OAC	FC	EC	NSI	GS	VISC
(a)						
OAC		−1.0*	0.95*	−0.98*	0.96*	0.69
FC	−1.0*		−0.93	0.97*	−0.95*	−0.64
EC	0.95*	−0.93		−0.96*	0.98*	0.86
NSI	−0.98*	0.97*	−0.96*		−0.92	−0.78
GS	0.96*	−0.95*	0.98*	−0.93		0.75
VISC	0.69	−0.64	0.86	−0.78	0.75	
(b)						
OAC		−0.7	0.96*	−0.98*	0.7	0.87
FC	−0.75		−0.91	0.85	−1.0*	−0.98*
EC	0.96*	−0.9		−0.99*	0.9	0.98*
NSI	−0.99*	0.8	−0.99*		−0.8	−0.94
GS	0.75	−1.0*	0.91	−0.85		0.98*
VISC	0.87	−1.0*	0.98*	−0.94	1.0*	
(c)						
OAC		−0.48	0.85*	−0.80*	0.85*	0.64
FC	−0.48		−0.52	0.11	−0.68	−0.05
EC	0.85*	−0.52		−0.85*	0.90*	0.82*
NSI	−0.80*	0.11	−0.85*		−0.71*	−0.86*
GS	0.85*	−0.68	0.90*	−0.71*		0.65
VISC	0.64	−0.05	0.82*	−0.86*	0.65	

Values with asterisks (*) are significantly correlated ($p < 0.05$).

displayed a main endothermic peak (considered as peak denaturation temperature) for both non-irradiated and irradiated protein concentrates in the range of 75.5–78.5 °C. These values fall within the range (75–90 °C) reported for the air-classified cowpea protein fraction (Sosulski, Hoover, Tyler, Murray, & Arntfield, 1985). The thermograms of FPC and PPC (not shown) were similar to those reported for heated faba bean protein (Arntfield & Murray, 1981), generally showing a progressive decrease in endothermic peak height with increasing irradiation dose.

The peak denaturation temperatures and enthalpies (Figs. 3 and 4, respectively) of non-irradiated FPC and PPC decreased progressively with increasing irradiation dose with the exception of PPC at 50 kGy. These general decreases may be indicative of partial protein denaturation by gamma irradiation in a dose-dependent manner. Decreases in transition temperatures and enthalpies are known to be associated with plant protein denaturation (Arntfield & Murray, 1981). The decreases in peak denaturation temperatures and enthalpies were more pronounced in FPC than in PPC at all doses studied. With the incorporation of water during preparation of cowpea pastes, prior to irradiation, the resultant secondary radiolytic effects of added water on proteins would have been expected to facilitate more protein denaturation. The DSC thermogram usually shows protein denaturation as an endothermic peak, which is a net value of endothermic and exothermic processes occurring in a highly cooperative manner (Harwalkar & Ma, 1996). Highly cross-linked proteins may require more energy for denaturation to occur during the assay than their less cross-linked counterparts. Therefore, the apparent increase in PGT, as well as the comparatively stable enthalpy at 50 kGy, in proteins isolated from cowpea pastes (Figs. 3 and 4, respectively), might have been due to extensive protein cross-linking occurring at this dose. The SDS-PAGE and SE-HPLC data revealed more extensive protein cross-linking in proteins from cowpea paste than from flour at 50 kGy.

Since endothermic peaks persisted after 50 kGy in both FPC and PPC, it may be inferred that irradiation up to this dose may not be expected to cause complete denaturation of proteins in multi-component cowpea flours and pastes. Arntfield and Murray (1981) reported that absence of endothermic peaks, in some commercial soy protein isolates, indicated that they were completely denatured.

3.4. Size exclusion high-performance liquid chromatography (SE-HPLC)

Irradiation caused changes in molecular weight distribution of proteins isolated from cowpea flours and pastes as determined by SE-HPLC (Fig. 1(a) and (b), Table 2).

Five peaks, corresponding to approximate molecular weights of 340, 140, 13, 3 and 1 kDa, were eluted in both FPC and PPC. The 140 kDa represented the major peak (ca. 70%) in non-irradiated and irradiated FPC and PPC samples. This peak compares reasonably well with the estimated major peak (150 kDa) reported by Rangel et al. (2003) for cowpea protein isolates and purified cowpea vicilins using SE-HPLC. In this study, a sixth peak (1700 kDa) was eluted in all PPC and in 10 and 50 kGy FPC samples after 7.4 min. The 1700 and 340 kDa peaks increased in percent area with increasing irradiation dose in both FPC and PPC at the expense of the 140 kDa peak (Table 2).

The decreases in the 140 kDa peak, coupled with the appearance and/or increase of the 1700 and 340 kDa peaks, may be due to cross-linking of polypeptides of the 140 kDa sub-fraction with increasing irradiation dose. Protein cross-linking is known to occur with irradiation under limiting oxygen conditions (as was the case in this research) due primarily to bityrosine cross-link formation (Giulivi et al., 2003).

3.5. SDS-Polyacrylamide gel electrophoresis

Fig. 2 shows SDS-PAGE of proteins prepared from irradiated and non-irradiated cowpea flours and pastes under non-reducing (a) and reducing (b) conditions. Cowpea FPC and PPC was comprised of several polypeptide bands, ranging from <14 kDa to about 100 kDa, and falls within the range reported for cowpea proteins. According to Khan et al. (1980), cowpea proteins are made up of several polypeptides, ranging from less than 22.5 to 300–400 kDa. Two major bands, of approximately 52 and 55 kDa, were visible under non-reducing and reducing conditions although Carasco et al. (1978) reported three major bands of approximately, 52, 54 and 56 kDa for cowpea proteins using SDS-PAGE. Under reducing conditions, some bands (mostly between 14.3 and 20.1 kDa) that were previously not visible with non-reducing conditions, appeared in all non-irradiated and irradiated FPC and PPC samples, possibly indicating the presence of disulphide bonds within their structures. However, the contribution of disulphide bonds to irradiation-induced protein cross-linking was not clearly evident. In fact the intensity of the major bands at 50 kGy (Fig. 2(b)) were visibly less in both FPC and PPC samples, suggesting that the protein polymers formed by irradiation, were not due, primarily, to disulphide bonds.

The 52 and 55 kDa bands decreased in intensity under both reducing and non-reducing conditions at 10 and 50 kGy, for both FPC and PPC. The observed decreases in intensity were apparently more pronounced at 50 kGy than at 10 kGy. This may indicate association of the 52 and 55 kDa polypeptides at 10 and 50 kGy, leading to higher molecular weight polymers that prob-

ably could not pass through the gradient gel. This finding is similar to the decreases observed in the major 140 kDa peak with increasing irradiation dose using SE-HPLC.

4. Conclusions

Our results indicate that most functional properties of cowpea proteins isolated from gamma-irradiated cowpea flours are affected by gamma irradiation at 2 kGy, whereas proteins from pastes are affected mainly at 10 and 50 kGy. In general, the changes in functional properties appear to be more drastic in FPC than in PPC samples, due partly to differences in the extents of protein denaturation and cross-linking under dry and wet conditions. Irradiation-induced decrease in NSI is accompanied by increase in OAC due, in part, to a probable increase in hydrophobicity, emanating from denaturation and cross-linking of cowpea proteins. Differential scanning calorimetry (DSC) and size exclusion high performance liquid chromatography (SE-HPLC) show that cowpea protein denaturation and cross-linking, respectively, may occur with irradiation. Reducing SDS-PAGE seems to suggest that disulphide bonds are not primarily responsible for irradiation-induced cowpea protein cross-linking.

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