

WHY GAMMA IRRADIATION MODIFIES COWPEA PROTEIN-RELATED FUNCTIONAL PROPERTIES

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INTRODUCTION

Gamma irradiation is usually applied to grains such as cowpeas to prevent or control insect and pest infestations (Diop, Marchioni, Ba & Hasselmann, 1997). 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 association of aromatic and heterocyclic residues (Cho, Yang & Song, 1999). The type and extent of these reactions depend on factors such as dose, pH, hydration state, presence or absence of oxygen as well as temperature during irradiation.

Significant changes in some protein-related functional properties such as decreased solubility and emulsifying properties of cowpea flours and pastes following their exposure to medium and high gamma irradiation doses have been reported (Abu *et al.*, 2005). The present objective was to study the molecular and thermal properties of proteins isolated from irradiated cowpea flours and pastes to help in understanding the reasons behind the observed changes in protein-related functional properties.

MATERIALS AND METHODS

Irradiation of cowpea flours and pastes

Cowpea flour and paste samples weighing approximately 200 g were sealed in polyethylene bags (ca 80 µm thick) and placed in ice-cooler boxes prior to and during irradiation. Samples were irradiated at 2, 10 and 50 kGy, respectively at Isotron S.A. in Isando, South Africa using a ⁶⁰Co source. Irradiation was carried out 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.

Protein preparation

All non-irradiated and irradiated cowpea flours and pastes samples were pooled per treatment prior to protein isolation. A three-step extraction process was used to prepare protein concentrates from irradiated cowpea flours (FPC) and pastes (PPC). 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.

Size Exclusion High Performance Liquid Chromatography (SE-HPLC)

Analyses were performed on a HPLC apparatus equipped with a C-10AD controller using a Superdex 200 column as described before (Abu et al., 2006).

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was conducted under reducing and non-reducing conditions as described previously (Abu et al., 2006).

Differential Scanning Calorimetry (DSC)

DSC was performed with a micro-DSC III system (Setaram, Caluire, France) as described before (Abu et al., 2006).

RESULTS AND DISCUSSION

The results of SE-HPLC revealed five peaks corresponding to approximate molecular weights of 340, 140, 13, 3 and 1 kDa in all protein samples (Figure 1). The 140 kDa represented the major peak (ca 70 %) in non-irradiated and irradiated samples. A sixth peak (1700 kDa) eluted in all PPC and in 10 and 50 kGy FPC samples after 7.4 min. The 1700 kDa and 340 kDa peaks increased in percent area with increasing irradiation dose in both flour and paste proteins at the expense of the 140 kDa peak due possibly to bityrosine cross-linking of this polypeptide peak (Guilivi *et al.*, 2003).

SDS-PAGE (Figure 2) revealed that isolated proteins comprised several polypeptide bands ranging from <14 kDa to about 100 kDa. Two major bands of approximately 52 and 55 kDa were visible under non-reducing and reducing conditions. The 52 and 55 kDa bands decreased in intensity under both reducing and non-reducing conditions more at 50 than at 10 kGy for both FPC and PPC. This may indicate association of the 52 and 55 kDa polypeptides at these doses leading to higher molecular weight proteins. Reducing SDS showed that the polypeptide cross-links formed with irradiation were not due to disulphide bonds.

The peak denaturation temperatures and enthalpies (data not shown) of non-irradiated proteins decreased progressively with increasing irradiation dose with the exception of paste proteins that increased slightly at 50 kGy. These 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 and Murray, 1981).

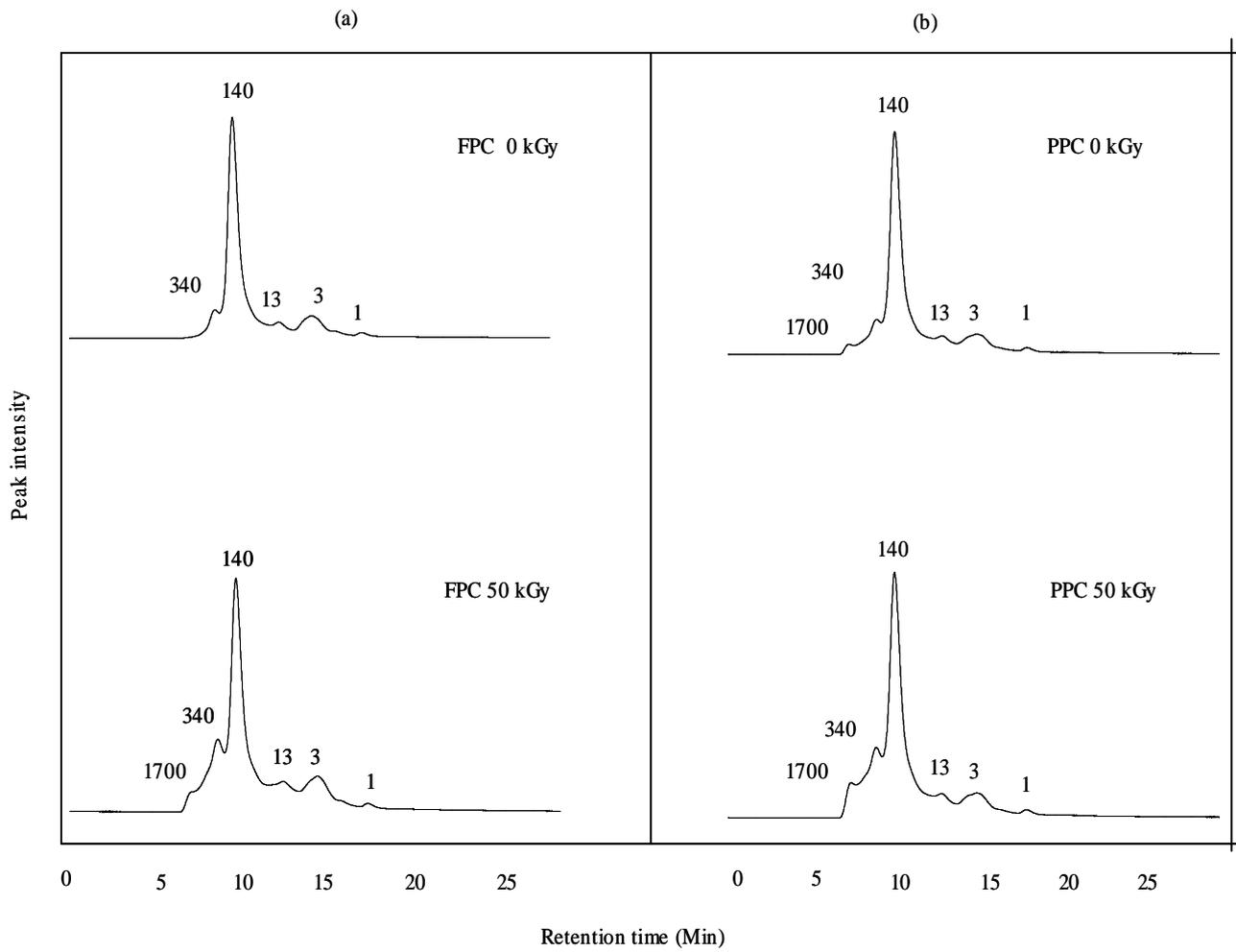


Figure 1. Effect of irradiation on molecular weight distribution (kDa) of proteins isolated from cowpea flours (FPC) (a) and pastes (PPC) (b) as determined by size-exclusion HPLC

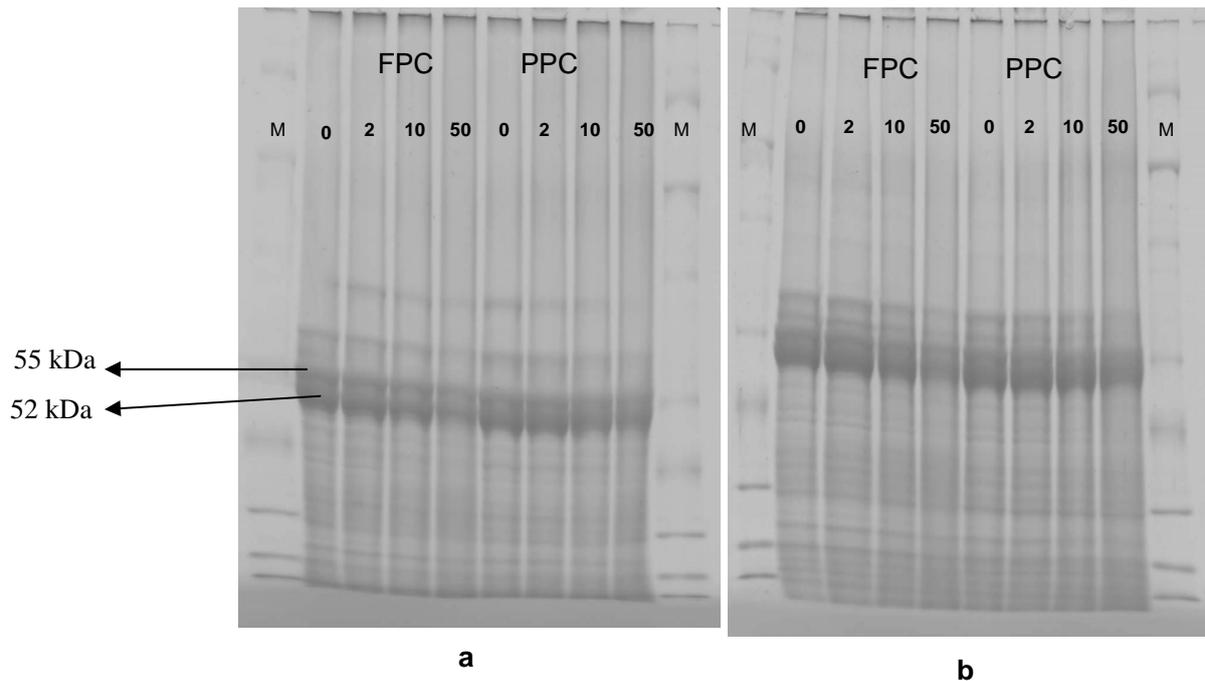


Figure 2. SDS-Polyacrylamide gel electrophoresis of proteins isolated from 0, 2, 10 and 50 kGy irradiated cowpea flours (FPC) and pastes (PPC) under non-reducing (a) and reducing (b) conditions (M= molecular markers)

CONCLUSIONS

Irradiation of cowpea flours and pastes mostly at 10 and 50 kGy result in protein denaturation (more in flour protein) and cross-linking (more in paste protein). These changes contributed to modifying protein-related functional properties of cowpea flours and pastes.

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