Food Science and Quality Management
ISSN 2224-6088 (Paper) ISSN 2225-0557 (Online)
Vol.64 2017

Pasting of G-Irradiated Proteins from Vigna Subterranea in Native Starch Models and the Surface Functional Properties of the Proteins

Pamela Esther Ayisi[1,2*], Isaac Williams. Ofosu[1],

1. Department of Food Science and Technology, Faculty of Biosciences, College of Science, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana
2. Baldwin College, Accra, Ghana.

Abstract:

This research was carried out to evaluate the surface functional properties of gamma irradiated Bambara groundnut protein isolates and to study the pasting characteristics of the modified protein in native starch models using the Brabender Viscoamylograph. Irradiation was done at five levels: 2.50, 5.00, 7.50, and 10.00 kGy; while the pasting of the Proteins (P) was run in Starch (S) models of three combinations; 30P:70S, 50P:50S, 70P:30S. The results showed significant ($p<0.05$) effects of increasing irradiation doses on protein related surface functional properties, while pasting characteristics of the irradiated protein in the experimental range showed no significant dose-dependent ($p<0.05$) changes. There was characteristic starch paste behaviour on the other hand with increasing starch:protein ratios. Conclusively, correlation studies suggested that the pasting properties depended solely on the starch concentration within the admixture models indicating the insignificant contribution of modified Bambara groundnut proteins to the pasting properties in the models. Enhanced surface functional properties of the gamma irradiated proteins make them potential foaming and emulsifying agents in food applications. The starch-protein admixture models may also serve as a potential protein based thickening agents for foods that require various degrees of viscosity modifying effects.

Key words: Bambara groundnut protein, modified protein, gamma irradiated proteins

1. Introduction

A recall of legumes, often without any hesitation includes soybean and cowpea to the neglect of other legumes that abound but are underutilized because of their usage problems. Many scientists have put forward various reasons why neglected and underutilized legumes do not feature in their research plan. Such problems as low essential amino acids, long cooking hours, the presence of anti-nutrients, namely, leptins, bitter principles and phytic acids and the presence of oligosaccharides implicated in flatulence, among others, pose serious usage problems. Fortunately, these orphan legumes have the potential of solving numerous rural based problems, though they are frequently overlooked by research considerations. No matter the reasons offered, it is clear that the lack of attention has meant that the potential values of these legumes are under-exploited and therefore placing them in danger of continued genetic erosion and ultimate disappearance. It is imperative that research be directed to the processing of these legumes to lift their usage potential taking advantage of the advances in the modification procedures available for instance in the grafting or polymerizing starches and proteins into new biopolymers. It has been anticipated that grafting or copolymerization can be carried out in such a way that the properties of the side chains can be added to those of the substrate polymers without greatly changing the latter. For instance, graft copolymerization of starch with acrylic acid has found extensive commercial applications, especially as hydrogels for personal care products, food packages, medical and agricultural applications (Athawale and Lele, 2001). Similarly, starch graft copolymers have been achieved by free radicalinitiated procedures where high energy ionization radiation, especially c-rays (Kiatkamjornwong *et al*., 2000), electron beam and Ultraviolet (UV) irradiation have been used to initiate grafting on starch (Fanta and Doane,1986). Researchers such as Abu *et al*. (2005) have also pointed out that at low dose irradiation (2 kGy), most of the protein-related functional properties of cowpea flours and pastes are not affected but only at higher doses of between 10 and 50 kGy where parameters such as Nitrogen Solubility Index (NSI), Oil Absorption Capacity (OAC), Foaming Capacity (FC) and Emulsion Capacity (EC) of cowpea flours and pastes. However, little or no investigation has been conducted using irradiation to fragment proteins studying their behaviour as pasted in starch models. Also, there is currently no data to show whether low dose radiation would be enough to fragment freeze-dried proteins that would copolymerize with such polymers as starch. Very little is also known as to the effect of starch-protein cogels food functionality as revealed by their pasting characteristics. Our objective was

to characterize fragmented freeze-dried proteins obtained by gamma irradiation as pasted in starch models and then study the surface functional properties of the gamma irradiated proteins.

2. Materials and methods

Bambara groundnuts obtained from the Crop Research Institute of the Plant Generic Resource Unit, Bunso, Ghana were sorted and solar-dried (to 12% moisture content) for a minimum of three days and milled. The dried flour was defatted using petroleum spirit in a ratio of meal to solvent ratio of, 1:10w/v, in a large scale Soxhlet's extractor, (PPC model, Saskatoon, SK, Canada) and solar-dried in solar tent for about two to three hours to expel the volatile extraction solvent.

2.1 Protein extraction

Alkaline extraction of protein from the dried defatted meal was done using 0.01 M NaOH with a meal to solvent ratio of 1:10 w/v. Agitation of solutions was carried out at 150 rpm at room temperature for two hours, using Environmental incubator shaker (Model G24, New Brunswick Scientific, Edison, NJ) and insoluble polysaccharides and residues were removed by centrifuging at 2500 rpm for 20 min. Supernatant was acidified to a pH range of 4.5-5.0 to allow protein precipitation. The resulting solution was centrifuged at 3000 rpm for 20 min to separate proteins from soluble polysaccharides. Centrifugation was repeated using distilled water, after which recovered proteins were freeze-dried using the HETO POWER DRY LL300 (Thermo Scientific Brand Products, Waltham, MA, US) freeze dryer.

2.2 Starch extraction

Starch was extracted from the solid fibrous portion obtained after the first round of centrifugation. Starch particles settled after filtrate was allowed to stand for 30-45 min and pure starch was obtained by repeated washing and subsequently solar dried to 10.44% moisture.

2.3 Irradiation of proteins

Five samples of freeze-dried proteins, each weighing 295.41 g, were separately placed in the inner region of the irradiation chamber (Gamma cell 220 ^{60}Co AECL, Canada) and calibrated with the Fricke Dosimetry System and exposed to 2.5, 5.0, 7.5 and 10.0 kGy of ^{60}Co gamma radiation, respectively, at 1.63 kGyh-1. The non-irradiated sample (0.0 kGy) was set as control.

2.4 Viscographic analysis

The method of Demiate *et al*. (2001) was used, but with slight modifications. Specific weights of starch-protein combinations depending on the moisture content was suspended in a specific volume of solvent and was analyzed with a Brabender viscoamylograph programmed to increase the temperature and to rotate the vessel at a fixed rate, 1.5 C/minute and 75 rpm, respectively. The procedure included an initial heating phase, from 50-95 C, in order to observe the viscosity features as: Beginning of gelatinization; maximum viscosity; start of holding period; start of cooling period; end of cooling period; end of final holding period; breakdown viscosity and setback viscosity. The influence of the different combinations of protein-starch blends on the pasting properties was studied. Results were recorded directly from the equipment as digitized viscoamylograph.

2.5 Physicofunctional properties

2.5.1 Determination of moisture content of irradiated proteins

Moisture content of samples was determined by the AOAC (1990) approved methods. Five grams of each protein was measured into separate crucibles and their respective gross weights taken. These were placed in the oven at 150^{o}C for 24 h and moisture content was expressed as percentage loss in weight of sample.

2.5.2 Water absorption capacity

Water absorption capacity was estimated by the method described by Wang and

Kinsella (1976). One gram each of irradiated protein samples was suspended in 10 ml distilled water in 15ml graduated centrifuge tubes and the weights taken before and after addition of distilled water. Samples were shaken for 30 min and centrifuged at 2500 rpm for 25 min at room temperature. The freed water was carefully decanted and weight of test tube plus content was taken. Water absorbed was calculated as the difference between the initial volume of water added to the sample and the volume of the supernatant. The water absorption capacity was expressed as the volume of water retained by one gram of the sample with density of water taken as 1.00 g/cm3.

2.5.3 Oil absorption capacity

Oil absorption capacity was measured by the method described by Wang and Kinsella (1976). In this process one gram each of irradiated protein samples was suspended in 10 ml vegetable oil in 15 ml graduated centrifuge tubes and the weights taken before and after addition of oil. Samples were shaken for 30 min and centrifuged at 2500 rpm for 25 min at room temperature. The freed oil was carefully decanted and weight of test tube plus content taken. Oil absorbed was calculated as the difference between the initial volume of oil added to the sample and the volume of the supernatant. The oil absorption capacity was expressed as the volume of oil retained by one gram of the sample with the density of oil taken as 0.89 g/cm^3.

2.5.4 Emulsifying activity and emulsifying stability

The procedure described by Volker and Kelin (1979) was used for emulsifying properties. In this study the emulsions were prepared with one gram of each protein sample using 50 ml distilled water and 50 ml vegetable oil. The mixtures were homogenized thoroughly at room temperature for 30 min. Each emulsified sample was divided equally into 50 ml centrifuge tubes. Content of one 50 ml tube was centrifuged directly at 3000x g for 30 min while the other centrifuged under the same conditions after heating in a water bath at 80oC for 30 min and cooling to 15^{o}C. The heights of the emulsified layers, as a percentage of the total height of the material in the unheated tubes was used to calculate the emulsifying activity and emulsifying stability using the appropriate formula as follows:

$$\text{Emulsifying activity (\%)} = \frac{\text{Height of emulsion}}{\text{Height of whole layer}} \text{ x } 100$$

$$\text{Emulsifying stability (\%)} = \frac{\text{Height of emulsion after heating}}{\text{Height of whole layer}} \text{ x } 100$$

2.5.5 Foaming capacity

To study the foam capacity according to the method of Lawhon *et al*. (1972) a volume of 50 ml of 0.01 M NaOH was added to five grams of protein samples to solubilize them. The mixtures were then thoroughly homogenized for 10 min using a laboratory homogenizer, (Silverson Machines USA, model L4R) set at 10,000 rpm at room temperature. Homogenized samples were poured into 100 ml measuring cylinders and the volume of foam after 30 sec was measured. The increase in volume of content of cylinder was expressed as a percent foam capacity. The procedure was repeated for a five gram egg white sample of the same moisture content.

2.5.6 Foaming stability

Foam stability was determined by measuring the decrease in volume of foam as a function of time at intervals of 10, 30, 60 and 120 min (Suliman *et al*., 2006).

2.6 Experimental design and statistical analysis

Functional properties were determined in duplicates. For all surface functional properties, sample variations effects were analyzed by one-way ANOVA (no blocking), while sample and treatment effects were analyzed by a two-way ANOVA (no blocking). Significant differences ($p<0.05$) between the means of sample variations and between variations and treatments were determined using the variance ratio (v.r). Correlations coefficients (r) of functional properties were obtained. The level of significance used was 95%.

3.0 Results and discussions

3.1. Viscographic analysis

3.1.1 Pasting temperature and peak time

The data obtained on pasting characteristics showed that there were no significant ($p<0.05$) dose-dependent increases in the gelatinization temperatures within the three levels of irradiated protein-native starch admixtures. However, of the three admixtures, the 70P:30S blends recorded the highest gelatinization temperatures of 88.20, 86.60, 91.50, 85.10 and 86.80 C, approximately, within 30 min for 0.00, 2.50, 5.00, 7.50 and 10.00 kGy levels of irradiated proteins. The 50P:50S blends recorded intermediate gelatinization temperatures while the 30P:70S blends had the least gelatinization temperatures. However, at irradiation levels 2.50 and 5.00 kGy, gelatinization temperatures for 30P:70S (76.20 C and 76.35 C) were higher than 50P:50S (64.15 C and 63.80 C), for reasons

that could not be explained in this research (Fig. 1). The high protein concentration in the 70P:30S blend may have retarded the gelling ability of the entire system, hence a requirement of higher temperatures of gelatinization for reasons that probably relate to increased protein-protein interaction and less protein-protein interaction. Correlation studies indicated that a decrease in starch concentration caused a corresponding increase in the gelatinization temperatures, while increases in protein concentration correlated positively with increases in gelatinization temperatures within the blends.

3.1.2 Peak viscosity

Maximum viscosity also decreased with decreases in starch and increases in protein concentrations (Fig. 2), evidenced by the significant negative correlation (r = -1.00) (Table 1) between increased protein-starch ratio and maximum viscosity. Peak viscosity ranged between 620.00 and 5.00BU. Blends constituted of 30% proteins and 70% starch attained the highest values for peak viscosity at all levels of irradiation. The least viscosity values were observed in the 70P:30S blends while the 50P:50S recorded intermediate values. For instance, at 2.50 kGy level

protein irradiation the viscosity values for the three blends, 30P:70S, 50P:50S, 70P:30S were 557.00, 288.00 and 64.00 BU, respectively. However, across the levels of irradiation peak viscosity did not differ significantly ($p<0.05$).

A significant negative correlation of protein with peak viscosity ($r = -0.863$, $p<0.01$) (Table 1) of corn flour had also been reported earlier by Sandhu and Singh (2007). Proteins did not gel when cooked and therefore exhibited very low to almost negligible viscosities. Possibly, the high maximum viscosity observed within the 30P:70S blend may be attributed to the high starch concentration and not to the proteins or the interaction between the two biopolymers. This observation also implies that the less starch within the blend, the less viscous the paste upon cooking at optimum temperatures. However, temperatures at which the different blends, each constituted of different modified proteins with corresponding attained maximum viscosities, did not differ significantly ($p<0.05$).

Table 1: Correlation Coefficient (r) of functional properties of irradiate Bambara groundnut proteins

Functional properties	SOL	WAC	OAC	FC	FS	EA	ES
SOL		-0.67	-0.21	-0.38	-0.04	-0.40	-0.67
WAC	-0.67		0.84*	0.92*	0.73	-0.14	0.31
FC	-0.38	0.92*		0.87*	0.92*	-0.62	-0.21

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

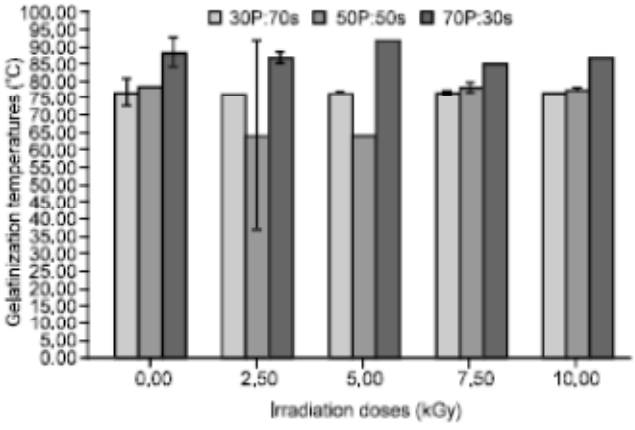

Figure 1: Gelatinization temperatures of protein-starch blends

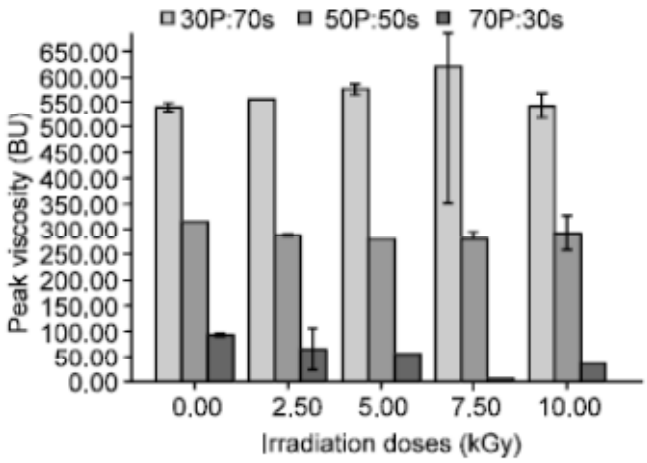

Figure 2: Peak viscosity of protein-starch blends

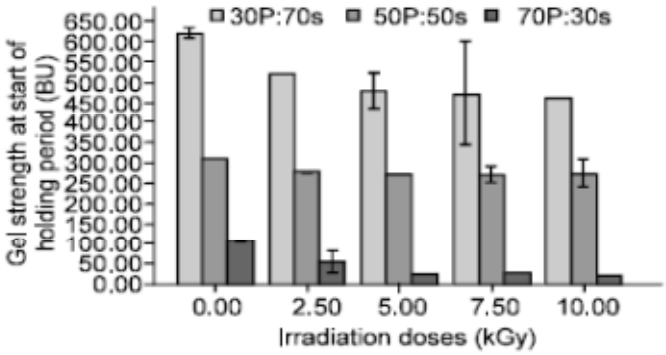

Figure 3: gel strength at start of holding period of protein-starch blends

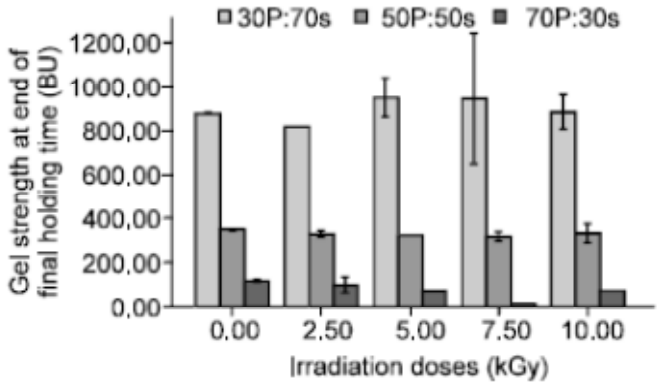

Figure 4: Gel strength at the end of final holding period of protein-starch blends

The temperature values ranged between 92.70 and 95.60oC. Again, all protein-starch admixtures exhibited increased viscosity during cooling at 50oC. Results showed that gel strengths decreased with decreasing starch

concentrations within blends. The reason advanced for this observation may be as a result of the direct consequence of the decreasing peak viscosities with decreased starch concentrations. Gel strength for all blends generally increased from start of holding period, through the cooling period, to the end of the final holding period. For instance, the gel strength of the 30P:70S blends constituted of 10.00 kGy proteins increased from 541.00BU at start of holding period to 896.00BU at the end of the final holding time (Fig. 3 and 4). Similar observations were made for the other two blends with proteins at the various levels of irradiation There were, however, no dose-dependent significant differences ($p<0.05$) in gel strengths with respect to the irradiated proteins used to constitute the three different protein-starch admixtures.

3.1.3 Breakdown

Breakdown viscosity values were between 0.00 and 42.00BU and were higher in the 50P:50S blends with all five levels of irradiated proteins compared with the 30P:70S and 70P:30S blends. There were dose-dependent significant differences ($p<0.05$) in breakdown viscosity with respect to the proteins used in constituting the 50P:70S and 70P:30S blends (Fig. 5). However, no viscosity breakdown was observed in the 30P:70S blend constituted of 7.50 kGy irradiated protein. These observations could not also be explained.

3.1.4 Setback and final viscosity

Setback viscosity, which is the measure of retrogradation accompanied by syneresis, ranged between 9.00 and 397.50 BU and had an increasing setback of 70P:30S, 50P:50S, 30P:70S.The reason for this observation might be that higher starch concentrations encouraged the formation of a more ordered structure, which in turn trapped enough water, forming stronger gels with higher viscosities. This phenomenon may have increased the tendencies for retrogradation accompanied by syneresis, hence the increasing setback viscosity. However, no dose dependent significant differences ($p<0.05$) were recorded for the setback viscosities with respect to the type of irradiated protein used in constituting the admixtures (Fig. 6). Results from the pasting characteristics of the protein-starch admixture models suggest the sole dependence of the pasting properties on the starch concentration within the blends, meaning that the contribution of the Bambara groundnut starch to the pasting properties of the blends was greater than that of the irradiated Bambara groundnut proteins. This seem to suggest that the modified proteins were simply unable to form strong gel matrices or gel networks with starches, perhaps, due to poor ionic interactions between the individual molecules and the poor networking between protein-protein and protein-solvent molecules.

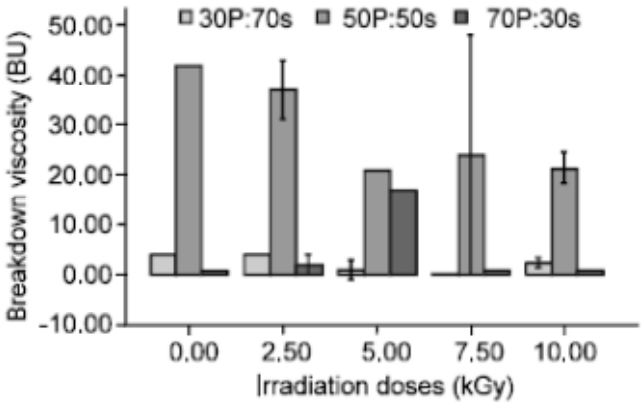

Figure 5: breakdown viscosities of protein-starch blends

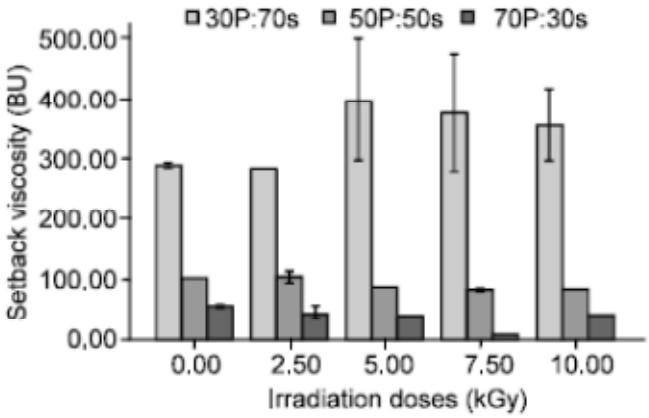

Figure 6: Setback viscosities of protein-starch blends

3.2 Physicochemical and functional properties

3.2.1 Moisture content

Moisture content of various irradiated protein samples ranged from 5.770-5.810% (Fig. 7). Clearly, there were no significant differences ($p<0.05$) among samples both before and after irradiation. This, however, is an indication that the irradiation procedure had no effect on the moisture content of the samples, evidenced by the negative correlation obtained ($r = -0.12$) (table 1) between irradiation dosage and moisture content of the irradiated Bambara groundnut protein samples.

3.2.2 Water and oil absorption capacities

Water and oil absorption generally increased across irradiation doses with the 10.00 kGy irradiated sample recording the highest for both Water Absorption Capacity (WAC) and Oil Absorption Capacity (OAC) - 18.45% and 10.09% respectively. The increase in the WAC may be as a result of the coupled effect of water adsorption via existing polar binding sites distributed over the protein surface, and molecular rearrangement leading to the exposure of more polar binding sites, following irradiation (Privalov, 1979). Increases in OAC following irradiation have been recorded for cowpea flours and pastes (Abu *et al*., 2005) and the trend is similar to what was observed for this work. Lipophilic tendencies of samples increased with increasing irradiation probably due to more exposed hydrophobic sites as scission and rearrangement of polypeptides occurred following a progressive increase in irradiation doses, compared to the non-irradiated proteins. Figure 8 shows higher values for WAC than OAC in Fig. 9. However, OAC values, unlike WAC, values did not vary significantly ($p<0.05$) between any two successive irradiation doses.

3.2.3 Emulsifying activity and emulsifying stability

Superior emulsifying properties are desired to make milk-like beverages and meat analogues (Friberg, 1976). In this

work it was observed that the irradiation caused a progressive decrease in Emulsifying Activity (EA) and Emulsifying Stability (ES) for Bambara groundnut protein samples (Fig. 10 and 11). The non-irradiated samples (0.00 kGy) recorded lower EA and ES values than the 2.5 kGy irradiated samples. The reason for this observation may be attributed to the predominantly hydrophilic nature of the non-irradiated proteins resulting from excess polar active sites residing on the protein surfaces thereby causing the bulk of the adsorbed and more compact proteins to reside within the water side of the interface, hence the reduced emulsion activity and stability (Friberg, 1976). However, irradiation at 2.50 kGy may have caused unfolding and fragmentation of proteins which possibly caused some previously exposed polar sites on the protein to be buried while exposing more non-polar sites, thus increasing the emulsifying properties only slightly.

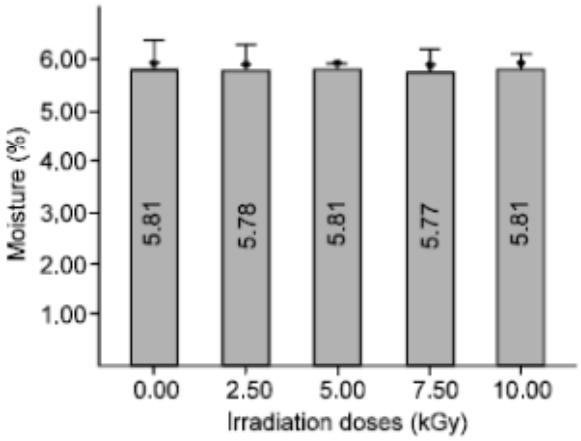

Figure 7: Moisture content of irradiated bambara groundnut protein isolates

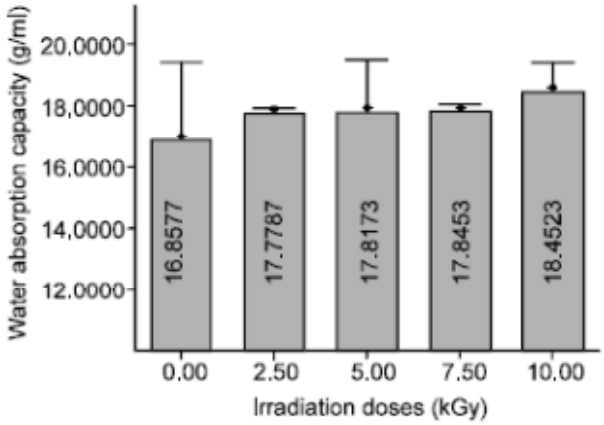

Figure 8: Water absorption capacities of irradiated bambara groundnut protein isolates

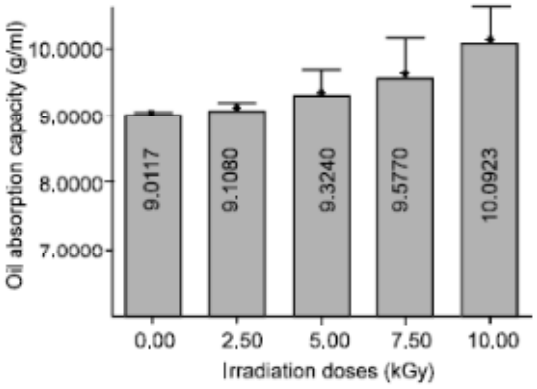

Figure 9: oil absorption capacities of irradiated Bambara groundnut protein isolates

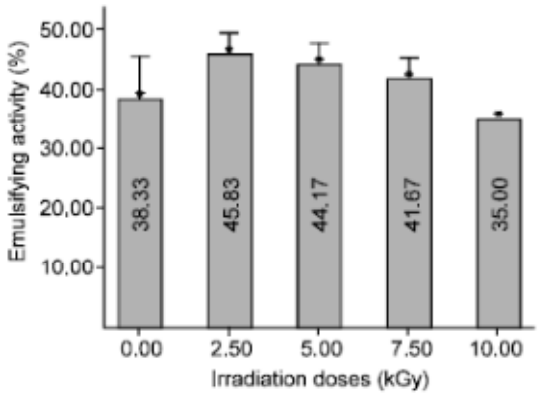

Figure 10: Emulsifying activities of irradiated Bambara groundnut protein isolates

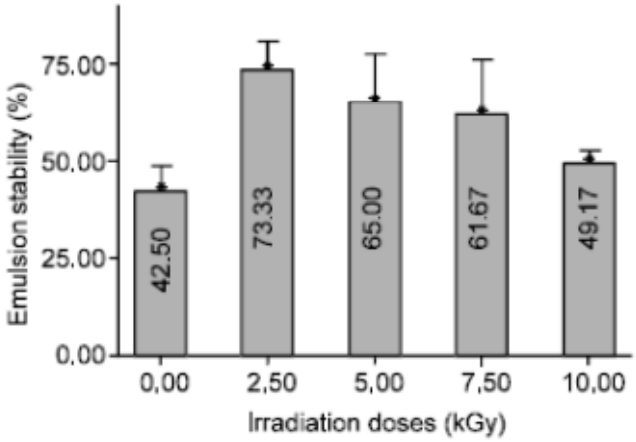

Figure 11: Emulsion stabilities of irradiated bambara groundnut protein isolates

As irradiation increased from 2.50 kGy to 10.00 kGy, the various degrees of protein unfolding and fragmentation again might have exposed either too many hydrophilic or hydrophobic active sites on protein surfaces, causing them to adsorb more, either within the water or the oil interface, thus reducing their emulsifying properties in a dose-dependent manner. A negative correlation ($r = -0.62$ and $r = -0.14$) (Table 1) was recorded between OAC and EA and WAC and EA, respectively, at all levels of irradiation for the same reason of irradiation-induced increases in either protein surface hydrophilicity or hydrophobicity.

3.2.4 Foaming capacity and stability

Irradiation caused a progressive increase in Foaming Capacity (FC) for all protein samples (Fig. 12). This observation may be due, in part, to increased diffusion of the dose-dependent unfolded and fragmented proteins towards the air/water interface. Increased unfolding and fragmentation of protein following irradiation, may have enabled the formation of more continuous phases of thin liquid layers which trapped air bubbles, hence the progressive increases in foaming capacity of irradiated protein samples. The egg white which has excellent foaming properties and therefore often used as the standard, recorded the highest FC (95%) and Foaming Stability (FS) values. The 10.00 kGy irradiated sample which recorded 80.00% FC exhibited a fairly high ability to foam, therefore could serve as much a foaming agent as the egg white in confectionery products, such as cakes and breads.

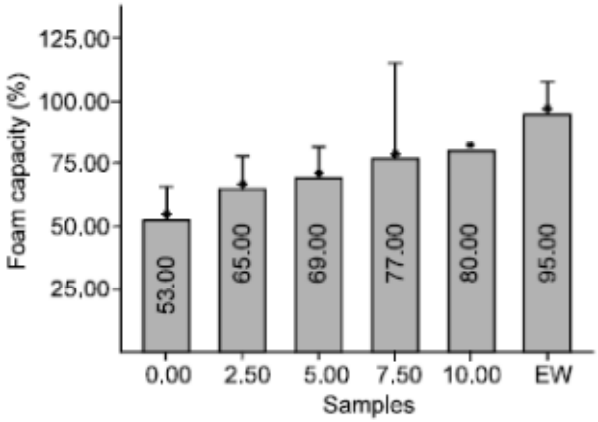

Figure 12: foam capacities of irradiated Bambara groundnut protein isolates

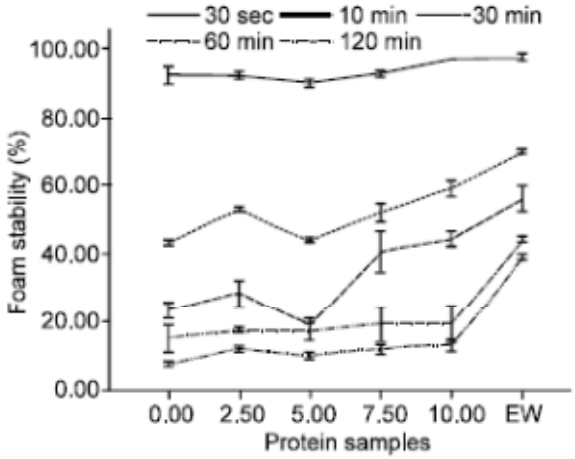

Figure 13: foam stabilities of irradiated Bambara groundnut protein isolates after 10 sec, 10, 30, 60 and 120 mins

Foam stability values for each sample decreased progressively from 30 sec to 120 min after whipping (Fig. 13). Breakdown of foams in a dose-dependent manner, resulting from drainage of lamella liquid, may have been due to the effect of gravitational force on the protein masses obtained from various degrees of unfolding and fragmentation after irradiation. However, foams of 2.50 kGy protein were more stable than those of 5.00 kGy over the two-hour period for reasons that could not be explained. The research showed significant ($p<0.05$) effects of increasing irradiation doses on some of the protein related functional properties, while pasting characteristics of admixtures showed no dose-dependent significant ($p<0.05$) changes. Increases in Water and Oil Absorption Capacities (WAC

and OAC) were dose-dependent, with samples showing significant differences ($p<0.05$). The 10.00 kGy samples recorded the highest values of 18.45% and 10.09% for WAC and OAC respectively. Foaming Properties increased across irradiation doses with some significant differences ($p<0.05$) among samples. However the 10.00 kGy irradiated samples compared to egg white, recorded lower values for foaming properties. Significant decreases ($p<0.05$) in emulsifying properties were also recorded after irradiation, with the 2.50 kGy sample recording the highest values of 45.83% and 73.33% for emulsifying activity and emulsion stability respectively. Pasting characteristics again increased significantly ($p<0.05$) with increasing starch:protein ratios. Of the three admixtures, the 70P:30S blends recorded the highest gelatinization temperatures. Blends constituted of 30% proteins and 70% starch attained the highest values for peak viscosity at all levels of irradiation. Pasting viscosities decreased with decreasing starch concentrations within blends, whereas breakdown viscosities were higher in the 50P:50S blends with all five levels of irradiated proteins compared with the other blends. Finally, setback viscosity was highest for the 30P:70S blends. Conclusively, correlation studies showed that the pasting

properties were solely dependent on the starch concentration within the blends, indicating the insignificant contribution of modified Bambara groundnut proteins to the pasting properties.

REFERENCES

Abu, J.O., K. Muller, K.G. Duodu and A. Minnaar, 2005. Functional properties of cowpea (*Vigna unguiculata* L. Walp) flours and pastes as affected by gamma irradiation. *Journal of Food Chemistry*, 93, 103-111.

AOAC, 1990. Official Methods of Analysis, 15th Edn., Arlington: Association of Official Analytical Chemists-Washington DC.

Athawale, V.D. and V.D. Lele, 2001. Recent trends in hydrogels based on starch-graft-acrylic acid: A Review. Starch/Staerke J., 53: 7-13.

Demiate, I.M., M. Oetterer and G. Wosiacki, 2001.Characterization of chestnut (castenea sativa, Mill) starch for Industrial Utilization. Brazilian Arch. Biol. Technol., Vol. 44.

Fanta, G.F. and W.M. Doane, 1986. Grafted starches. In O.B. Wurzburg (Ed.), Modified starches: Properties and uses (pp: 149-196). Boca Raton, FL: CRC Press.

Friberg, S., 1976. Food Emulsions, Marcel Dekker, Inc., New York.

Kiatkamjornwong, S., W. Chomsaksakul and M. Sonsuk, 2000. Radiation modification of water absorption of cassava starch by acrylic acid/acrylamide. *Radiation Physics and Chemistry*, 59, 413-427.

Lawhon, J.T., C.M. Gater and K.F. Mattil, 1972. Whippable extract from glandless cottonseed flour. *Journal of Food Science*, 37, 317-321.

Privalov, P.L., 1979. Stability of proteins, in Advances in Protein Chemistry, Vol. 33, Anfinsen, C.B., J.T. Edsall and F.M. Richards (Eds.), Academic Press, New York, pp: 167-241.

Sandhu, K.S. and N. Singh, 2007. Some properties of corn starches II: Physicochemical, gelatinization, retrogradation, pasting and gel textural properties. *Journal of Food Chemistry*, 101, 1499-1507.

Suliman, M.A., A.H. El Tinay and E.O. Elkhalita, 2006. Solubility as influenced by pH and NaCl concentrations and functional properties of Lentil protein isolates. *Pakistani Journal of Nutrition*, 5, 89-593.

Volker, M.A. and B.P. Kelin, 1979. Protein dispersability and emulsion characteristics of flour soy products.

Journal of Food Science, 44, 43-96.

Wang, J.C. and J.E. Kinsella, 1976. Functional properties of novel proteins (Alfalf leaf protein). *Journal of Food Science*, 41, 286-292.