Modification of the functional properties of hard-to-cook cowpea seed flours and cooked prepared pastes by γ-irradiation

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Abstract

Cowpeas are an inexpensive source of quality protein but their utilisation is limited by long seed cooking time. This is exacerbated by development of the hard-to-cook (HTC) defect, which also adversely affects starch and protein functionality. Gamma-irradiation can eliminate cowpea seed insect infestation and affects seed functional properties, including reducing cooking time. Hence, the potential of γ-irradiation to modify the starch- and protein-related functionalities of HTC cowpeas was investigated. Gamma-irradiation at approximately 11 kGy was applied to the seeds of two cowpea varieties, differing in HTC susceptibility, where HTC had been induced by high-temperature, high-humidity (HTHH) storage. HTHH storage increased flour pasting peak viscosity by up to 40% in the less susceptible variety and by more than 100% in the more susceptible variety. Gamma-irradiation at least completely reversed this effect, due to starch depolymerisation and debranching. Gamma-irradiation also positively impacted on some protein-related properties adversely affected by HTC; partially reversing the reduction in flour and cooked paste nitrogen solubility index of the HTC-susceptible cowpea, as a result of protein depolymerisation. The multiple benefits of γ-irradiation: disinfection, cooking time reduction and reversing some adverse effects of HTC on functional properties could make it a viable process for improving HTC cowpea quality.

Keywords Cowpeas; Gamma-irradiation; Hard-to-cook defect; Pasting properties; Nitrogen solubility index

Abbreviations

BV Breakdown viscosity

HTC Hard-to-cook

HTHH High temperature, high humidity

NSI Nitrogen solubility index

PV Pasting viscosity

SP Swelling power

SV Setback viscosity

WAC Water absorption capacity

WSI Water solubility index

Introduction

Cowpeas are an important food grain legume in arid regions of sub-Saharan Africa, South America and south Asia as they provide a relatively inexpensive source of high quality protein (Kyei-Boahen et al., 2017). However, cowpea utilisation is limited by its long cooking time, which can be greatly exacerbated by the development of the hard-to-cook (HTC) defect.

The HTC defect occurs in legume seeds that have been stored at high temperature (>25ºC) and high relative humidity (>65%) (HTHH conditions) (Liu et al., 1992). Two main hypotheses have been postulated to explain HTC development: the phytase-phytate-pectin hypothesis (Galiotou-Panayotou et al., 2008) and the lignification theory (Hinks and Stanley, 1987). However, also of relevance is the intracellular proteins and starch hypothesis, based on research using cowpeas (Liu et al., 1992; Lui, 1997). This proposes that under HTHH storage, changes occur to the cotyledon storage proteins. These changes result in the proteins denaturing at a low temperature, around 56ºC, prior to starch gelatinisation when the seeds are cooked. Consequently, starch granule water absorption and swelling are restricted. A similar effect of legume cotyledon protein denaturation limiting starch granule hydration has been observed when Bambara groundnuts were subjected to soaking and micronization (infrared heat treatment) (Ogundele et al., 2017).

The starch in cooked cowpeas seeds and their pastes is critically important as it is primarily responsible for product texture (Mangwela et al., 2007; Falade and Kolawole, 2013). In HTC cowpeas, starch granule expansion is limited by poor water uptake by the seeds (Jombo et al., 2018). Furthermore, there is some evidence that the granules exhibit differences from those in non-HTC legume seeds. Starch granules from HTC common bean have been found to have much stronger birefringence than those from non-HTC beans, indicating a higher degree of crystallinity (Garcia and Lajolo, 1994). Additionally, these authors observed that the gelatinisation temperature of the starch increased by 6.9°C in beans that had been stored under HTHH conditions for 5 years. More recently, starch from HTC common beans was observed to have a higher proportion of small size granules (Parmar et al., 2017).

Gamma-irradiation has been widely studied as a means of seed insect disinfestation. With cowpeas, Darfour et al. (2012a) obtained promising disinfestation effects by γ-irradiation of cowpeas infected with the cowpea weevil (*Callosobruchus maculates* F.). However, γ-irradiation has been found to depolymerise the starch in legumes (Rombo et al., 2004; Chung and Liu, 2010). Hence, its effects on the functional properties of cowpea without the HTC defect have been investigated in some detail. Gamma-irradiation has been shown to reduce flour pasting peak trough and final viscosities (Abu et al., 2005; Abu and Minnaar, 2009; Darfour et al., 2012b; Falade and Kolawole, 2013). It also has been shown to have some clear beneficial effects on cowpea functionality. In cowpeas without the HTC defect, γ-irradiation at doses of 2 and 10 kGy has been found significantly reduce seed cooking time, possibly through irradiation degradation of starch and pectic substances (Abu and Minnaar, 2009). The effect of γ-irradiation on HTC cowpeas has been much less studied. Gamma-irradiation of HTC susceptible and HTC less-susceptible cowpeas at 11 kGy reduced cotyledon cell wall thickness and increased pectin solubility in both types (Jombo et al., 2018). It also increased starch granule size and importantly reduced cooking time in the less susceptible variety. Therefore, the potential of γ-irradiation to improve the flour and paste related functionalities of cowpeas exhibiting the HTC defect was investigated in this study.

Materials and methods

Cowpeas

Two cowpea varieties were studied that had been found to differ in susceptibility to the HTC defect: Bechuana White (less susceptible) and Agrigold (more susceptible) (Jombo et al., 2018). After harvesting, the seeds were stored at 8°C and 61% Relative Humidity (RH) prior to the research study.

HTC defect induction by accelerated HTHH storage

This was performed as described (Jombo et al., 2018). In brief, the HTC defect was effectively induced by storing the cowpea seeds at 40°C and 80% RH (HTHH conditions) in airtight containers for 20 and 40 days. HTHH storage for 20 and 40 days increased the cooking time of the Bechuana White cowpeas to 84 and 234 min, respectively, compared to the non-HTC induced control's cooking time of 54 min. For Agrigold cowpeas, HTC induction increased their cooking time to >540 min compared to the control's cooking time of 92 min.

Gamma-irradiation

The HTHH stored and control stored cowpea seeds were vacuum sealed in low-density polyethylene bags, which were packed in cardboard boxes and then subjected to γ-irradiation at a temperature of 22^{o}C. The dose was on average 11.2 kGy, achieved at a dose rate of 1.7 kGy/h. This irradiation dose was used as it had been found to exert measurable physicochemical changes in HTC cowpea seeds and reduced their cooking time to some extent (Jombo et al., 2018).

Preparation of cowpea flours and cooked pastes

After HTHH storage followed by γ-irradiation, the cowpea seeds were manually dehulled and either dry milled to a flour maximum particle size of 500 µm, as described (Jombo et al., 2018) or prepared as a cooked moinmoin-type paste, essentially as described by Olopade et al. (2003). Cooked pastes were obtained by first soaking the dehulled cowpea seeds in distilled water (22ºC) for 3 h at a ratio of 1:3 (m:v), followed by cooking at 95ºC for 2 h in the water. The drained cooked dehulled cowpeas were then formed into a paste using a pestle and mortar. The paste was freeze dried, then milled to a maximum particle size of 500 µm prior to analyses.

Moisture determination

Seed and flour moisture contents were determined by AACC air-oven method 44-15A (American Association of Cereal Chemists, 2000).

Pasting properties

Pasting was performed as described by Abu et al., 2005) with slight modification. Pasting properties of the dehulled cowpea seed flours and cooked freeze-dried paste flours were determined using an Anton Paar Rheometer (Physica MCR 101, Ostildern, Germany). Flour (approx. 1.7 g db) weighed accurately was suspended in distilled water and adjusted to a total mass of 17 g. Stirring was at 960 rpm at 50°C for 30 s. and then at 160 rpm for the rest of the cycle. The suspension was heated from 50°C to 91°C (heating rate of 5.5°C/min) and held at this temperature for 15 min before cooling to 50°C (cooling rate of 5.5°C/min). The parameters recorded were the peak viscosity (PV), breakdown viscosity (BV) and setback viscosity (SV).

Water absorption capacity (WAC)

WAC was determined according to AACC method 56-20 (American Association of Cereal Chemists, 2000) with slight modifications. Raw cowpea flours or cooked freeze dried pastes (1 g) (M0) were accurately weighed and dispersed in 20 mL deionised water at 22°C and vortexed for 10 min. The suspensions were then centrifuged (1000 *g*, for 15 min at 20°C) and the supernatant decanted. The centrifuge tubes were then inverted for 5 min on a paper towel and residue weighed (M1). WAC was calculated as M0 minus M1/M0 and expressed as a percentage.

Water Solubility Index (WSI) and Swelling Power (SP)

WSI and SP were determined essentially as described (Ocloo et al, 2014). Flour (125 mg db) was heated in 20 mL distilled water at 95°C for 30 min in shaking water bath. The samples were then cooled and centrifuged at ambient temp. The clear supernatant was decanted and evaporated in a forced draught air-oven at 105°C for 16 h. WSI was determined as the mass ratio of the dried supernatant flour weight and expressed as a percentage. The residue obtained after centrifugation was then weighed to obtain the SP, which was calculated as mass ratio of the final residue to the initial dry sample weight.

Nitrogen Solubility Index (NSI)

NSI was determined according to AACC Method 46-23 (American Association of Cereal Chemists, 2000) with modification. Cowpea flour and freeze dried paste (1 g) were dispersed in 20 mL 0.1 M NaCl and stirred continuously for 1 h at 30°C. The suspension was centrifuged (7000 *g*, for 15 min, at 4°C) and the supernatant filtered through Whatman No. 1 filter paper. The residue from the suspension was re-washed twice in 10 mL 0.1 M NaCl. The total filtrate was frozen and then freeze dried. Its nitrogen content was determined by Dumas combustion analysis, AACC Method 46-23 (American Association of Cereal Chemists, 2000). NSI was expressed as a percentage of the total nitrogen content the flour (dry basis).

Thermal properties

Cowpea seed thermal properties were determined using a Metler Toledo HPDSC-827 differential scanning calorimeter (Schwerzenback, Switzerland). Cowpea flour (10 mg) was accurately weighed into a 100 µL aluminium sample pan, and 30 mg distilled water added. The pans were hermetically sealed and the samples were equilibrated at 22°C. The instrument was calibrated using indium, and an empty aluminium pan was used as reference. Thermal properties were measured using a heating rate of 10ºC min^{-1} over 30-120ºC. The following parameters were determined: The melting enthalpy (ΔH J/g) peak onset (T_o), peak (T_p) and peak end (T_c) temperatures.

Statistical analyses

All experiments were repeated. The effects of irradiation on thermal and functional properties were analysed by both one-way and multifactor analysis of variance (MANOVA) with the means separated using Tukey's honest significant difference (HSD) test.

Results and discussion

Effects on pasting properties

MANOVA revealed that cowpea variety, HTHH storage and γ-irradiation all had highly significant effects ($P \leq 0.001$) on the pasting peak viscosity of the dehulled cowpea seed flours (Table 1). HTHH storage significantly ($P \leq 0.05$) increased the peak viscosity of both varieties, with Bechuana White the less HTC susceptible variety by 14.8% and 40.7% after 20 and 40 days, respectively. With the more HTC susceptible Agrigold, peak viscosity increased by 101.1% and 108.1% after 20 and 40 days, respectively, i.e. a much greater increase with HTHH storage. This finding is broadly in agreement with Parmar et al. (2017) that harder-to-cook kidney (common) beans had a higher pasting peak viscosity than easier-to-cook varieties. In general terms, pasting peak viscosity is indicative of starch granule water binding (Shimelis et al., 2006). The increase in peak viscosity during storage was probably due to interactions of water with calcium ion stabilised pectin and starch, which can result in gel formation (Fu et al., 2001). Our previous work on these two cowpea varieties suggested the specific involvement of alkali-soluble, ester bonded pectins in the HTC defect (Jombo et al., 2018).

Gamma-irradiation reduced the peak viscosity of the Agrigold and Bechuana White dehulled seed flours by 70.3% and 53.9% and by 62.2% and 64.8%, after 20 and 40 days of HTHH storage, respectively (Table 1). The magnitude of reduction was such that the peak viscosity of Agrigold 40 days HTHH stored was only 698 mPa.s, similar to that of the Agrigold and Bechuana White controls (not HTHH stored nor irradiated treatments) and that of the Bechuana White 40 days HTHH stored was 325 mPa.s, half that of its control. In fact, γ-irradiation generally more than completely reversed the increase in peak pasting peak

Table 1 Effects of HTHH storage at 40ºC and 80% RH and γ-irradiation on the peak, breakdown and setback viscosities of dehulled Bechuana White and Agrigold cowpea flours and their cooked prepared pastes

Cowpea variety and form[1]	Storage Time (days)	Peak Viscosity (PV) (mPa.s)					Breakdown Viscosity (BV) (mPa.s)					Setback Viscosity (SV) (mPa.s)				
		Irradiation dose (kGy)					Irradiation dose (kGy)					Irradiation dose (kGy)				
		0	% Change due to HTHH storage	11	% Change due to HTHH storage	% Change due to Irradiation	0	% Change due to HTHH storage	11	% Change due to HTHH storage	% Change due to Irradiation	0	% Change due to HTHH storage	11	% Change due to HTHH storage	% Change due to Irradiation
Bechuana White (HTC defect less susceptible) (Flour)	0	655.5^{b} (3.5)[2]		210.0^{a} (8.5)		-68.0	31.5^{a} (7.8)		40.5^{a} (7.8)		+28.6	492.0^{b} (33.9)		152.0^{a} (26.9)		-69.1
	20	752.5^{b} (78.5)	+14.8	284.5^{a} (24.7)	+35.5	-62.2	91.5^{a} (77.1)	+190.4	82.0^{a} (21.2)	+102.7	-10.4	529.5^{b} (55.9)	+7.6	112.0^{a} (90.5)	-26.3	-78.8
	40	922.5^{c} (37.4)	+40.7	325.0^{a} (26.2)	+54.8	-64.8	169.5^{a} (58.7)	+438.1	49.5^{a} (2.1)	+22.2	-70.8	548.0^{b} (53.7)	+11.4	181.0^{a} (5.6)	+19.1	-67.0
Agrigold (HTC defect more susceptible) (Flour)	0	728.0^{b} (50.9)		381.5^{a} (88.4)		-47.6	86.5^{a} (14.8)		139.5^{a} (57.3)		+61.3	310.0^{b} (63.6)		120.0^{a} (2.7)		-61.3
	20	1464.5^{c} (2.1)	+101.1	434.5^{a} (34.6)	+13.9	-70.3	840.0^{c} (12.7)	+871.1	198.5^{a} (27.6)	+42.3	-76.3	341.0^{b} (5.7)	+10	164.0^{a} (4.2)	+36.7	-51.9
	40	1515.0^{c} (60.8)	+108.1	698.0^{b} (17.0)	+83.0	-53.9	858.0^{c} (19.8)	+891.9	502.0^{b} (10.0)	+259.9	-41.5	327.5^{b} (9.2)	+5.6	145.0^{a} (0.0)	+20.8	-55.7
Bechuana White (HTC defect less susceptible) (Paste)	0	16.5^{a} (2.1)		17.5^{a} (3.5)		+6.1	5.0^{a} (1.4)		5.5^{a} (3.5)		+10	3.0^{a} (0.0)		2.0^{a} (0.0)		-33.3
	20	14.5^{a} (2.1)	-12.1	15.5^{a} (3.5)	-11.4	+6.9	4.5^{a} (2.1)	-10	4.5^{a} (2.1)	-18.2	0	3.0^{a} (0.0)	0	1.5^{a} (0.7)	-25.0	-50.0
	40	18.0^{a} (4.2)	+9.1	17.5^{a} (3.5)	0	-2.8	7.0^{a} (4.2)	+40	7.0^{a} (1.4)	+27.3	0	2.5^{a} (0.7)	-16.7	1.5^{a} (0.7)	-25.0	-40.0
Agrigold (HTC defect more susceptible) (Paste)	0	23.5^{a} (7.8)		25.0^{a} (2.8)		+6.4	10.0^{a} (8.4)		14.0^{a} (1.4)		+40.0	3.5^{a} (0.7)		2.5^{a} (2.1)		-28.5
	20	20.0^{a} (2.8)	-14.9	13.0^{a} (1.4)	-48.0	-35.0	7.0^{a} (2.8)	-30	3.0^{a} (0.0)	-78.6	-57.1	1.5^{a} (0.7)	-57.1	1.5^{a} (0.7)	-40.0	0
	40	25.0^{a} (2.8)	+6.4	13.5^{a} (0.7)	-46.0	-46.0	10.5^{a} (3.5)	+5	4.0^{a} (1.4)	-71.4	-61.9	2.5^{a} (0.7)	-28.6	2.2^{a} (0.0)	-12.0	-9.2

Table 1 continued

MANOVA Flours main treatment effects						
	Peak Viscosity (PV)		Breakdown Viscosity (BV)		Setback Viscosity (SV)	
Treatment	**Degrees of freedom**	***P* value**	**Degrees of freedom**	***P* value**	**Degrees of freedom**	***P* value**
Variety	1	0.000	1	0.000	1	0.000
HTHH storage days	1	0.000	1	0.341	1	0.341
Irradiation	1	0.000	1	0.000	1	0.000
MANOVA Pastes main treatment effects						
Treatment	**Degrees of freedom**	***P* value**	**Degrees of freedom**	***P* value**	**Degrees of freedom**	***P* value**
Variety	1	0.036	1	0.098	1	1.000
HTHH storage days	1	0.053	1	0.114	1	0.129
Irradiation	1	0.100	1	0.487	1	0.028

[1] For cowpea seeds or paste, for each parameter, means followed by different letters in each block are significantly different at $P \leq 0.05$.
[2] Means ± standard deviations (in brackets) of two independent experiments.

viscosity induced by HTHH storage. These effects of γ-irradiation are attributable to hydrolysis of internal glucosidic bonds in amylopectin to form shorter molecular units (Rombo et al., 2004; Cieśla and Eliasson (2007), which have a lower hydrodynamic volume and hence low paste viscosity.

MANOVA revealed that cowpea variety, HTHH storage and γ-irradiation also all had highly significant effects ($P \leq 0.001$) on the breakdown viscosity of the cowpea seed flours. With HTHH stored Agrigold there was a greater reduction in paste viscosity on holding at 91^{o}C (sheer thinning) compared to Bechuana White (Figure 1). Hence, Agrigold had a higher breakdown viscosity, particularly after HTHH storage (Table 1). Breakdown viscosity is inversely related to the stability of a starch paste (Moorthy, 1985) and primarily due to starch granule disruption (Whistler, and BeMiller, 1997). It would therefore appear that the calcium ion, pectin and starch interactions in Agrigold highlighted earlier, were not stable on disruption of the starch granules under shear, resulting in the observed great reduction in paste viscosity. The breakdown viscosity of the γ-irradiated HTHH stored Agrigold was significantly ($P \leq 0.05$) reduced. In other words, the pastes were relatively more stable on holding (less susceptible to sheer thinning) compared to the not irradiated controls. This effect was presumably a reflection of the great reduction in pasting peak viscosity brought about by γ-irradiation.

MANOVA showed that only variety and γ-irradiation had a significant effect ($P \leq 0.001$) on the setback viscosity of the cowpea seed flours. Agrigold, the more HTC susceptible variety, had a substantially lower setback than Bechuana White (Table 1 and Figure 1). Setback is generally regarded as a measure of the retrogradation tendency of starch (Shelton and Lee,

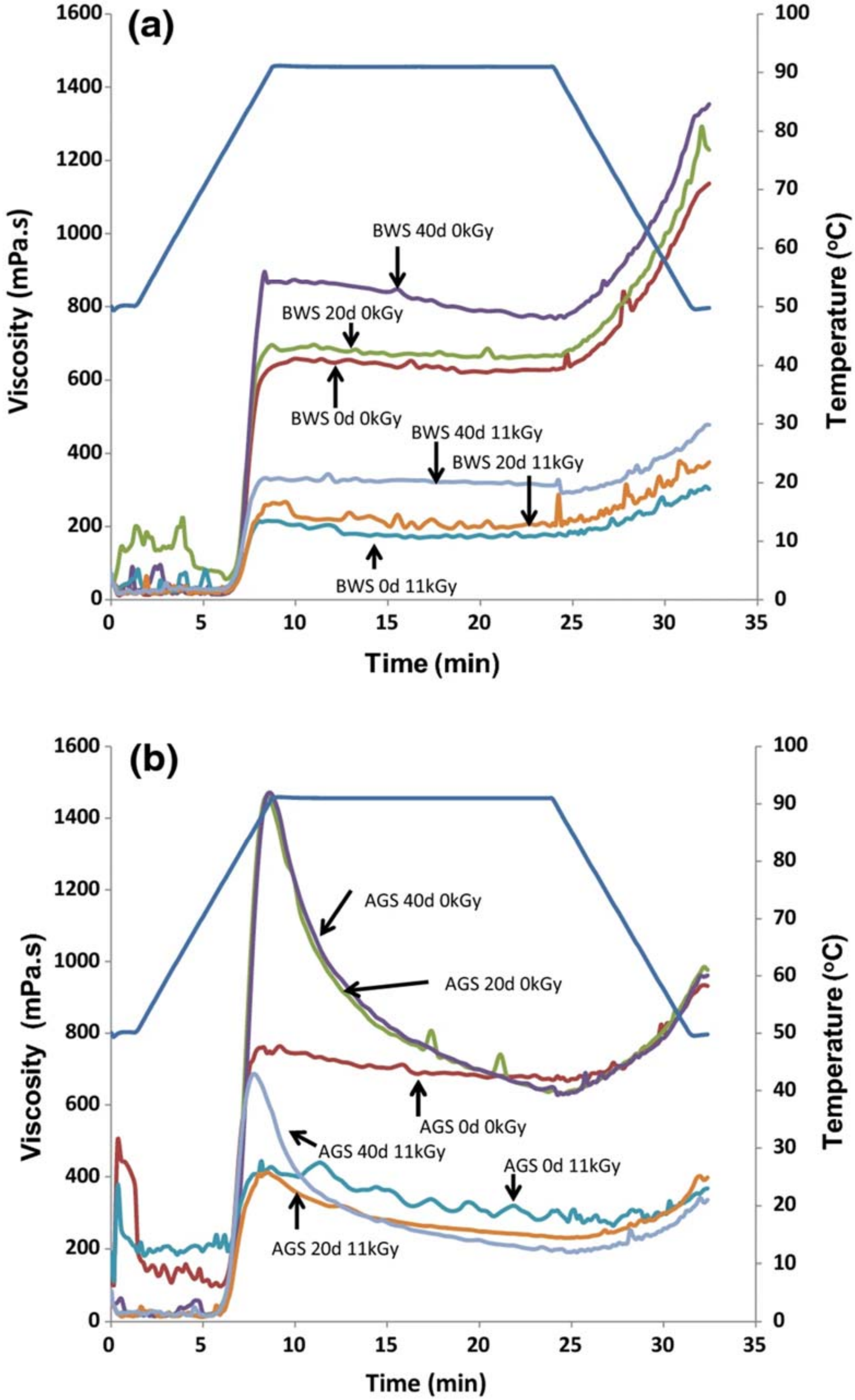

Figure 1. Modification of the functional properties of hard-to-cook cowpea seed flours and cooked prepared pastes by γ-irradiation.

Table 2 Effects of HTHH storage days at 40°C and 80% RH and γ-irradiation on the nitrogen solubility index of dehulled Bechuana White and Agrigold cowpea flours and their cooked prepared pastes

Cowpea variety and form[1]	Storage time (days)	Nitrogen Solubility Index (NSI)				
		Irradiation dose (kGy) 0		11		
			% Change due to HTHH storage		% Change due to HTHH storage	% Change due to Irradiation
Bechuana White (HTC less susceptible) (Flour)	0	48.3^{c} (0.6)[2]		37.2^{b} (2.5)		-23.0
	20	39.4^{b} (3.6)	-18.4	34.2^{ab} (0.1)	-8.1	-13.2
	40	33.8^{ab} (2.5)	-30.0	28.0^{a} (0.7)	-24.7	-17.1
Agrigold (HTC defect more susceptible) (Flour)	0	43.4^{c} (3.9)		37.2^{bc} (3.4)		-14.3
	20	15.8^{a} (0.3)	-63.6	30.1^{b} (0.4)	-19.1	+90.5
	40	15.0^{a} (1.6)	-65.4	13.0^{a} (0.7)	-65.1	-13.3
Bechuana White (HTC defect less susceptible) (Paste)	0	13.6^{a} (3.9)		9.9^{a} (0.2)		-27.2
	20	11.0^{a} (1.1)	-19.1	8.9^{a} (2.2)	-10.1	-19.1
	40	10.8^{a} (1.3)	-20.6	7.5^{a} (0.1)	-24.2	-30.1
Agrigold (HTC defect more susceptible) (Paste)	0	12.1^{cd} (0.0)		10.7^{cd} (0.2)		-11.6
	20	8.0^{b} (0.2)	-33.9	12.7^{d} (0.0)	+18.7	+58.8
	40	5.8^{a} (0.1)	-52.1	10.1^{c} (1.2)	-5.6	+74.1

MANOVA Flours main treatment effects

Treatment	Degrees of freedom	*P* value
Variety	1	0.000
HTHH storage days	1	0.000
Irradiation	1	0.011

MANOVA Pastes main treatment effects

Treatment	Degrees of freedom	*P* value
Variety	1	0.555
HTHH storage days	1	0.005
Irradiation	1	0.705

[1]For cowpea flours or pastes, means followed by different letters in a block across are significantly different at $P \leq 0.05$.

[2] Means ± standard deviations (in brackets) of two independent experiments.

($P \leq 0.05$) all had significant effects on the NSI of the cowpea flours (Table 2). HTHH storage generally significantly reduced ($P \leq 0.05$) the NSI of both the Bechuana White, and Agrigold non-irradiated flours and their cooked prepared pastes, the exception being Bechuana White dehulled cooked cowpea pastes. The reduction in NSI was generally much greater with the Agrigold, the more HTC susceptible variety. These results are broadly in agreement with the findings of Lui et al. (1992). These authors observed dramatic decreases in protein extractability in water and protein thermal transition temperature with HTHH storage of cowpeas. As stated, it has been proposed that cotyledon protein denaturation is partly responsible for the HTC defect by limiting starch gelatinisation (granule water uptake) (Lui, 1997). In this present work, it is significant that the NSIs of all the pastes were far lower than their corresponding flours, also illustrating the effect of protein thermal denaturation. Furthermore, HTHH storage still significantly reduced ($P \leq 0.05$) the NSI of the pastes made from Agrigold, but not of Bechuana White, the less HTC susceptible variety; indicating that the protein in Agrigold had been more denatured. Also notable was that γ-irradiation in combination with HTHH storage generally caused a significant ($P \leq 0.05$) increase in NSI with Agrigold flours and pastes but not with Bechuana White. This increase was probably caused by γ-irradiation induced depolymerisation (Kuan et al., 2013) and hence partial solubilisation of the cowpea storage proteinsthat had been denatured by HTHH storage (Lui et al., 1992; Lui, 1997).

Effects on WAC, SP and WSI

Overall, cowpea variety, HTHH storage and γ-irradiation all had significant effects ($P \leq 0.001$) on raw cowpea flour WAC (Table 3). HTHH storage significantly increased ($P \leq 0.05$) the WAC of the more HTC susceptible Agrigold, by 47.8% and 73.1% after 20 and 40 days

Table3 Effects of HTHH storage at 40ºC and 80% RH and γ-irradiation on the water absorption capacity, swelling power and water solubility index of Bechuana White and Agrigold cowpea flours and their cooked prepared pastes

Cowpea variety and form[1]	Storage time (days)	Water absorption capacity (WAC) (g/100)					Swelling power (SP) (g/g)					Water solubility index (WSI) (g/100 g)				
		Irradiation dose (kGy)					Irradiation dose (kGy)					Irradiation dose (kGy)				
		0		11			0		11			0		11		
			% Change due to HTHH storage		% Change due to HTHH storage	% Change due to Irradiation		% Change due to HTHH storage		% Change due to HTHH storage	% Change due to Irradiation		% Change due to HTHH storage		% Change due to HTHH storage	% Change due to Irradiation
Bechuana White (HTC defect less susceptible) (Flour)	0	86.1^{a} (1.5)[2]		85.4^{a} (2.8)		-0.8	8.3^{bc} (0.2)		7.1^{a} (0.1)		-14.5	71.2^{ab} (6.5)		61.1^{a} (1.0)		-14.2
	20	91.7^{ab} (3.6)	+6.5	100.3^{bc} (3.4)	+17.4	+9.4	7.8^{ab} (0.6)	-6	6.8^{a} (0.2)	-4.2	-12.8	76.2^{b} (2.0)	+7	67.2^{ab} (1.8)	+10.0	-11.8
	40	92.2^{ab} (2.0)	+7.1	108.1^{c} (1.3)	+26.6	+17.2	9.3^{c} (0.2)	+12	6.7^{a} (0.0)	-5.6	-39.8	72.1^{ab} (2.4)	+1.3	69.5^{ab} (1.6)	+13.7	-2.3
Agrigold (HTC defect more susceptible) (Flour)	0	84.7^{a} (0.8)		97.4^{b} (1.6)		+15.0	8.1^{bc} (0.1)		6.6^{a} (0.1)		-18.5	74.2^{a} (3.4)		63.6^{a} (2.7)		-14.3
	20	125.2^{d} (3.6)	+47.8	112.1^{c} (0.1)	+15.1	-10.5	8.9^{c} (0.2)	+9.9	7.0^{ab} (0.3)	+6.1	-21.3	76.4^{a} (0.3)	+3	64.6^{a} (4.3)	+1.6	-15.4
	40	146.6^{e} (0.9)	+73.1	149.7^{e} (4.2)	+53.7	+2.1	9.1^{c} (0.6)	+12.3	7.2^{ab} (0.0)	+9.1	-20.9	78.6^{a} (8.2)	+5.9	67.6^{a} (2.0)	+6.3	-14.0
Bechuana White (HTC defect less susceptible) (Paste)	0	153.0^{ab} (6.0)		166.9^{b} (6.8)		+9.1	5.3^{a} (0.0)		5.5^{a} (0.3)		+3.8	84.2^{a} (1.4)		85.4^{a} (1.2)		+1.4
	20	148.5^{ab} (2.9)	-2.9	154.2^{ab} (10.7)	-7.6	+3.8	5.5^{a} (0.6)	+3.8	5.2^{a} (0.0)	-5.5	-5.5	87.0^{a} (0.9)	+3.3	84.2^{a} (0.3)	-1.4	-3.2
	40	140.0^{a} (1.8)	-8.5	138.9^{a} (7.4)	-16.8	-0.8	5.2^{a} (0.2)	-1.9	5.1^{a} (0.2)	-7.3	-1.9	86.7^{a} (1.4)	-3	87.0^{a} (1.4)	+1.9	+0.3
Agrigold (HTC defect less susceptible) (Paste)	0	160.0^{cd} (8.5)		163.9^{d} (1.9)		+2.4	5.8^{b} (0.2)		5.4^{ab} (0.0)		-6.9	85.7^{d} (0.7)		84.7^{a} (0.4)		-1.2
	20	129.3^{b} (0.1)	-19.2	145.4^{c} (1.7)	-11.3	+12.5	4.7^{a} (0.3)	-19.0	4.9^{a} (0.1)	-9.2	+4.3	84.3^{a} (4.4)	-1.6	80.3^{a} (0.1)	-5.2	-4.7
	40	111.5^{a} (0.5)	-30.3	164.9^{d} (0.5)	+0.6	+47.9	4.6^{a} (0.3)	-20.7	5.2^{ab} (0.2)	-3.7	+13.0	86.3^{a} (2.5)	+0.6	83.6^{a} (4.6)	-1.3	-3.1

Table 3 continued

MANOVA Flours main treatment effects						
	Water absorption capacity (WAC)		**Swelling power (SP)**		**Water solubility index (WSI)**	
Treatment	**Degrees of freedom**	***P* value**	**Degrees of freedom**	***P* value**	**Degrees of freedom**	***P* value**
Variety	1	0.000	1	0.274	1	0.415
HTHH storage days	1	0.000	1	0.007	1	0.081
Irradiation	1	0.001	1	0.000	1	0.000
MANOVA Pastes main treatment effects						
Treatment	**Degrees of freedom**	***P* value**	**Degrees of freedom**	***P* value**	**Degrees of freedom**	***P* value**
Variety	1	0.065	1	0.027	1	0.088
HTHH storage days	1	0.000	1	0.001	1	0.241
Irradiation	1	0.000	1	0.805	1	0.105

[1] For cowpea flours or pastes, for each parameter, means followed by different letters in a block are significantly different at $P \leq 0.05$.

[2] Means ± standard deviations (in brackets) of two independent experiments.

HTHH storage, respectively. In contrast, HTHH storage had no significant effect (P>0.05) on the WAC of Bechuana White. WAC represents the ability of a substance to associate with water in restricted conditions (Singh, 2001). The higher WAC of Agrigold is in agreement with its much higher pasting peak viscosity (Table 1). The increase in WAC is also likely to be due to $Ca^{2+}$and pectin interaction (Galiotou-Panayotou et al., 2008), as the starch was not gelatinised. Gamma-irradiation did not have a large effect on the WAC of either variety, unlike the dramatic reduction in pasting viscosity that occurred with γ-irradiation (Table 1), probably because the starch was not gelatinised.

With the cooked prepared pastes, overall HTHH storage and γ-irradiation had highly significant effects (P≤0.001) on WAC, but not variety (Table 3). The WAC of the non-irradiated pastes were much higher than those of the flours, approximately double, as a result of the starch being gelatinised. Generally, there was a small reduction in WAC with HTHH storage. Since the NSI of the cooked pastes were far lower than those of raw flours (Table 2), this reduction was presumably due to protein denaturation (Lui et al., 1992; Lui, 1997; Ogundele et al., 2017). Protein denaturation could have affected the WAC of the pastes because protein also plays a role in water absorption in legumes (Du et al., 2014). Gamma-irradiation significantly increased (P≤0.05) the WAC of Agrigold pastes after HTHH storage but had no significant effect (P>0.05) on the WAC of Bechuana White pastes (Table 3). This is in general agreement with the large increase in NSI observed with Agrigold and the smaller decrease with Bechuana White, indicating that the changes in WAC were as a result of effects of γ-irradiation on the protein, probably depolymerisation, as described.

Overall, HTHH storage and γ-irradiation both had significant effects ($P \leq 0.01$) on the SP of the flours, but not variety (Table 3). The effects of HTHH storage on SP were small with generally a slight increase with storage time. However, the SP of the flours of both varieties were significantly reduced by γ-irradiation ($P \leq 0.05$), essentially eliminating the effect of HTHH storage. SP indicates the ability of starch to hydrate and swell (Kaur et al., 2007). Since flour swelling behaviour is attributed to the amylopectin fraction (Tester and Morrison, 1990), the reduction in SP was probably due to depolymerisation and debranching of amylopectin, as described above. In contrast, overall, variety and HTHH storage but not γ-irradiation significantly affected ($P \leq 0.05$) the SP of the cooked prepared pastes. However, the effects of variety and HTHH storage were only small. Of more significance was that the cooked pastes had substantially lower SP than the raw flours, for example a mean of 32% lower for both varieties not stored nor irradiated. The low SP was presumably due to thermal denaturation of the storage proteins, as described.

Overall, only γ-irradiation significantly affected ($P \leq 0.001$) the WSI of the flours (Table 3). WSI reflects the amount of soluble solids and is generally used to indicate starch hydrolysis and dextrinisation (Dogan and Karwe, 2003). There was a small but consistent decrease in WSI with γ-irradiation. Overall, neither variety, nor HTHH storage, nor γ-irradiation affected the WSI of the cooked pastes ($P > 0.05$).

Effects on thermal properties

Overall, variety had a significant effect ($P \leq 0.01$) on the thermal properties of the cowpea flours, but not HTHH storage, nor γ-irradiation (Table 4). There were no consistent effects of

Table 4 Effects of HTHH storage at 40°C and 80% RH and of γ-irradiation on the thermal properties of dehulled Bechuana White and Agrigold cowpea flours

Cowpea variety[1]	**Storage time (days)**	**Onset temp. T_o (°C)**					**Peak temp. T_p (°C)**					**Endset temp. T_c (°C)**					**ΔH (J/g)**				
		Irradiation dose (kGy)					**Irradiation dose (kGy)**					**Irradiation dose (kGy)**					**Irradiation dose (kGy)**				
		0		**11**			**0**		**11**			**0**		**11**			**0**		**11**		
			% Change due to HTHH storage		**% Change due to HTHH storage**	**% Change due to Irradiation**		**% Change due to HTHH storage**		**% Change due to HTHH storage**	**% Change due to Irradiation**		**% Change due to HTHH storage**		**% Change due to HTHH storage**	**% Change due to Irradiation**		**% Change due to HTHH storage**		**% Change due to HTHH storage**	**% Change due to Irradiation**
Bechuana White (HTC defect less susceptible)	0	73.3^a (1.1)[2]		72.8^a (0.2)		-0.7	76.4^a (0.5)		76.6^a (0.4)		+0.3	78.6^a (0.6)		79.6^a (1.5)		+1.3	17.2^a (10.5)		29.5^a (2.0)		+71.5
	20	72.2^a (0.2)	-1.5	72.9^a (0.6)	+0.1	+1.0	77.4^a (1.2)	+1.3	76.5^a (0.0)	-0.1	-1.3	80.0^a (0.8)	+1.8	78.6^a (3.1)	-1.3	-1.8	26.5^a (10.7)	+54.1	23.3^a (9.9)	-21.0	-12.1
	40	73.3^a (1.6)	0	72.9^a (0.1)	+0.1	-0.8	77.9^a (1.4)	+4.6	76.4^a (0.1)	-0.2	-1.9	78.7^a (3.8)	+0.1	79.0^a (1.3)	-0.8	+0.4	29.6^a (8.5)	+72.1	22.3^a (9.5)	-33.9	+14.5
Agrigold (HTC defect more susceptible)	0	70.4^a (3.0)		70.6^a (2.5)		+0.4	75.8^a (2.4)		75.7^a (2.6)		-0.1	80.3^a (1.4)		79.8^a (1.9)		-0.6	9.6^a (7.7)		10.5^a (3.3)		+9.4
	20	68.2^a (0.6)	-3.1	70.7^a (0.5)	+0.1	+3.7	72.7^a (0.2)	-4.1	73.8^a (0.3)	-2.5	+1.5	77.4^a (2.3)	-3.6	77.0^a (1.0)	-3.5	-0.5	5.7^a (0.9)	-40.7	10.1^a (7.3)	-3.8	+94.7
	40	69.9^a (0.3)	-0.7	71.2^a (2.2)	+0.8	+1.9	74.1^a (1.3)	-2.2	74.4^a (2.6)	-1.7	+0.4	78.3^a (3.3)	-2.5	78.9^a (1.2)	-1.3	+0.8	8.6^a (9.8)	-10.4	7.3^a (1.2)	-30.5	-15.1

Table 4 continued

MANOVA Main treatment effects								
	Onset temp. T_o		Peak temp. T_p		Endset temp. T_c		ΔH	
Treatment	**Degrees of freedom**	***P* value**	**Degrees of freedom**	***P* value**	**Degrees of freedom**	***P* value**	**Degrees of freedom**	***P* value**
Variety	1	0.001	1	0.001	1	0.626	1	0.000
HTHH storage days	1	0.452	1	0.377	1	0.449	1	0.991
Irradiation	1	0.309	1	0.822	1	0.942	1	0.782

[1] For each parameter, means followed by different letters in each block are significantly different at $P \leq 0.05$.

[2] Means ± standard deviations (in brackets) of two independent experiments.

these factors on the thermal properties of the cooked prepared pastes (data not shown). With the flours, Agrigold, the more HTC susceptible variety, showed a lower onset (T_o) and peak (T_p) starch gelatinisation temperature than Bechuana White; means over the storage period of 69.5 and 74.2°C versus 72.9 and 77.2°C, respectively. Agrigold also had a much lower gelatinisation enthalpy (ΔH); mean over storage 8.0 J/g versus 24.4 J/g for Bechuana White. These data suggest that when the Agrigold seeds were milled into flour, denaturation of the storage proteins did not impact on starch gelatinisation or ΔH, presumably because the cotyledon cellular structure had been disrupted. They also confirm that the HTC character greatly affects the starch-related properties of cowpeas, as has been previously demonstrated in legumes (Paredes-López, 1988; Yousif et al., 2007).

Conclusions

Application of γ-irradiation at a dose of approx. 11 kGy to HTC susceptible and less susceptible cowpea seeds positively affects some flour starch-related properties that are adversely affected by the HTC defect. It dramatically reduces the pasting peak viscosities of their flours and at least reverses the considerable increase in peak viscosity caused by HTHH storage. Similarly, γ-irradiation reverses the small increase in flour swelling power caused by HTHH storage. Gamma-irradiation also positively affects some protein-related properties cowpeas that are adversely affected by the HTC defect. It increases the nitrogen solubility index of flours and cooked pastes of HTC-susceptible cowpea.

Hence, γ-irradiation of cowpea seeds has potential to not only to disinfest the seeds (Darfour et al., 2012a) and reduce cooking time (Abu and Minnaar, 2009; Jombo et al., 2018) but also to reverse some of the adverse effects of the HTC defect on cowpea functionality. These multiple beneficial effects of γ-irradiation could make it a viable process for improving the quality of cowpeas exhibiting the HTC defect.

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Compliance with ethical standards

Conflict of interest The authors report no conflicts of interest.

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