Effects of gamma-irradiation on cotyledon cell separation and pectin solubilisation in hard-to-cook cowpeas

Talknice Z Jombo[a], Amanda Minnaar[a], John RN Taylor[a]*

*Correspondence to: John RN Taylor, Institute for Food, Nutrition and Well-being and

[a]Department of Food Science, Private Bag X20, Hatfield 0028, South Africa,

email:john.taylor@up.ac.ac.za; Tel.: +27 124204296; Fax:+27 12 420 2839

Abstract

BACKGROUND: Cowpeas stored under high temperature and humidity develop the hard-to-cook defect (HTC). This defect greatly increases cooking times and energy costs. To better understand the mechanisms involved in the HTC defect development, the effects of γ-irradiation on cotyledon cellular structure and pectin solubility in two cowpea cultivars with different susceptibility to HTC defect were investigated.

RESULTS: Gamma-irradiation decreased cotyledon cell wall thickness, increased cell size intercellular spaces in both cowpea cultivars and reduced cooking time of the less HTC susceptible cultivar. However, it did not reverse the HTC defect in the susceptible cultivar. Gamma-irradiation also increased the levels of cold water- and hot water-soluble pectin. The irradiation effects were thus mainly due to hydrolysis of pectin fractions in the cell walls. However, chelator-soluble pectin (CSP) solubility was not affected.

CONCLUSION: As the cell wall changes brought about by γ-irradiation were associated with pectin solubilisation, this supports the phytate-phytase pectin theory as a major cause of the HTC defect. However, the non-reversal of the defect in HTC susceptible cowpeas and the absence of an effect on CSP indicate that other mechanisms are involved in HTC defect development in cowpeas, possibly the formation of alkali–soluble, ester bonded pectins.

Keywords: cotyledon cell wall; cowpea; gamma-irradiation; hard-to-cook defect; pectin

INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp) is one of the most important grain legumes (pulses) in Africa, Latin America and Asia in the fight against food insecurity and malnutrition, and in improving human health.[1] It offers a relatively cheap source of protein compared to animal protein, which can improve the diets of poor households.[2] However, its utilisation is limited by its long cooking time, which translates into high energy and time demands. In rural Africa, firewood is the most used source of energy, but its increasing use has a severe impact on the environment.[3] Importantly, cowpea utilisation is also specifically limited by the development of the hard-to-cook (HTC) defect, which further increases the cooking time of the seeds up to greater than 4 hours.[4] The HTC defect occurs in legumes which are stored under tropical conditions of high temperature (>25^{o}C) and high relative humidity (>65%),[5] referred to as high temperature, high humidity (HTHH) conditions.

Several theories have been proposed to explain the HTC defect: (1) the phytase-phytate-pectin theory;[6] (2) the lignification theory;[7] (3) protein and starch interactions[8] and (4) a multiple mechanism theory.[9] Despite much research, the mechanisms responsible have not been completely elucidated. The most widely accepted is the 'phytase-phytate-pectin' theory.[6] According to this theory, increased activity of phytase due to the HTHH storage conditions degrades phytate leading to release of divalent ions which crosslink pectin, rendering it insoluble which causes failure of cell separation during wet cooking.[10] With cowpea, this theory is supported by the fact that both cold water soluble pectin and hot water soluble pectin have been shown to decrease with increase in storage time under HTHH conditions.[4]

Pectin is one of the main components of the plant cell wall middle lamella., It is responsible for “cementing” adjacent cells together.[11] During the cooking of legumes, tissue softening occurs due to the disintegration of the middle lamella, which allows cell separation.[12] Ultrastructural and histochemical studies have revealed that the cotyledon cells in HTC seeds are poorly separated, showing failure of cell separation.[13] This restricted cell separation leads to extended cooking time of the seeds.[14]

Gamma-irradiation could be a valuable tool to help better understand the mechanisms responsible for the HTC defect. Gamma-irradiation hydrolyses polysaccharides such as starch, cellulose, and pectin via cleavage of glycosidic bonds.[15.16] This is important in legumes since, as indicated, the middle lamella which is critical for cell separation during cooking is pectin rich.[17] Gamma-irradiation at 2-10 kGy has been shown to decrease the cooking time in cowpeas.[18] More recently, γ-irradiation has been investigated to modify physical, functional and pasting properties of cowpea seeds.[19] The effects of γ-irradiation on the legumes seeds were found to be related mainly to changes in the major macromolecules, i.e. starch and protein.

The application of γ-irradiation to study the mechanisms involved in the development of HTC defect in legumes has not been previously reported. This study investigated the effects of γ-irradiation on cotyledon cellular structure and pectin solubility in two HTC cowpea cultivars with different susceptibility to the HTC defect.

MATERIALS AND METHODS

Cowpeas

Two cowpeas cultivars were selected for this investigation which had been found to have different susceptibility to the HTC defect (unpublished data): Agrigold (more susceptible) and Bechuana White (less susceptible). The two cowpea cultivars were cultivated in South Africa. After harvesting, they were stored in polypropylene plastic containers at 8°C and 61% Relative Humidity (RH) until analysis.

Accelerated HTHH storage conditions

HTC defect was induced by incubating the cowpea seeds (3.6 kg) at 40°C and 80% RH for 20 and 40 days in airtight polypropylene plastic containers. To obtain the desired RH, saturated potassium chloride solution was used according to ASTM E104-02.[20] Temperature and RH were monitored using a humidity/temperature logger, which was placed inside the plastic container.

Irradiation

The HTHH stored and control stored (at 8°C) cowpea seeds were then vacuum sealed in low density polyethylene bags and placed into cardboard boxes and subjected to γ-irradiation. They were irradiated by Synergy Sterilisation SA (PTY) Ltd (Isando, South Africa) using a ^{60}Co source. The target dose was 11 kGy. The actual dose delivered was an average of 11.2 kGy at a dose rate of 1.7 kGy h^{-1}, measured using a Harwell Perspex dosimeter (Didcot, UK). The un-irradiated cowpea seeds (0 kGy) were used as controls.

Preparation of cowpea flours

After HTC defect induction and control storage (as applicable) and irradiation treatment (as applicable), the cowpeas were ground to pass through a 0.25 mm opening screen using an air cooled laboratory hammer mill (Falling Number hammer mill 3100, Perten Instruments, Huddinge, Sweden. The cowpea flours were packaged in zip-lock type polyethylene bags. The flours from the cowpeas where HTC had been induced were stored at ambient temperature (22ºC), whereas flours from the control cowpeas were stored at 4ºC. Different storage temperatures were used because reversibility of the HTC defect (decreased cooking time) has been observed by storage of HTC cowpea seeds under refrigerated conditions (6.5°C, 71% RH).[21]

ANALYSES

Moisture

Moisture content was determined using AACC method 44-15A air-oven method.[22]

Cooking time

Cooking time was determined using a Mattson bean cooker, as described by Mwangwela *et al*.[23] For each treatment, 25 cowpeas were positioned on the perforations in the cooker, placed in an aluminium pan with deionised water and boiled at 95ºC (the boiling temperature at 1,200 m altitude where the research was conducted). The cooking time of the cowpeas was recorded as the time when 80% of the pins (pin weight 50 g) had penetrated the cowpeas and plunged through the holes in the base of the cooker.

Isolation of pectin fractions

Three pectin fractions were isolated:

Cold water soluble pectin (CWSP) - comprising pectic substances that are not strongly associated with the cell walls[24] (bonding interactions involve weak bonds/van der Waals forces;[25] **Hot water soluble pectin (HWSP)** - comprising pectic substances associated with the cell walls by intensive hydrogen bonding interactions[25] plus the CWSP; **Chelator soluble pectin** (**CSP) -** comprising pectin cross-linked by ionic bonds, particularly involving Ca^{2+} ions.[26]

Cowpea flour (5 g) was mixed with 30 mL 746 g kg^{-1} ethanol for 10 minutes at ambient temperature (22ºC) to remove soluble sugars. The mixture was centrifuged at 17300 × g for 10 minutes. Aqueous ethanol extraction was repeated twice. The final extraction was performed with absolute ethanol. The residual pellet (alcohol-insoluble solids) (AIS) was vacuum dried and stored in a desiccator at 22ºC. AIS (1 g) was extracted three times with 10 mL cold distilled water for 10 minutes at 22ºC and the combined extracts were considered as CWSP.[4] HWSP was determined according to Bernal-Lugo *et al*.[27] by extraction of 1 g AIS with hot distilled water at 80ºC. CSP was determined essentially as described by Hentges *et al*.[28] After CWSP extraction, the resulting pellet was extracted three times with 10 cm^3 aliquots of 5 g kg^{-1} ethylenediaminetetraacetic acid (EDTA) for 10 minutes at 22ºC. The three pectin fractions were each analysed for their galacturonic acid content using the metahydroxydiphenyl method of Blumenkrantz and Asboe-Hansen,[29] with galacturonic acid (Sigma-Aldrich, catalogue no. G2125, Johannesburg, South Africa) as the standard.

Phytate

Phytate was determined using an indirect quantitative analysis method by measuring organic phosphate, as described by Frubeck *et al*.[30] Anion exchange chromatography using Dowex 1; anion-exchange resin-AG 1 x 4, 4% cross-linkage, chloride form, 100-200 mesh (Sigma-

Aldrich) was applied to remove inorganic phosphate. Phosphate was determined colorimetrically at 500 nm based on the pink colour of Wade reagent.

Confocal laser scanning microscopy (CLSM)

Tissue blocks (approx. 1 mm^3) were cut from the inside edge of the cowpea seed cotyledons. These were fixed in 2.5 g kg^{-1} (w/v) formaldehyde overnight. The fixed cotyledon tissues were rinsed three times in 0.075 M sodium phosphate buffer for 10 minutes. The tissues were dehydrated at 22^{o}C for 15 minute periods in a graded series of aqueous ethanol (236 g kg^{-1}, 393 g kg^{-1}, 550 g $kg,^{-1}$ 707 g kg^{-1}, and 785 g kg^{-1}). The dehydrated tissues were infiltrated with 500 g kg^{-1} (v/v) London Resin White (LR) in ethanol for 1 hour and then with 1000 g kg^{-1}(LR overnight at 4^{o}C. The tissues were then placed in gelatin capsules, filled with LR and polymerised for 48 hours. Sections of 2.0 µm thickness were cut and placed on a glass slide. They were then stained with the fluorescent stain Calcoflour white MR2 (Sigma-Aldrich) for 5 minutes, to identify the cell walls. The stained sections were washed in running water and dried. Sections were then viewed using a Zeiss LSM 510 META Confocal Laser Scanning Microscope (Zeiss, Jena, Germany). The microscope system was equipped with a Plan-Apochromat 20 x/0.75 objective lens. Excitation was at 405 nm and emission at 514 nm using a 420 nm long pass filter, with a pinhole set at 48 µm. The digital images obtained were processed using Zeiss LSM image browser software.

Scanning electron microscopy (SEM)

The tissue blocks embedded in gelatin used for CLSM (above) were also used for SEM. The gelatin capsules were cut into 10 mm long pieces and mounted on an aluminium stub using double sided carbon tape. After which, they were sputter coated with carbon. The coated

samples were viewed at 1.0 kV using a Zeiss Gemini Ultra Plus Field Emission SEM (Oberkochen, Germany).

Statistical analysis

All experiments were repeated at least twice. The data were subjected to one-way ANOVA using Statistica® 8.0 and the means separated using Fisher's least significant difference (LSD) test at a 95% level of probability ($P \leq 0.05$).

RESULTS AND DISCUSSION

Effects of HTHH storage and irradiation on cooking time

The storage of both cowpeas cultivars under HTHH conditions substantially ($P \leq 0.05$) increased cooking time, indicating successful development of the HTC defect (Table 1). HTHH storage increased cooking time of Bechuana White cowpeas by 45% and 303% after 20 and 40 days of storage, respectively. With Agrigold cowpeas, which are more susceptible to development of HTC defect, the cooking time increased by greater than 600% after 20 days of storage.

Gamma-irradiation significantly ($P \leq 0.05$) reduced the cooking time of both cowpea cultivars . Cooking time of Bechuana White cowpeas was reduced by 25% and 36% after 20 and 40 days of HTHH storage, respectively. Gamma-irradiation reduced initial cooking time of Agrigold cowpeas by 30% before HTHH storage. However, it did not evidently result in a reduction in cooking time of HTC Agrigold after 20 and 40 days of storage and cooking time remained extremely long, greater than 540 minutes.

Table 1. Effects of HTHH storage at 40ºC and 80% RH and γ-irradiation on the cooking time of Bechuana White and Agrigold cowpea varieties

Cowpea variety[1]	Storage time (days)	Cooking time (minutes)				
		Irradiation dose (kGy)				
		0		11		
			% Change due to HTHH		% Change due to HTHH	% Change due to Irradiation
Bechuana White	0	$58^{a} \pm 7$[2]		$45^{a} \pm 2$		(-22)
	20	$84^{b} \pm 9$	(+45)	$63^{a} \pm 5$	(+40)	(-25)
	40	$234^{d} \pm 22$	(+303)	$149^{c} \pm 12$	(+231)	(-36)
Agrigold	0	$92^{b} \pm 4$		$71^{a} \pm 8$		(-23)
	20	$>540^{c}$	(> 487)	$>540^{c}$	(> 661)	(0)
	40	$>540^{c}$	(>487)	$>540^{c}$	(> 661)	(0)

[1] For each cowpea variety, means followed by different letters are significantly different at $P \leq 0.05$.
[2] Means (±) standard deviations of three independent experiments

Effects of HTHH storage and irradiation on cotyledon cellular structure

The development of HTC defect in both cultivars of cowpeas by HTHH storage resulted in closely packed cotyledon cells, which showed only limited cell separation after cooking for 60 and 120 minutes, as viewed by CLSM (Fig 1e,g Bechuana White, Fig 2e,g Agrigold). This is in contrast to the situation where after 120 minutes of cooking of both cowpea varieties which had not been subjected to HTHH storage, there was more general cell separation (Fig 1c,d Bechuana White, Fig 2c,d Agrigold).

Gamma-irradiation also caused visible cell elongation in 120 minute cooked cowpeas that had not been subjected to HTHH storage (Fig 1d Bechuana White, Fig 2d Agrigold). However, there was no cell elongation with both cowpea cultivars that had been subjected to HTHH storage (Fig 1f,h Bechuana White, Fig 2f,h Agrigold). This is despite the fact that irradiation substantially reduced the cooking time of HTHH stored Bechuana White (Table 1). Furthermore, it was observed that γ-irradiation generally reduced cotyledon cell wall thickness ($P \leq 0.05$) in both cowpea cultivars that had been cooked for 60 minutes. For the cowpeas which had not been subjected to HTHH storage, the reductions were from an average of 4.0 μm to 3.8 μm in Bechuana White (Fig 1a and 1b) and from 2.5 μm to 2.0 μm in Agrigold (Fig 2a and 2b). For the cowpeas that had been subjected to 40 days HTHH storage the reductions were from 4.0 μm to 3.5 μm in Bechuana White (Fig 1e and 1f) and from 3.1 μm to 2.8 μm in Agrigold (Fig 2e and 2f). In addition, γ-irradiation substantially increased the size of the intercellular spaces between the cotyledon cells for both cowpea cultivars . For Bechuana White, the increase in intercellular space was from an average of 7.1 μm to 18.5 μm (Fig 1e,f) after storage, and 13.5 μm to 22 μm (Fig 2a,b) with Agrigold. There was also a significant ($P \leq 0.05$) increase in cell size after γ-irradiation and cooking for 120 minutes for both cowpea varieties. With Bechuana White, the increase in cell size was from an average of 60.3 μm to 82.6 μm (Fig 1c,d) and 57.2 to 80.6 μm for Agrigold cowpeas (Fig

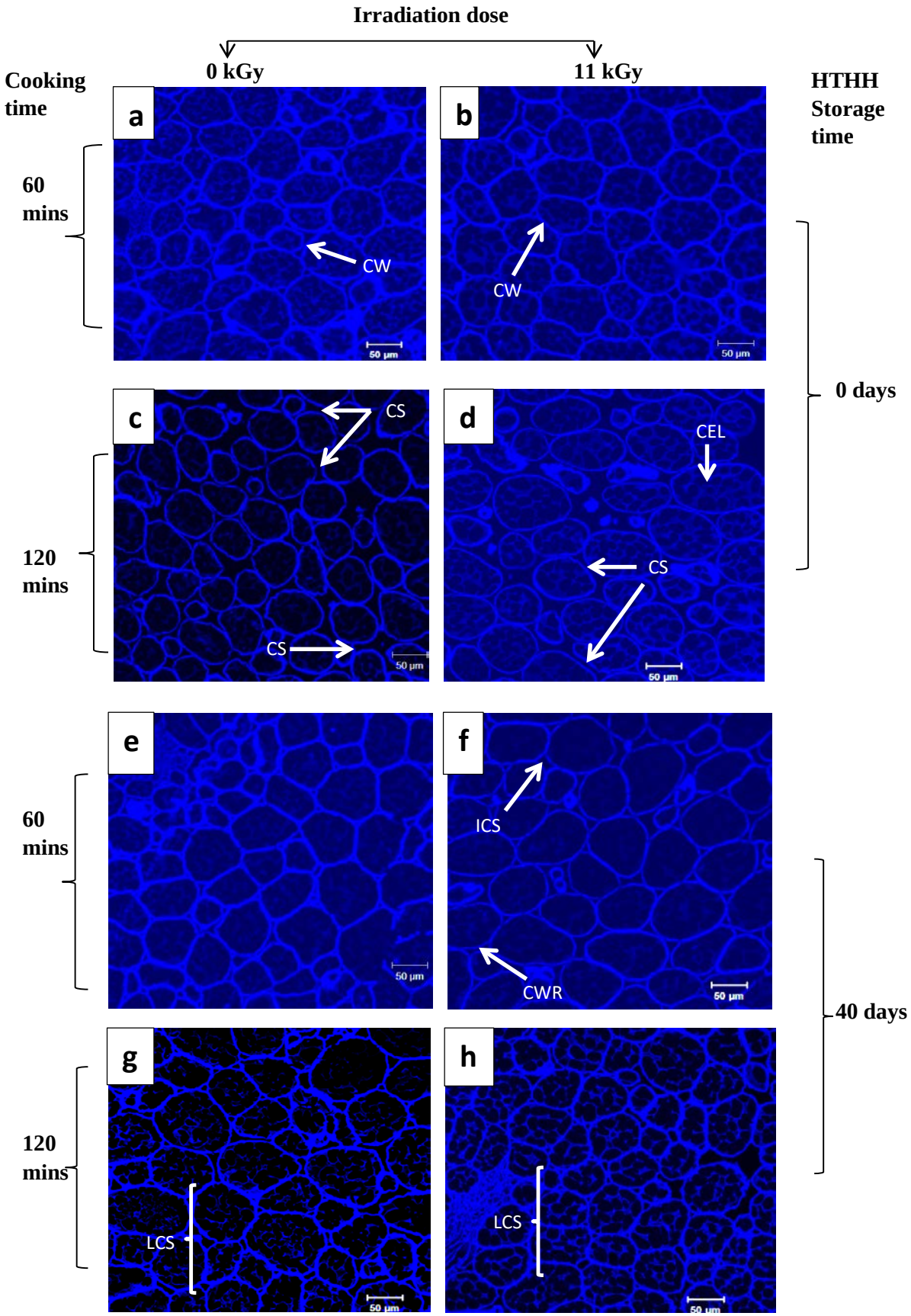

Figure 1. Confocal laser scanning microscopy illustrating effects of cooking for 60 and 120 minutes on cell separation in high temperature high humidity stored and irradiated Bechuana White cowpeas. CS- Cell separation, CW- Cell wall, LCS - Limited cell separation, CWR - Cell wall reduction, CE - Cell Expansion, CEL – Cell Elongation, ICS - Intercellular space

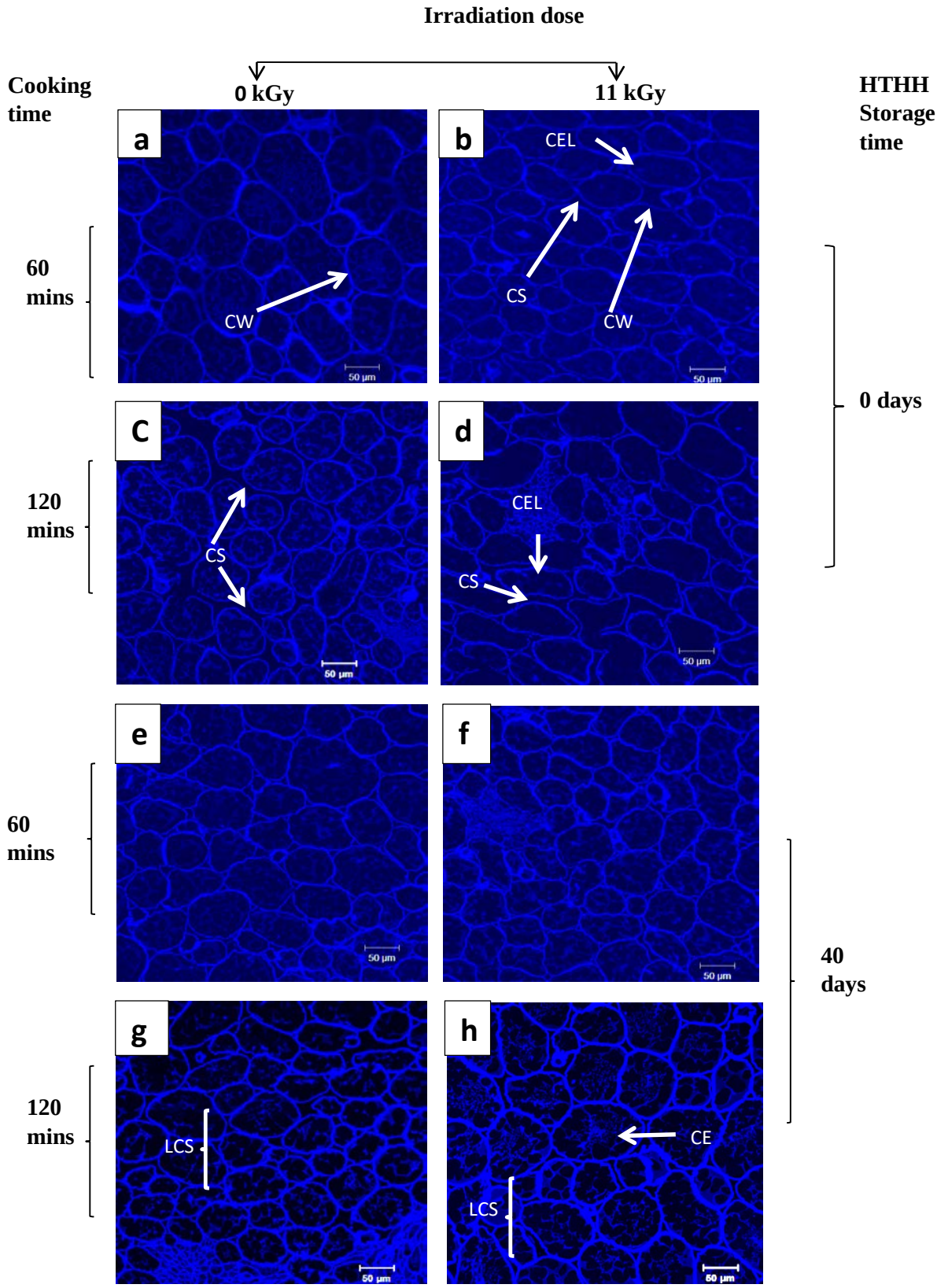

Figure 2. Confocal laser scanning microscopy illustrating effects of cooking for 60 and 120 minutes on cell separation in high temperature high humidity stored and irradiated Agrigold cowpeas. CEL - Cell Elongation, CS- Cell separation, LCS - Limited cell separation, CWR – Cell wall reduction

2c,d) which had not been subjected to HTHH storage. Legume cotyledon cell expansion during cooking as result of water uptake normally occurs in advance of cell separation[31] and the separation of cells is enhanced by disintegration of the middle lamella.[32] The reduction in cell wall thickness, increase in intercellular space size and cell expansion with γ-irradiation suggests that it was causing partial disintegration of the middle lamella.

To better understand the changes in cellular structure brought about by γ-irradiation observed by CLSM, the cowpea seed tissues were also studied using SEM, which provides a 3-dimensional surface image. Generally, after HTHH storage and γ-irradiation treatment, starch granules increased in size and were more clearly defined in both cowpea cultivars. With the seeds cooked for 120 minutes that had not been subjected to HTHH storage but had been subjected to γ-irradiation treatment, the starch granules in Bechuana White were more clearly defined and had increased in size to approximately 15 μm (Fig 3d) from 11 μm (Fig 3c) with the corresponding treatment without irradiation. For Bechuana White which had been HTHH stored for 40 days and subjected to γ-irradiation and then cooked for 120 minutes, the starch granules were also clearly defined and had increased in size to approximately 16 μm (Fig 3h) from 12 μm (Fig 3g) with the corresponding treatment without γ-irradiation. This shows that the starch granules had enhanced water uptake as a result of the γ-irradiation treatment and supports the data in Table 1 that γ-irradiation reduced cooking time. However, with Agrigold which was HTHH stored for 40 days and cooked for 120 minutes, there was no clear difference in starch granule appearance between those that had been subjected to γ-irradiation treatment (Fig 4h) and the corresponding treatment without (Fig 4g). With both treatments the starch granules were of a similar size, approximately 14 μm. This is a clear reflection of the greater susceptibility of the Agrigold variety to the HTC defect and suggests that changes had taken place in the middle lamella which prevented water uptake.

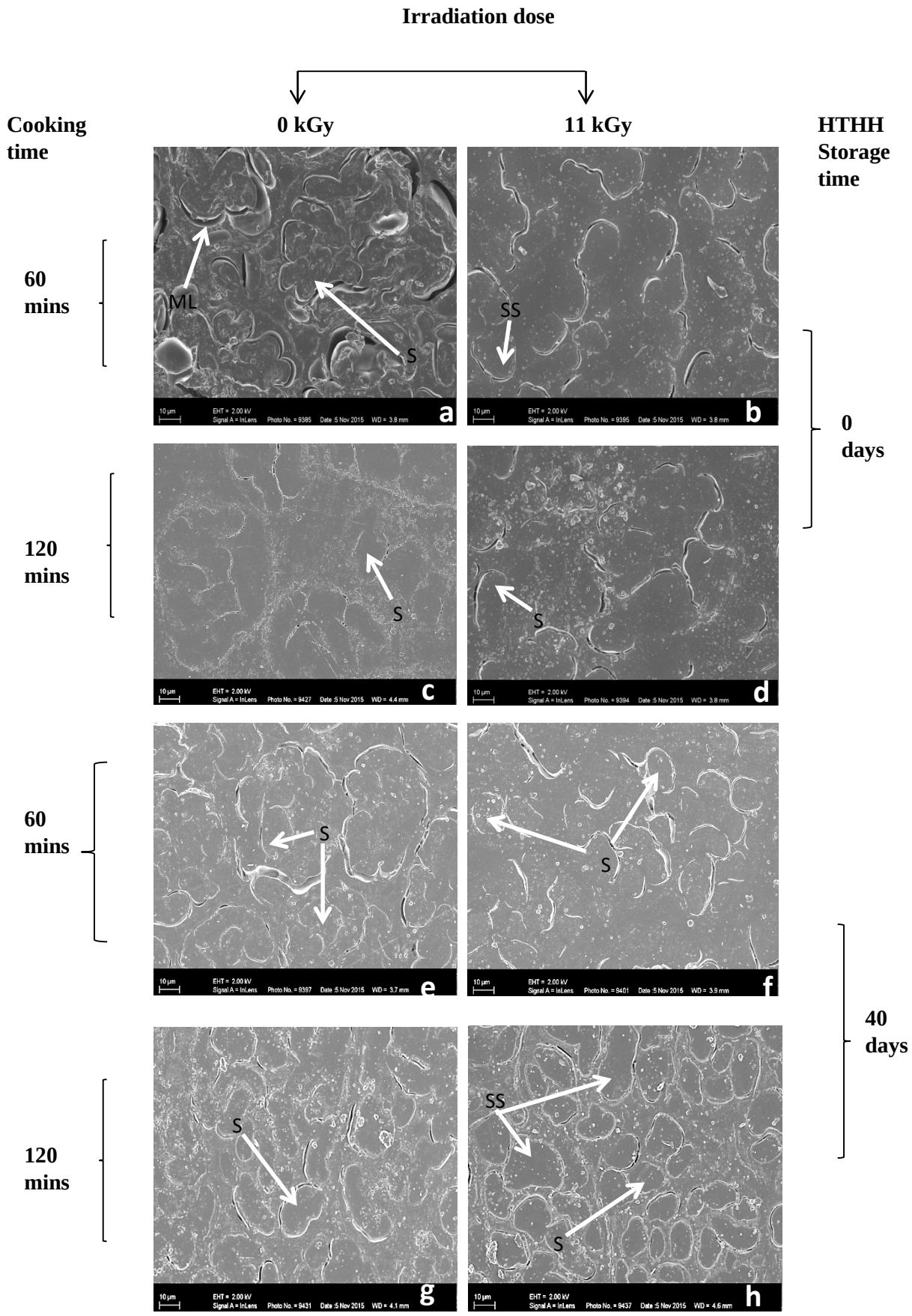

Figure 3. Scanning electron microscopy illustrating effects of cooking for 60 and 120 minutes on microstructure of high temperature high humidity stored and irradiated Bechuana White cowpeas. M-Middle Lamella; S-Starch granule; SS-Starch swelling

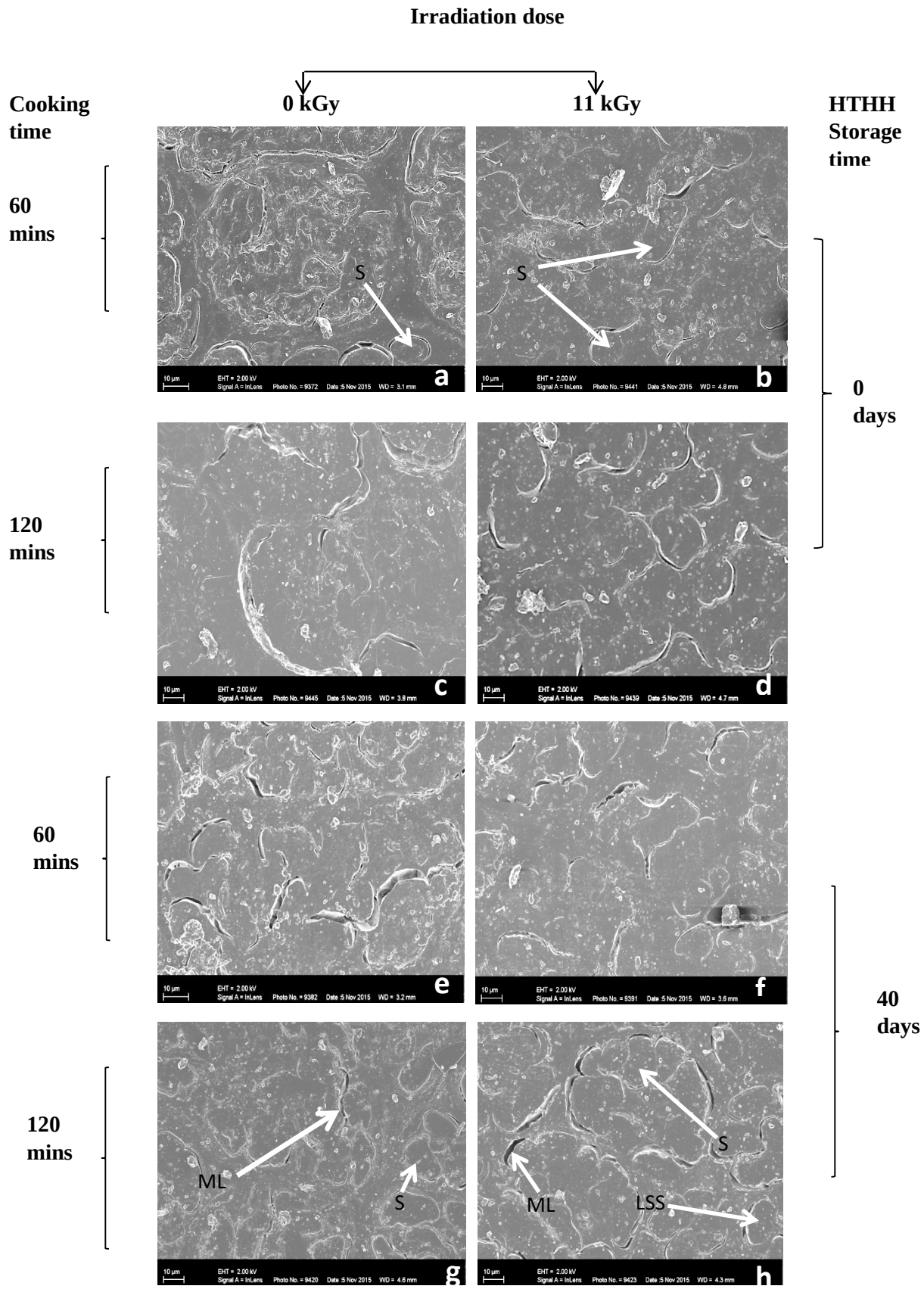

Figure 4. Scanning electron microscopy illustrating effects of cooking for 60 and 120 minutes on microstructure of high temperature high humidity stored and irradiated Agrigold cowpeas. M-Middle Lamella, S – Starch granule, LSS-Limited starch swelling

Effects of HTHH storage and irradiation on phytate and pectin

To explain the visual changes in the cotyledon cells caused by HTHH storage and γ-irradiation, the levels of phytate, CWSP, HWSP and CSP (middle lamella components) were determined in the variously treated samples. Concerning phytate, overall there was a decrease in phytate level in both cowpea varieties with HTHH storage (Table 2). The decreases were 8% and 10% for Bechuana White and 10% and 18% for Agrigold of storage, respectively. The decrease was probably due to increased activity of phytase during HTC development which hydrolyses phytate releasing divalent minerals, inorganic phosphates and produces lower level inositol phosphates.[33]

With γ-irradiation treatment there were some small reductions in the level of phytate as result of irradiation treatment (Table 2). The reduction of phytate by irradiation has been previously reported in soya beans.[34] Its action is probably by the cleavage of phytate inositol ring.[35] However, generally the γ-irradiation treatment had only a limited effect on the phytate levels after HTHH storage for both cowpea varieties. This is presumably because after the development of the HTC defect the amount of available phytate had been reduced by action of the phytase. This is supported by the fact that the highest reduction in phytate (11%) was with the Agrigold cowpeas that had not been subjected to HTHH storage.

Regarding pectin, overall HTHH storage generally caused reductions ($P \leq 0.05$) in the levels of CWSP (pectic substances bound by interactions involve weak bonds/van der Waals forces[25] and not strongly associated with the cell walls[24]) and HWSP (pectic substances associated with the cell walls by intensive hydrogen bonding interactions[25] plus the CWSP) in both cowpea varieties, but not CSP (pectin cross-linked by ionic bonds, particularly

Table 2. Effects of HTHH storage at 40ºC and 80% RH and γ-irradiation on the phytate content of Bechuana White and Agrigold cowpea varieties

Cowpea variety[1]	Storage time (days)	Phytate (g kg^{-1} dry basis)				
		Irradiation dose (kGy)				
		0		11		
			% Change due to HTHH		% Change due to HTHH	% Change due to Irradiation
Bechuana White	0	$9.67^{c} \pm 0.29$[2]		$9.63^{c} \pm 0.19$		0
	20	$8.87^{b} \pm 0.08$	(-8)	$8.72^{b} \pm 0.04$	(-9)	(-2)
	40	$8.72^{b} \pm 0.04$	(-10)	$8.15^{a} \pm 0.05$	(-15)	(-7)
Agrigold	0	$12.07^{d} \pm 0.15$		$10.78^{bc} \pm 0.09$		(-11)
	20	$10.83^{c} \pm 0.21$	(-10)	$10.28^{bc} \pm 0.09$	(-5)	(-5)
	40	$9.84^{a} \pm 0.28$	(-18)	$9.95^{a} \pm 0.30$	(-8)	(+1)

[1] For each cowpea variety, means followed by different letters are significantly different at $P \leq 0.05$.
[2] Means (±) standard deviations of two independent experiments

involving Ca^{2+} ions) which increased slightly but significantly ($P \leq 0.05$) with the cowpea seeds that had not been subjected to γ-irradiation. The reduction in the levels of pectin that were solubilised with hot and cold water with HTHH storage can be attributed to the formation of water insoluble Ca and Mg pectates in accordance with the phytase-phytate-pectin theory.[6] During the development of the HTC defect in cowpeas Ca and Mg have been shown to migrate to concentrate around the cell wall-middle lamella[36] where they most probably bind pectin. This in part explains the slight increase in the CSP fraction. However, the reduction of CWSP and HWSP cannot be directly explained by the phytase-phytate-pectin theory. It is probable that the decrease in the amount of pectin solubilised with hot and cold water was due to formation of alkali–soluble, ester bonded pectins.[37,38] The authors found that these difficult to extract alkali-soluble, ester bonded pectins, increased with HTC development in common beans (*Phaseolus vulgaris*).

Gamma-irradiation increased the CWSP fraction in both cowpea varieties (Table 3). Likewise, γ-irradiation of Bechuana White resulted in increased HWSP fraction. In contrast, γ-irradiation had no effect on the HWSP fraction in Agrigold. The increase in pectin that was solubilised by cold and hot water was probably as a result of hydrolysis of glycosidic bonds by γ-irradiation,[15] which results in readily soluble lower molecular weight fragments. This would be the cause of the indicative partial disintegration of the middle lamella, as evidenced by reduction in cotyledon cell wall thickness, increase in intercellular space size and cell expansion as observed by CLSM (Figs 1 and 2). Interestingly, as shown in Table 3 γ-irradiation affected CWSP and HWSP solubilisation differently in the two cowpea cultivars . More of these pectin fractions was solubilised with Bechuana White than with Agrigold. This is probably related to the fact that Bechuana White (which is less susceptible to the HTC defect than Agrigold) showed a decrease in cooking time after γ-irradiation treatment (Table

Table 3. Effects of HTHH storage days at 40°C and 80% RH and for γ-irradiation on the content of different pectin fractions of Bechuana White and Agrigold cowpea varieties

Cowpea Variety[1]	Storage Time (days)	Cold Water Soluble Pectin (CWSP) (g kg^{-1} dry basis)					Hot Water Soluble Pectin (HWSP) (g kg^{-1} dry basis)					Chelator Soluble Pectin (CSP) (g kg^{-1} dry basis)				
		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	0	1.16^{d} ±0.02[2]		1.39^{e} ± 0.04		(+20)	1.28^{d} ±0.01		1.39^{e} ±0.01		(+9)	0.35^{a} ±0.00		0.36^{b} ±0.01		(+3)
	20	1.03^{b} ±0.01	(-11)	1.19^{d} ±0.01	(-14)	(+16)	1.14^{a} ±0.01	(-11)	1.37^{e} ±0.00	(-1)	(+20)	0.36^{b} ±0.00	(+3)	0.36^{b} ±0.00	(0)	(0)
	40	1.10^{c} ±0.01	(-5)	0.90^{a} ±0.00	(-35)	(-18)	1.18^{b} ±0.01	(-8)	1.25^{c} ±0.01	(-10)	(+6)	0.36^{b} ±0.00	(+3)	0.36^{b} ±0.00	(0)	(0)
Agrigold	0	1.29^{d} ±0.01		1.31^{d} ±0.01		(+2)	1.39^{abc} ±0.01		1.44^{c} ±0.02		(+4)	0.39^{a} ±0.00		0.39^{a} ±0.00		(0)
	20	1.06^{b} ±0.04	(-18)	1.18^{c} ±0.03	(-10)	(+11)	1.40^{b} ±0.01	(+1)	1.40^{bc} ±0.08	(-3)	(0)	0.40^{c} ±0.00	(+3)	0.40^{c} ±0.00	(+3)	(0)
	40	0.92^{a} ±0.01	(-29)	0.95^{a} ±0.01	(-27)	(+3)	1.32^{ab} ± 0.00	(-5)	1.31^{a} ±0.00	(-9)	(-1)	0.39^{a} ±0.00	(0)	0.39^{a} ±0.00	(0)	(0)

[1] For each cowpea variety, means followed by different letters are significantly different at $P \leq 0.05$ for each pectin type.
[2] Means (±) standard deviations of two independent experiments

1). The fact that γ-irradiation can modify pectin resulting in a decrease in cowpea cooking time after HTHH storage strongly supports the concept that pectin modification plays a key role in the development of the HTC defect in cowpeas. Furthermore, the absence of an effect in the case of HWSP in Agrigold (the variety which was more susceptible to the HTC defect) after γ-irradiation treatment was likely due to a difference in pectin composition.

Also, γ-irradiation of the cowpeas had no significant (P>0.05) effect on the CSP fraction. CSP is the pectin fraction which is usually bound to cell wall via calcium bridges.[26] Since γ-irradiation did not modify this pectin fraction in either cowpea variety, this further supports the earlier proposed concept that formation alkali–soluble, ester bonded pectins also be involved in the development of the HTC defect. This is because Bechuana White had a reduction in cooking time even though γ-irradiation did not affect the proportion of this fraction after HTHH storage.

CONCLUSIONS

The application of γ-irradiation has helped to further understand the mechanisms by which the HTC defect prevents cotyledon cell wall separation in cowpeas during cooking. It reduces cotyledon cell wall thickness, increases cell size and intercellular spaces in both highly and less susceptible HTC defect type cowpeas. It does not, however, reverse the HTC defect in cowpeas that are very susceptible to the HTC defect. The effects of γ-irradiation are as result of hydrolysis of pectin fractions in the cell walls. The CWSP, HWSP and CSP are affected differently, with large increases in CWSP and HWSP particularly in the cowpeas that are less susceptible to HTC development but essentially no change in CSP. The fact that the cell wall changes brought about by γ-irradiation were associated with pectin solubilisation supports the phytate-phytase pectin theory to some extent. However, the non-reversal of the HTC defect

in HTC susceptible cowpeas and the absence of an effect on CSP indicate that other mechanisms are also involved in the HTC development in cowpeas, possibly the formation of alkali–soluble, ester bonded pectins.

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