

Effect of Ionising Radiation and Thermal Processing on Anti-Nutritional Factors of Vegetable Cowpea (*Vigna Sesquipedalis*) Seeds

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ABSTRACT

*Vegetable cowpea (*Vigna sesquipedalis*) seeds were subjected to gamma radiation at various doses (2, 5, 10, 15 and 20 kGy). The irradiated samples were divided into three. One portion was cooked, another portion was roasted and the other portion was autoclaved. The effect of irradiation and the various thermal processing methods – cooking and autoclaving – on anti-nutritional factors and proximate composition were investigated. A decrease in phytic acid, hydrogen cyanide, trypsin inhibitor activity and oligosaccharides resulted from increase in radiation doses and processing time. Also a radiation dose dependent increase in tannins was observed. The crude carbohydrate values showed no significant changes for irradiated samples but crude protein increased with increase in radiation doses. The crude fibre, moisture content, crude lipid, and ash content decreased with increases in radiation doses. Irradiation enhanced the ability of vegetable cowpea (*Vigna sesquipedalis*) seeds to serve as sources of food with good nutritional value.*

1. INTRODUCTION

The Confederation of British Industry (2007) reported that radiation has been employed in the food industry for controlling insect infestations and the numbers of pathogenic or spoilage microorganisms; arrest of autolysis in fresh fruits, vegetables; reduction of sprouting rate in tubers and germination time in bulbs. Nutritional composition of food products is a major parameter that is commonly considered by food processors. Radiation as a food processing method has been used in the food industry to alter the nutritional composition of food materials that would have otherwise been harmful to human health (Chung *et al.*, 2002). Radiation processing has been used as a good means of food preservation and in the lowering of anti-nutritional factor that are found in some legumes (Bhat *et al.*, 2007; Alothman *et al.*, 2009). According to Andress *et al.* (2005), the Food and Drug Administration (FDA) in United States of America (US) noted that irradiation of foods up to 20 kGy can safely be used to maintain their nutritional qualities.

Anti-nutritional factors inhibit growth and slow the rate of digestion and absorption of essential nutrients required by the body to function efficiently. Anti-nutritional factors include phytate, trypsin inhibitor, tannin, hydrogen cyanide and oligosaccharides (Udensi *et al.*, 2007). These anti-nutritional factor

components of foods which are lowered by different processing methods can be found in different classes of food including legumes (Singh, 1988).

Vegetable Cowpea (*Vigna sesquipedalis*) is a member of the leguminous family with the sub – family as *Papilionaceae* (Enwere, 1998). The cropping system in the Igbo speaking regions of Nigeria where vegetable cowpea is part of the popular diet, is not complete without their cultivation. Locally amongst the Igbos vegetable cowpea is called “*akidior akidi Oji*” depending on the locality. Vegetable cowpea is an important source of nutrient among Igbos and is commonly eating either as vegetables when the buds are young and immature or dried seeds when buds are matured (Udensi *et al.*, 2007).

The aim of this research is to evaluate the effect of ionising radiation and thermal processing on anti-nutritional factors of vegetable cowpea (*vigna sesquipedalis*) seeds. The effect of irradiation and the various thermal processing methods – cooking and autoclaving – on anti-nutritional factors and proximate composition were investigated.

2. METHODOLOGY

2.1 Raw Materials

Dried seeds of the vegetable cowpea were purchased at Nkwo Mbala-Isuochi a local market in Umunneochi Local Government Area of Abia State. The seeds that were observed to be immature and damaged were sorted out. Surface dirt on the seeds was removed by cleaning with fine muslin cloth.

2.2 Preparation of Samples

Vegetable Cowpea seeds weighing 500 g were irradiated in a Cobalt-60 Gamma cell unit (Model GS 1000, Category 4, Panorama Wet storage Source, Siemen, Germany). The seeds were treated to 2, 5, 10, 15 and 20kGy doses of radiation at a temperature of $25\pm1^{\circ}\text{C}$. The absorbed doses were confirmed using Alanine dosimeters. Seeds (500 g) that serve as control samples were not treated to radiation and were packed in polyethylene pouches.

2.3 Thermal Processing

Boiling

The method used by Udensi *et al.*, (2005) was applied during boiling. Distilled water was used to boil five hundred grammes (500g) portions each of the irradiated and non-irradiated seeds at a constant temperature of 100°C in the ratio of 1:10 w/v for 15, 30, 45 and 60 min; after which they were drained and oven dried at 60°C to 14% moisture content. The seeds were dehulled and a local attrition milling machine was used to mill them into flour to obtain 1mm mesh size particles. The flour was analysed for anti-nutritional factors.

Autoclaving

The method adopted by Udensi *et al.*, (2005) was used during autoclaving. Five hundred grammes (500g) portions each of the irradiated and non-irradiated seeds were autoclaved at a temperature of 120°C and pressure of 718Nm^{-2} for 15, 30, 45 and 60 min respectively after which they were drained and oven dried at 60°C to 10% moisture content. The seeds were dehulled and a local attrition milling machine was used to mill them into flour to obtain 1mm mesh size particles. The flour was analysed for anti-nutritional factors.

2.4 Determination of Anti-Nutritional Factors

Reddy *et al.* (1982) method as modified from Wheeler and Ferrel (1971) was used to determine the phytic acid content. Kakade *et al.* (1974) method adopted by Tresina and Mohan (2012) was used to determine the trypsin inhibitor activity. The tannin contents were determined by the modified vanillin-HCl method (Burns, 1971; Price *et al.* (1978). Oligosaccharides were extracted by the method of Somiari and Balogh

(1993). Alkaline titration procedure by Anhwange *et al.* (2004) was used to determine the hydrogen cyanide.

2.5 Proximate Analysis

Crude protein was determined by the micro-Kjeldahl method (Tresina and Mohan 2012). Soxhlet extraction method was used to estimate the crude lipid using diethyl ether, crude fibre by acid and alkaline digestion and ash was determined gravimetrically on incineration in a muffle furnace at 550°C as described by Association of Official Analytical Chemists (AOAC, 2005). The formulae used to calculate by difference the crude carbohydrates were by Tresina and Mohan (2012) as shown in equation 1:

$$\text{Crude carbohydrates (\%)} = 100 - [(\text{crude protein (\%)} + \text{crude lipid (\%)} + \text{crude fibre (\%)} + \text{ash (\%)})] \quad (1)$$

3. RESULTS AND DISCUSSION

3.1 Anti-Nutritional Factors

Phytic Acid content

The effect of boiling and autoclaving on the level of phytic acid in non-irradiated and irradiated vegetable cowpea (*Vigna sesquipedalis*) is given in Table 1. The seeds of vegetable cowpea showed a radiation dose-time dependent loss of phytic acid. It was noted in the study that as the radiation dose and processing time increased the phytic acid content reduced. Udensi *et al.* (2005) reported the loss of phytate in non-irradiated *Mucuna Sloaniea* leguminous plant. He also noted that phytates are heat labile and they also form insoluble complexes with other components during boiling. The ease with which phytates form bonds may be the reason why thermal processing reduced the phytic acid content in the study. According to Raboy (2001), phytic acid influences the digestive enzymes in the gut and intestines by binding minerals before they are absorbed thus, reduction in the phytic acid content is desirable.

Table 1: Effect of Boiling and Autoclaving on the Phytic Acid of Non-Irradiated and Irradiated Vegetable Cowpea (*Vigna sesquipedalis*)

Treatment (min)	Dose (kGy)					
	Non-irradiated	2	5	10	15	20
Raw seed	523.40	519.16	470.24	459.32	438.13	428.45
Boiling						
15	514.44	511.76	466.94	443.39	423.18	412.54
30	510.21	506.56	450.44	431.45	418.63	408.39
45	504.78	500.37	445.82	427.39	418.63	404.75
60	496.12	481.53	410.24	399.62	401.68	387.45
Autoclaved						
15	223.47	216.51	202.43	199.23	173.30	158.41
30	217.98	216.51	200.25	187.32	161.87	132.54
45	198.54	195.83	177.19	169.81	150.13	120.47
60	157.41	143.56	132.70	127.10	111.48	108.29

Trypsin Inhibitor Activity

Effect of boiling and autoclaving on the trypsin inhibitor activity of non-irradiated and irradiated vegetable cowpea (*Vigna sesquipedalis*) is presented in Table 2. Thermal processing and irradiation led to reduction in trypsin inhibitor activity (TIA) in the study. In the study, there was a time-dependent reduction of TIA. As the time increased with corresponding increase in temperature of processing the TIA decreased. Autoclaving at 45 min completely eliminated the TIA. Khokhar and Chauhan (1986) noted that trypsin

inhibitors are heat labile in nature, this may be the reason for the result obtained in the present study. Cooking and autoclaving as reported by Udensi *et al.* (2004) can be used to inactivate protease inhibitors in legumes. Trypsin inhibitor in human makes the absorption and utilisation of trypsin and plasmin almost impossible by forming equimolar complexes when it acts upon both trypsin and chymotrypsin. Thus, the destruction of trypsin inhibitors increases the nutritive value of vegetable cowpea and allows for its more utilisation in food formulations.

Table 2: Effect of Boiling and Autoclaving on the Trypsin Inhibitor Activity of Non-Irradiated and Irradiated Vegetable Cowpea (*Vigna sesquipedalis*)

Treatment (min)	Dose (kGy)					
	Non-irradiated	2	5	10	15	20
Raw seed	46.23	45.10	44.29	40.26	36.70	32.94
Boiling						
15	41.63	40.50	41.53	39.47	36.29	31.46
30	41.34	37.26	36.19	33.12	26.04	20.11
45	39.32	32.21	29.77	23.26	21.02	14.69
60	31.11	27.63	24.01	20.90	18.47	10.51
Autoclaved						
15	18.76	16.45	13.29	10.40	8.31	4.27
30	12.57	10.57	8.52	7.24	6.29	4.20
45	0.00	0.00	0.00	0.00	0.00	0.00
60	0.00	0.00	0.00	0.00	0.00	0.00

Tannins

The result presented in Table 3 shows the effect on tannin by cooking and autoclaving of non-irradiated and irradiated vegetable cowpea (*Vigna sesquipedalis*) seeds. The study showed decrease in tannin content during boiling and autoclaving as the processing time increased. Polyphenols of which tannins is a member have been reported by Kumar *et al.* (1979) and Chung *et al.* (1998) to be water soluble in nature. During cooking and autoclaving these water soluble tannins leached out into their surrounding medium (Vijayakumani *et al.*, 1992). The reduction in tannin content during cooking and autoclaving in the study may be due to leaching. Singh (1988) reported that tannins are mostly found in the coats of seeds and are heat labile. The reduction in tannins as observed in the study may also be due to the fact that tannins in the seed coat were degraded by heat during processing.

Oligosaccharides

The result of the study as shown in Table 4 indicated that after 60 minutes of autoclaving, the highest reduction in oligosaccharides level was obtained. The study observed a time-dose dependent reduction in oligosaccharides. Increases in processing time and radiation dose resulted in the decrease in oligosaccharides values. Oligosaccharides have been shown by Oligbinde and Akinyele (1983) to form simple disaccharides and monosaccharide during heat hydrolysis. Thermal processing involves heat hydrolysis and may have caused the decrease in the levels of oligosaccharides during boiling and autoclaving. Oligosaccharides that are easily found in food are most times associated with causing flatulence in man with the common oligosaccharides being raffinose, stachyose, ciceritol, and verbascose (Udensi *et al.*, 2005). Low level of these oligosaccharides would reduce flatulence in humans.

Table 3: Effect of Boiling and Autoclaving on the Tannins of Non-Irradiated and Irradiated Vegetable Cowpea (*Vigna sesquipedalis*)

Treatment (min)	Dose (kGy)					
	Non-irradiated	2	5	10	15	20
Raw seed	0.31	0.41	0.49	0.56	0.60	0.67
Boiling						
15	0.28	0.37	0.40	0.48	0.53	0.61
30	0.28	0.32	0.38	0.44	0.49	0.58
45	0.26	0.22	0.38	0.40	0.47	0.51
60	0.23	0.19	0.29	0.34	0.41	0.46
Autoclaved						
15	0.13	0.22	0.28	0.32	0.33	0.38
30	0.05	0.15	0.24	0.31	0.32	0.34
45	0.05	0.11	0.20	0.26	0.29	0.30
60	0.03	0.06	0.11	0.19	0.21	0.27

Table 4: Effect of Boiling and Autoclaving on the Oligosaccharides of Non-Irradiated and Irradiated Vegetable Cowpea (*Vigna sesquipedalis*)

Treatment (min)	Dose (kGy)					
	Non-irradiated	2	5	10	15	20
Raffinose	0.58	0.52	0.48	0.43	0.36	0.29
Stachyose	1.63	1.49	1.21	1.06	0.72	0.44
Boiling						
15	0.43	0.41	0.40	0.37	0.32	0.23
	1.38	1.31	1.18	1.02	0.67	0.40
30	0.41	0.37	0.36	0.33	0.29	0.18
	1.36	1.27	1.15	1.00	0.55	0.36
45	0.38	0.35	0.36	0.31	0.26	0.18
	1.31	1.28	1.12	0.93	0.48	0.23
60	0.30	0.27	0.25	0.21	0.19	0.10
	1.29	1.21	1.09	0.87	0.40	0.16
Autoclaved						
15	0.38	0.33	0.32	0.32	0.23	0.13
	1.26	1.22	1.16	0.97	0.50	0.32
30	0.34	0.32	0.31	0.25	0.17	0.10
	1.17	1.15	1.11	0.81	0.43	0.27
45	0.30	0.29	0.26	0.20	0.15	0.12
	1.10	1.07	0.89	0.54	0.31	0.21
60	0.27	0.22	0.18	0.12	0.09	0.02
	0.92	0.74	0.53	0.41	0.20	0.16

Hydrogen Cyanide

A significant loss of hydrogen cyanide (HCN) content was observed in the non-irradiated and irradiated vegetable cowpea (*Vigna sesquipedalis*) by the study as shown in Table 5. Tresina and Mohan (2012) showed that free and bond cyanides are both water soluble. The high solubility HCN makes them to leach out easily during boiling and autoclaving. The reduction of hydrogen cyanide (HCN) due to boiling and autoclaving as observed in the study may be as a result of leaching. The result of the study is in agreement

with that by Udensi *et al.* (2005) in their study of *Mucuna Sloanie*. Chung *et al.* (1998) noted that the nervous system is affected by the consumption of food rich in hydrogen cyanide. Udensi *et al.* (2007) reported 36 mg/100 mg as the lethal level for hydrogen cyanide. The low value of HCN obtained in the study indicates that vegetable cowpea could be safely used in food formulations.

Table 5: Effect of Boiling and Autoclaving on the Hydrogen Cyanide of Non-Irradiated and Irradiated Vegetable Cowpea (*Vigna sesquipedalis*)

Treatment (min)	Dose (kGy)					
	non-irradiated	2	5	10	15	20
Raw seed	3.47	3.44	3.41	3.36	3.31	3.28
Boiling						
15	1.66	1.57	1.53	1.47	1.29	1.16
30	1.30	1.26	1.19	1.12	1.04	1.01
45	1.32	1.26	1.17	1.12	1.05	0.99
60	1.11	1.03	1.01	0.90	0.74	0.51
Autoclaved						
15	0.76	0.65	0.62	0.40	0.31	0.27
30	0.76	0.57	0.62	0.41	0.29	0.20
45	0.52	0.57	0.51	0.36	0.27	0.20
60	0.40	0.21	0.23	0.19	0.14	0.10

3.2 Proximate Analysis

The result of the proximate composition of irradiated and non-irradiated vegetable cowpea (*Vigna sesquipedalis*) seeds is presented in Table 6. There was a significant decreased in the moisture content of the irradiated seeds when compared to the non-irradiated seeds. The crude protein content increased with increase in radiation doses and the crude protein value of non-irradiated seed samples was in the same range (20-30) as other *Vigna spp.* (Arinathan *et al.*, 2003). Increase in radiation doses resulted in a decrease in the crude fibre, crude lipid and ash contents. The crude lipid in raw vegetable cowpea (*Vigna sesquipedalis*) seeds was observed to be higher when compared to crude lipid in other non-irradiated *Vigna spp.* as reported by in Bravo *et al.* (1999) and Kala *et al.* (2010). Balogun and Fetuga (1986) noted that low crude fibre is desirable nutritionally as foods low in fibre has higher values of proteins and carbohydrates. The decrease in fibre can be associated with depolymerization and delignification of the plant matrix (Sande and Karaivanov, 2007). The crude carbohydrate values in vegetable cowpea (*Vigna sesquipedalis*) seeds showed no significant changes in values for the irradiated samples. These values for crude carbohydrates showed a trend similar to those of other legumes that were investigated by Tresina and Mohan (2012).

Table 6: Proximate Composition of Non-Irradiated and Irradiated Vegetable Cowpea Seeds

Component	Dose (kGy)					
	Non-irradiated	2	5	10	15	20
Moisture	6.23±0.11 ^a	6.14±0.16 ^a	5.87±0.17 ^a	5.45±0.12 ^a	5.09±0.15 ^b	5.02±0.15 ^b
Crude protein	21.90 ±0.16 ^a	24.04±0.14 ^c	24.97±0.19 ^c	25.56±0.49 ^c	26.23±0.34 ^c	27.47±0.20 ^c
Crude lipid	6.65±0.03 ^a	5.37±0.06 ^a	4.10±0.09 ^{ab}	3.66±0.08 ^b	3.71±0.04 ^b	3.28±0.02 ^c
Crude Fibre	5.45±0.04 ^a	5.18±0.07 ^b	4.73±0.04 ^b	4.39±0.06 ^b	4.22±0.09 ^{bc}	3.72±0.08 ^c
Ash	4.61±0.01 ^a	4.17±0.02 ^a	3.34±0.04 ^b	3.16±0.02 ^{bc}	2.88±0.03 ^c	2.13±0.01 ^c
Crude Carbohydrate	55.16±2.91 ^a	55.10±1.68 ^a	56.99±1.37 ^a	57.78±2.06 ^a	57.87±3.19 ^a	58.38±2.09 ^a

Means ± SE (N = 3) means in the row with same superscript differ not significantly (p<0.05)

4. CONCLUSION

Results from the study showed that the combined effect of gamma irradiation and thermal processing increased the nutritional quality of the seed samples by lowering the values of anti-nutritional factors with autoclaving completely removing trypsin inhibitors after 45 min of processing. However, increase in the doses of radiation resulted in increase in crude protein and decrease the crude fibre, moisture content, crude lipid, and ash content. Therefore, the crude carbohydrate values did not show any significant changes with increase in doses.

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