EFFECTS OF DIFFERENT PROCESSING METHODS ON THE ANTI-NUTRIENT CONTENTS IN TWO IMPROVED VARIETIES OF COWPEA

[2]OSUNBITAN, S. O. [1]TAIWO, K. A. AND [1]GBADAMOSI, S. O.

[1]Department of Food science and Technology, Obafemi Awolowo University, Ile-Ife. Nigeria
[2]Institute of Agricultural Research and Training, Obafemi Awolowo University. Moor Plantation, Ibadan. Nigeria
Corresponding author: Email:Sundaygbenga15@yahoo.com

ABSTRACT

Two cowpeas IT90K-277-2 and IT97K-568-18 were evaluated for their anti-nutrient contents (tannin, phytate and trypsin inhibitor activities-TIA) before and after different processing methods. (soaking, cooking, pressure cooking and dehulling). Unprocessed beans had tannin contents in the range 10.5 – 12.0 mg 100 g^{-1}, phytate, 20 – 22 mg 100 g^{-1}, and (TIA) 8.46 – 12.97 mg g^{-1}. For different processing times, soaking achieved 5.83 – 54.3% reduction in tannin, 6.66 – 59.50% in phytate and 2.96 – 63.70% in TIA. Dehulling caused reduction of 12.50 – 76.19% in tannin, 16.00 – 74.55 % in phytate and 2.31- 74.56% in TIA. Atmospheric cooking achieved reduction of 93.0 – 95.26 % in tannin, 98.0 – 98.54 % in phytate and 80.03 – 92.08 % in TIA, while pressure cooking accounted for reduction of 91.43 – 98.33 % in tannin and 85.58 – 98.23 % in TIA. Effect of time and temperature were significant ($P < 0.05$) for up to 4 h of soaking. The relationship between the anti-nutrient content retained and time is linear with R^2 values of 0.95, 0.93 and 0.85, for room temperature, 50 and 60 ^{0}C, respectively.

Key words: anti-nutrient contents. Improved varieties of cowpea, soaking, atmospheric cooking, Pressure cooking

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INTRODUCTION

Legumes are less expensive sources of food protein in the diet of many developing countries. The protein content of legumes, especially cowpea is between 20 – 23 %. The presence of anti-nutritional factors is one of the main drawbacks limiting the nutritional and food quality of legumes, especially cowpea. The common anti-nutrients found in legumes, especially cowpea are trypsin inhibitors, tannin, phytate, lectins, and saponin (Vidivel and Jonardhanen, 2003). Trypsin inhibitors prevent digestion of protein and suppresses release of amino- acid. Tannin forms complex compounds with protein thereby reducing its digestibility. It also inhibits activities of some enzymes e.g trypsin and amylase. Phytate combines with di and trivalent metals and this reduces mineral bioavailability. However, several processing methods can be used to eliminate or reduce anti-nutrients in cereals and legumes, thus enhancing the bio-availability of minerals and nutrients in plant based food. A study on the effect of soaking time on proximate, mineral and anti-nutrient contents of yellow maize was investigated, yellow maize seeds were soaked in de-ioned water for 12, 24, 36 and 48h followed by draining, drying and milling. All the anti-nutrient contents investigated reduced significantly with soaking time. Phytate in unprocessed maize was 1.06mg/g reduced to 0.52mg/g after 48h of soaking. Tannin in unprocessed maize was reduced from 1.34mg/g to 0.46mg/g after 48h, while trypsin inhibitor activity was 3.67mg/g reduced to 0.83mg/g after 48h (Obasi and Wogu, 2008). A study was carried out on anti-nutrient contents of cowpea (Sesquipedalis) seeds during thermal processing. The reduction of trypsin inhibitor was found to be highest (100%) from 1113 Tiug/g in unprocessed seed to 0 with autoclaving for 60min. Boiling was more effective in reducing phytate (68.34%) from 4.25mg/100g in unprocessed seed to 1.35mg/100g for 60min. Tannin was reduced by boiling (75%) from 0.40 to 0.10% for 60min. (Udensi et al., 2007)

Over two million tonnes of cowpea are produced annually in Nigeria, but a high percentage suffers wastage due to pest attack and diseases (Rachie *et al*.,2000) hence, new cowpea varieties were developed to address some of these constraints. In recent times, newer varieties have emerged and there is a dearth of information on the effect of processing on their anti-nutrient contents of these new varieties; hence, this study. Eating cowpea helps to meet the nutritional daily protein requirement, but if this is limited by anti-nutrients, it should be addressed.

MATERIALS AND METHODS

Source of materials

Two varieties of improved cowpea: IT90K-277-2 (white seed) with rough seed coat and IT97K-568-18 (brown seed) with rough seed coat were obtained from the International Institute for Tropical Agriculture (IITA), Ibadan.

Sample Preparation

Soaking of beans: Using the method of Alfonso (2006), 100 g of the two bean varieties were soaked in distilled water (1:5 $^{w}/_{v}$) in a glass beaker placed in water bath at room temperature (27 ± 2 ^{0}C, 50 ^{0}C and 60 ^{0}C) for 1, 2 4 and 6 h. At the end of each soaking time, the samples were removed from the beaker and surface water blotted with a tissue paper. Weight of the beans was noted.

Dehulling of seed coat: After each soaking period, some of the cowpea seeds were dehulled as described by EI-Beltagy (1996), while some remained as whole bean seeds. The seed coats were removed manually by rubbing the wet beans between the palm several times after soaking the beans in water at (27 ± 2 ^{0}C, 50 ^{0}C and 60 ^{0}C) for 1, 2, 4 and 6 h.

Drying of cowpea seeds: At the end of soaking, after blotting the beans with tissue paper to remove adhering water, the beans were oven-dried at 80 ^{0}C for 24 h to constant weight. The dried seeds were milled into flour using laboratory grinding machine (poly mix PX-MFC 90D, Switzerland) and stored in airtight plastic container at 4 ^{0}C until used.

Cooking of cowpea seeds: The beans were cooked in distilled water (1:10 $^{w}/_{v}$) for 10, 20 and 30 min at atmospheric pressure in a stainless steel pot with lid and at reduced pressure using a domestic pressure cooker (Portable Steam Steriliser, 7500 Series) at 15psi for 10, 20 and 30 min. The beans were drained, dried at 80 ^{0}C for 24 h and milled into flour for subsequent testing (Uzoechuna, 2008).

Chemical Analysis.

Anti-nutritional Factors: The tannin contents of the bean flours was determined using the procedure of Makkar and Goodchild (1996). Phytate contents of the bean flours were determined

using the method of Maga (1982). Trypsin inhibitor contents was determined using the method of Kakade *et al*., (1974).

Statistical Analysis Analysis of variance was used to test the anti-nutritional values of tannin, phytate and trypsin inhibitor activities (TIA) and difference of means was tested using Duncan test using SPSS 200x software.

RESULTS AND DISCUSSION

Effect of processing variables on tannin content of soaked beans

The tannin contents in the raw bean seeds were 12 and 10.5 mg 100 g^{-1} for IT90K-277-2 and IT97K-568-18, respectively. Table 1 shows the effect of time on leaching of tannin from whole beans during soaking. The tannin content reduced with soaking time. The percentage reduction ranged from 5.83 – 47.50 % for variety IT90K-277-2 depending on the temperature, although the amount lost reduced after the fourth hour. This loss may be attributed to leaching of tannin from the bean into the soaking medium. The reduction observed agrees with the work of Onwuka (2006) who reported a decrease in tannin content of pigeon pea and cowpea with increase in soaking time.

Effect of dehulling on tannin contents is shown in Table 1 where whole bean seeds had lower % reduction in tannin content (5.83 to 47.50) compared to dehulled seeds.(12.50 to 70.83). The difference was significant ($P < 0.05$). The higher % reduction in tannin content in the dehulled beans may be due to the absence of the seed coat (Reddy and Pierson, 1994; Kumar *et al*., 1979) It is believed that a high proportion of the anti nutrients reside in the seed coat.

Effects of soaking temperatures on tannin are shown in Fig 1.Tannin content was significantly ($p<0.05$) influenced by the seed variety IT90K-277-2 , while there was no significant ($p>0.05$) difference in the tannin content in the seed variety IT97K-568-18 at different temperatures. At higher soaking temperatures,(50- 60^{o}C) the tannin content in the soaked beans became lower

implying more tannin was released into the soaking medium and possibly degraded by the heat from soaking medium..

Table 1. Percent reduction of tannin after soaking of whole and dehulled bean of IT90K-277-2

SAMPLE	Soaking Time (H)	Room Temperature	50^{o} C	60^{O} C
Whole bean	1	5.83±0.02	13.30±0.01	33.30±0.02
	2	12.50±0.03	20.80±0.02	41.70±0.04
	4	16.70±0.01	38.30±0.03	45.00±0.03
	6	22.50±0.04	41.70±0.02	47.50±0.03

Sample	Soaking Time (H)	Room temperature	50^{o} C	60^{o} C
Dehulled beans	1	12.50±0.02	27.50±0.03	41.66± 0.01
	2	37.50±0.03	46.66± 0.04	53.33± 0.02
	4	46.66±0.04	51.66± 0.02	60.00± 0.01
	6	52.50±0.03	63.33± 0.02	70.83± 0.01

Effect of processing variables on phytate contents of soaked beans

The phytate content in the raw beans were 22 and 20mg 100 g^{-1} for the bean varieties IT90K-277-2 and IT97K-568-18**,** respectively. Table 2 shows the effect of time on leaching of phytate from the whole beans. The phytate content reduced with time. The percentage reduction ranged from 6.66 – 54.30 % for bean variety IT97K-568-18 depending on the temperature although the rate of loss became insignificant ($p>0.05$) after the fourth hour. This reduction in loss may be due to increase in phytate content in the soaking medium (approaching saturation point) because more phytate was leached from the bean with longer hour of soaking. This is in agreement with

the report of Onimawo and Akubor (2005) that phytate reduced during longer soaking time of maize grain.

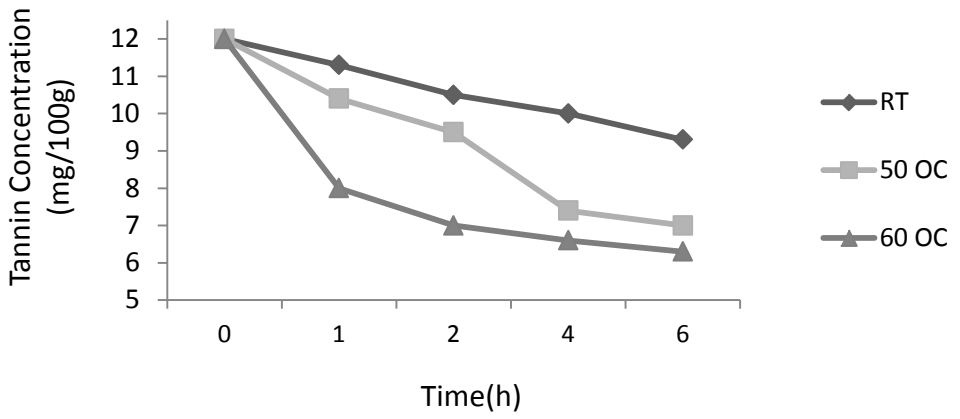
Table 2 shows the % reduction of phytate on dehulled beans of IT97K-568-18 after soaking at room temperature, 50 and 60 ^{0}C. Dehulled bean seeds had higher % reduction in phytate contents (16.00 to 72.00 %) compared to whole bean.(6.66 to 50.50 %). The higher % reduction in phytate content may be attributed to the absence of seed coats.

Table 2 Percent reduction of phytate after soaking of whole and dehulled beans of IT97K 568-18 at different temperatures and soaking time

Sample	**Soaking Time (H)**	**Room Temp.**	**50^{0}C**	**60^{0}C**
Whole seed	1	6.66±0.02	16.20±0.03	23.80±0.03
	2	18.10±0.01	20.00±0.04	23.80±0.03
	4	33.30±0.02	37.10±0.03	50.50±0.01
	6	36.20±0.02	44.80±0.04	54.30±0.03

Sample	**Soaking Time (H)**	**Room Temp.**	**50^{0}C**	**60^{0}C**
Dehulled seed	1	16.00±0.02	20.00±0.03	30.00±0.01
	2	32.00±0.04	40.00±0.03	50.00±0.02
	4	46.00±0.04	56.00±0.04	63.50±0.01
	6	60.00±0.03	66.50±0.02	72.00±0.01

(a)



(b)

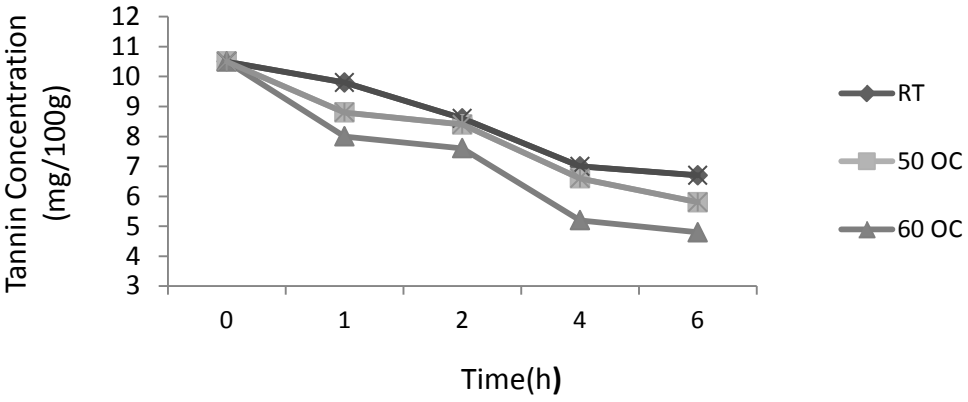
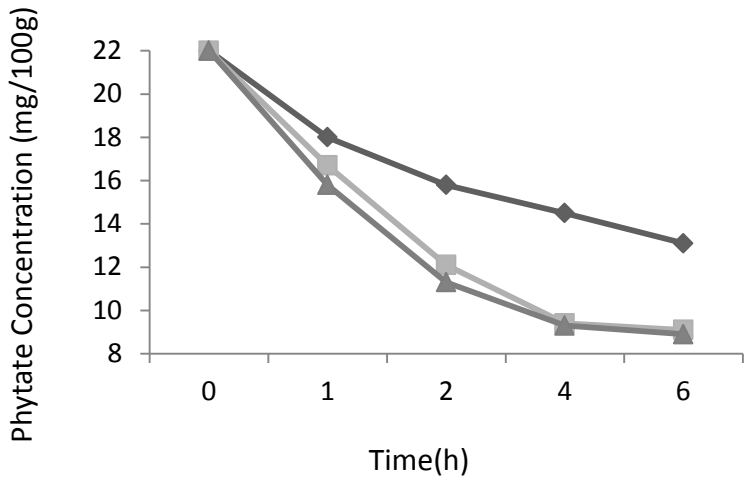


Fig.1: Effect of soaking temperature and time on tannin content of whole (a) IT90K-277-2 and (b) IT97K-568-18 bean varieties.

(i)



(ii)

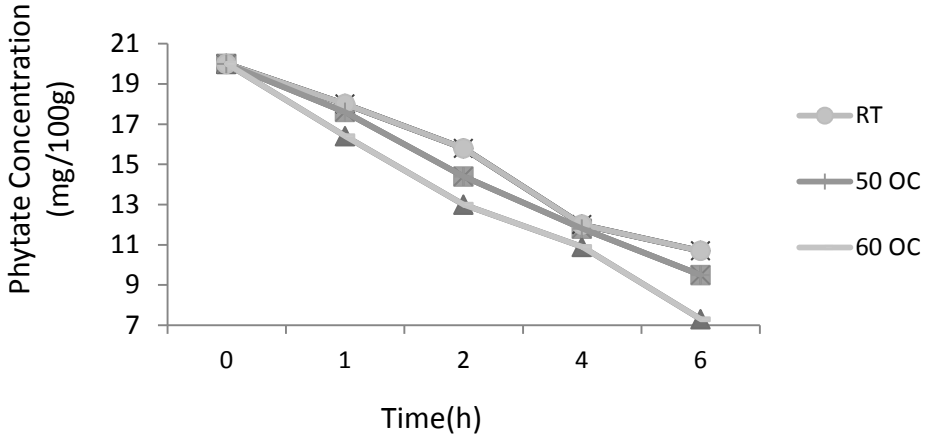


Fig.2: Effect of temperature and time on phytate content of whole beans(i) IT90K-277-2 and (ii) IT97K-568-18 bean varieties.

Fig 2 shows the effect of temperature on the phytate content of the two whole bean varieties. All the soaking conditions at room temperature, 50 ^{0}C and 60 ^{0}C reduced phytate content. At room temperature, the values ranged between 13.10 and 18.18 mg 100 g^{-1} and 10.7 and18.10 mg 100 g^{-1} denoting a reduction of 18.2 to 40.5 % and 10.0 to 46.5 % for the bean seeds of IT90K-277-2

and IT97K-568-18, respectively. Soaking at 50 ^{0}C, the phytate content ranged from 8.9 and 15.8 mg 100 g^{-1} and 7.3 and 16.4 mg 100 g^{-1} denoting a reduction of 28.10 to 59.90 and 18.0 to 63.5 % for IT90K-277-2 and IT97K-568-18, respectively. At 60 ^{0}C, more phytate was removed from whole bean seeds compared to other soaking temperatures. This may be due to the formation of insoluble complexes between phytate and other components in the beans (Udensi *et al.*, 2007). The effect of temperature was variety influenced. In variety IT90K-277-2, there was no significant difference ($p>0.05$) in the phytate content of samples soaked at 50 ^{0}C and those soaked at 60 ^{0}C. Samples soaked at room temperature had higher phytate content. This suggests that phytate is destroyed by heat

Effect of processing variables on trypsin inhibitor contents of soaked beans

The mean trypsin inhibitor activity (TIA) values for the two cowpea varieties in the raw seeds were 8.46 and 12.97 mg g^{-1} for bean seeds IT90K-277-2 and IT97K-568-18, respectively. Fig 3 shows the TIA value for whole bean seeds of the two cowpea varieties. The TIA decreased with increase in soaking time. The percentage reduction ranged from 2.96 to 25.41 % and 1.93 to 19.43 % for varieties IT90K-277-2 and IT97K-568-18, respectively for 6hrs of soaking. The rate of leaching of TIA into soaking water reduced after the fourth hour, except at 60 ^{0}C for the bean variety IT90K-277-2. TIA is retained higher in IT97K-568-18 than in IT90k-277-2 as the effect of leaching was influenced by variety.

Effect of processing variables on tannin contents of cooked beans

Tables 3 and 4 show the effect of cooking time on the leaching rate of tannin from whole beans. The tannin contents reduced with cooking time. The percentage reduction ranged from 93.0 to 94.75% and 89.9 to 95.24 % for bean varieties IT90K-277-2 and IT97K-568-18 respectively. This reduction may probably be due to its solubility and leaching into liquid media (Reddy and Pierson, 1994; Kumar *et al*., 1979).

Pressure cooking at 121 ^{0}C, and 15 psi for a time of 10 to 30 min gave lower tannin contents in the beans. The values ranged from 0.20 and 0.62 mg 100 g^{-1} denoting a reduction of 94.83 to 98.33 % and 0.30 and 0.90 mg 100 g^{-1} denoting a reduction of 91.43 to 97.14 % for bean varieties IT90K-277-2 and IT97K-568-18, respectively. Rehman and Shah (2005) reported that tannin content in red and white kidney bean significantly reduced after pressure cooking at 121

^{0}C for 20 min. The percentage reduction of tannin contents of atmospheric and pressure cooked beans stated above are higher than percentage reduction of tannin contents of soaked beans (5.83 to 45.00 %).

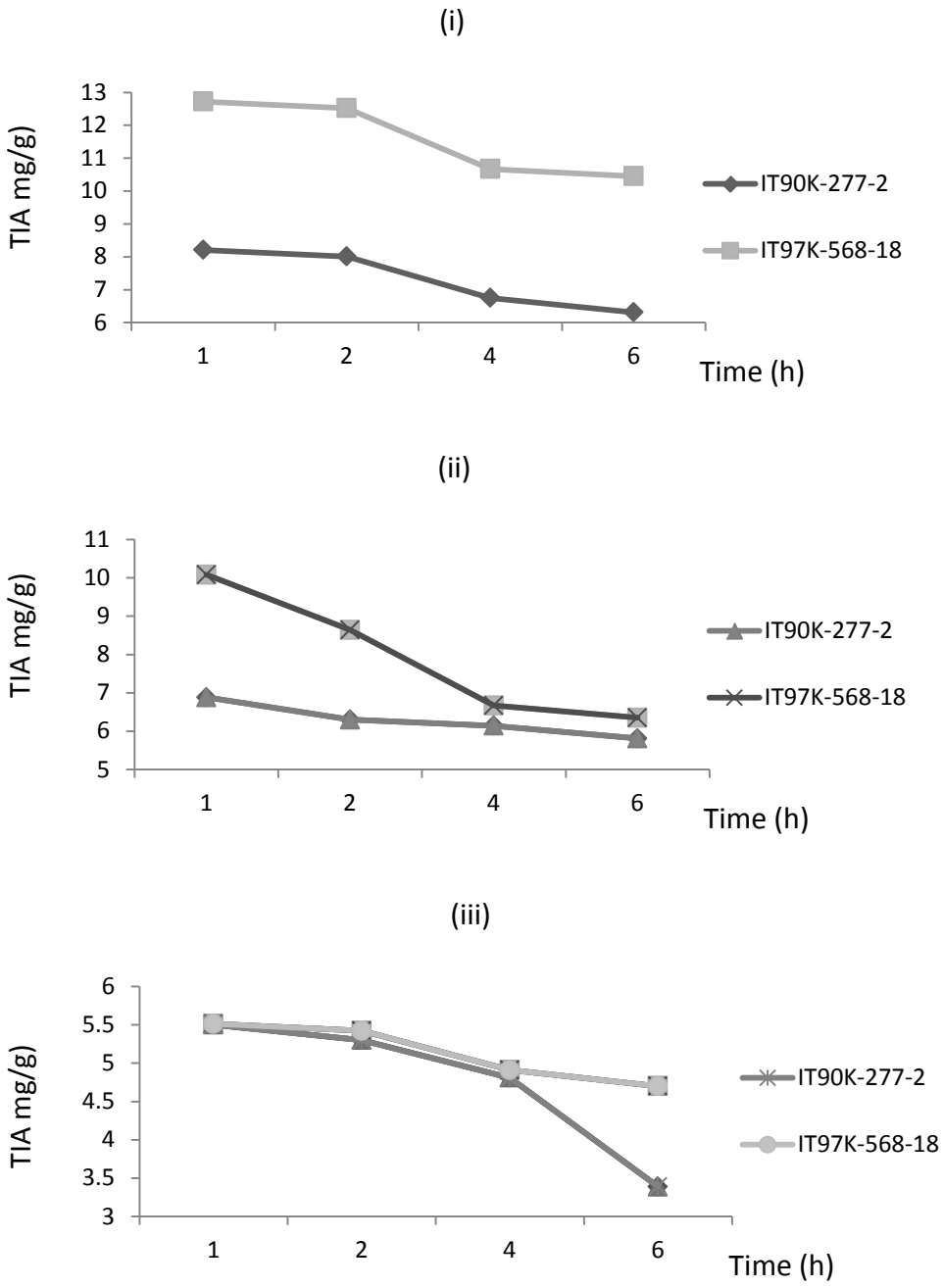


Fig. 3: Effect of soaking time on TIA of the whole bean at (i) room temperature; (ii), 50 ^{O}C; and (iii), 60^{O}C.

Effect of processing variables on phytate contents of cooked beans

The phytate contents of cooked beans reduced with increase in cooking time from 10 – 30 min as shown in Tables 3 and 4. The values ranged from 0.32 to 0.33 mg 100 g^{-1} indicating a reduction of 98.48 to 98.54 % and 0.40 to 0.41 mg 100 g^{-1} indicating reduction of 97.95 to 98.0 % for bean varieties IT90K-277-2 and IT97K-568-18, respectively. The high % reduction value obtained may be due to its leaching into cooking water.

Pressure cooking between 10 – 30 min resulted in lower phytate contents than atmospheric cooking at 100 ^{0}C for 10 – 30 min. The values ranged from 0.21 to 0.23 mg 100 g^{-1} denoting a reduction of 98.96 to 99.03 % and 0.29 to 0.33 mg 100 g^{-1}denoting a reduction of 98.5 to 98.55 % for bean varieties IT90K-277-2 and IT97K-568-18, respectively. This implies that atmospheric cooking is adequate to eliminate phytate because there is no significant difference ($p<0.05$) in the phytate contents in the two varieties. Also varying cooking time under pressure did not affect amount of phytate lost. This suggests that cooking for 10mins is adequate to remove the antinutrient but because beans require long cooking time to become edible and digestible, cooking has to be for longer duration.

Effect of processing variables on trypsin inhibitor activity (TIA) **contents of cooked beans varieties**

Tables 3 and 4 show the effect of cooking time on TIA contents of cooked bean seed varieties. TIA contents reduced with increase in cooking time from 10 to 30 min. The initial values ranged from 8.46 to 12.97 mg g^{-1} before cooking but after cooking the values were between 0.67 to 0.98 mg 100 g^{-1} denoting a reduction of 88.42 to 92.08 % and 1.74 to 2.57 mg g^{-1} denoting a reduction of 80.03 to 86.58 % for bean varieties IT90K-277-2 and IT97K-568-18, respectively. The decrease in TIA during boiling may be due to the heat-labile nature of trypsin inhibitor (Udensi *et al*., 2004) and also probably due to the formation of irreversible chemical reaction between trypsin enzymes and trypsin inhibitor complexes (Pugalenthi *et al*., 2005).

The effects of cooking methods are also shown in Tables 3 and 4. Pressure cooking at 121 ^{0}C, 15 psi for 10 – 30 min resulted into lower TIA contents than at atmospheric cooking at 100 ^{0}C for the same time period.For pressured cooked beans,the values ranged between 0 15 and 0.50 mg g^{-1} denoting a reduction of 94.09 to 98.23 % and 1.25 to 1.87 mg g^{-1} denoting a reduction of 85.58

to 90.36 % for bean varieties IT90K-277-2 and IT97K-568-18, respectively. Although pressure cooked samples lost more (TIA) than samples cooked at atmospheric pressure the difference was not significant. Also the percentage reduction of TIA was not different in the two varieties.

Table 3: Anti nutritional contents of the variety IT90K-277-2 using different cooking methods

Parameter	Atmospheric cooking (min)			Pressure cooking (min)		
	10	20	30	10	20	30
Tannin$_{(mg/100g)}$	0.84± 0.02	0.70± 0.03	0.63±0.04	0.62±0.02	0.42±0.04	0.20±0.01
(%)	(93.00)	(94.17)	(94.77)	(94.83)	(96.50)	(98.33)
Phytate$_{(mg/100g)}$	0.333±0.03	0.328±0.04	0.322±0.02	0.228±0.01	0.219±0.02	0.213±0.03
(%)	(98.48)	(98.51)	(98.54)	(98.96)	(99.00)	(99.03)
TIA (mg/g)	0.98±0.04	0.87±0.03	0.67±0.02	0.50±0.01	0.28±0.04	0.15±0.03
	(88.42)	(89.72)	(92.08)	(94.09)	(96.69)	(98.23)

Values are mean of duplicates. Values in parenthesis are percentage reduction.

CONCLUSION

Effects of time and temperature were significant ($P < 0.05$) on tannin, phytate and trypsin inhibitor activities (TIA) for up to four hours of soaking. The higher the temperature and time, the higher the reduction of anti-nutritional factors obtained. Dehulling increased percentage reduction in all the anti-nutrients studied. Cooking methods and time affected percentage reduction of TIA and tannin in the two bean varieties. Pressure cooking gave higher percentage reduction in anti-nutrient contents than atmospheric cooking. There was no significant ($P > 0.05$) difference in percentage reduction values of phytate using either pressure cooker or atmospheric cooking.

Table 4: Anti nutritional content of variety IT97K-568-18 using different cooking methods

Parameter	Atmospheric cooking (min)			Pressure cooking(min)		
	10	20	30	10	20	30
Tannin (mg/100g)	1.06±0.02	0.80±0.03	0.50±0.04	0.90±0.02	0.75±0.04	0.30±0.01
(%)	(89.90)	(92.38)	(95.24)	(91.43)	(92.86)	(97.14)
Phytate(mg/100g)	0.411±0.03	0.405±0.04	0.401±0.02	0.301±0.01	0.295±0.02	0.291±0.03
(%)	(97.95)	(97.98)	(98.00)	(98.50)	(98.53)	(98.55)
TIA (mg/g)	2.59±0.04	2.28±0.03	1.74±0.02	1.87±0.01	1.60±0.04	1.25±0.03
	(80.03)	(82.42)	(86.56)	(85.58)	(87.66)	(90.36)

Values are mean of duplicates. Values in parenthesis are percentage reduction.

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