EFFECT OF AUTOCLAVING ON THE ANTI-NUTRITIONAL FACTORS OF COWPEA VARIETIES.

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ABSTRACT

Some antinutritional factors were extracted and quantified from raw and autoclaved cowpea varieties. All the cowpea varieties in the raw state contained trypsin inhibitors, lectins HCN, tannic acid and phytic acid. Autoclaving destroyed all the trypsin inhibitors and lectins but significantly reduced HCN level while tannic and phytic acids were heat-stable. In the raw samples, values obtained for these compounds, in sequence, were 10.88 to 33.74 TIU/mg protein, 28.56 to 76.57 HU/mg protein, 1.55 to 3.90mg/100g, 0.21 to 0.39mg/g and 422.27 to 543.42mg/100g respectively. Autoclaved samples contained 0.73 to 1.32mg/100g, 0.17 to 0.36mg/g and 314.97 to 420.54mg/100g HCN, tannic acid and phytic acid respectively. The amount of total phosphorus that remained bound to phytic acid which is unavailable nutritionally ranged from 29.60 to 33.15%. Antinutritional factors concentrations differed between varieties ($P < 0.001$).

Keywords: Autoclaving, heat-labile, heat-stable, Anti-nutritional factors, cowpea varieties.

INTRODUCTION

Apart from knowing the nutrient composition of a biological substance, information on the bioavailability of nutrients must also be known. Although legumes are nutritionally promising, they have been found to contain some inherent constituents which limit their nutritive value by exerting antinutritional effects. Such effects include lowering of the bioavailability of sulphur amino acid with respect to trypsin inhibitors (Kakade *et al.*, 1974), hemagglutinating effect of lectin (Liener, 1974), lowering of mineral bioavailability by phytins (Maga, 1982), the

ability of tannins to form insoluble complexes with proteins thus interfering with the digestion process by inactivating the digestive enzymes (Bate Smith, 1973), and release of HCN by cyanogenic glycosides when the latter is hydrolysed (Montgomery, 1980). Legumes can nonetheless be safely eaten provided they are well prepared especially by adequate heat treatment (Liener, 1962; Aykroyd and Doughty, 1982; Bender, 1983). It is against this background that autoclaving was the only processing method employed.

This study was designed to quantify the antinutritional factors as they occurred in raw and autoclaved cowpea varieties and classified as Heat-stable and heat-labile.

MATERIALS AND METHODS

Cowpea varieties used for this study are described in Table 1

TABLE 1: DESCRIPTION OF COWPEA VARIETIES.

Variety	Coat Colour	Coat texture
TVU 3000	White	Rough
IT 84E-1-108	Cream	Smooth
IT 82D-889	Red	Smooth
IT 81D-1137	White	Rough
IT 84E-124	Brown	Rough

Preparation of Cowpea flour:

Five varieties of air-dried raw cowpea seeds were milled to pass through a 40 mesh sieve and put in screw-capped bottles and stored in deep freezer prior to use.

Autoclaving of samples:

Milled raw cowpea seeds were autoclaved at 1.2kgfcm^{-2} pressure for 30 minutes. Autoclaving temperature was 105°C . Cool autoclaved samples were then stored in screw-capped bottles at 4°C

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Extraction and quantification of trypsin inhibitor:

Extracts of the five cowpea varieties (raw and autoclaved) were obtained by defatting 10g of cowpea powder using 100ml. petroleum ether at room temperature.

The method of Kakade *et al.* (1969) was employed to quantify the trypsin inhibitor. Trypsin inhibitor activity (TIA) was evaluated on the basis of the extent to which a portion of an aqueous extract of the cowpea inhibited the action of trypsin on benzoyl - DL - arginine - p - nitroanilide as substrate.

Extraction and quantification of lectin:

Phytohemagglutinins (lectins) in raw and autoclaved cowpea samples were extracted in a step-wise manner and the lectins quantified according to the modified method of Liener (1955).

Estimation of phytic acid and phytic acid phosphorus:

Eight grams of defatted finely ground (raw and autoclaved) cowpea samples were soaked in 200 ml of 2% HCl solution for 3 hours and then filtered through two layers of hardened filter paper. In a 400ml beaker, 50ml of the filtrate was placed and 107ml distilled water was added to give it the proper acidity and thereafter titrated with standard ferric chloride solution containing 19.5mgFe/ml until a brownish yellow colour persisted for 5 mins. Phytic acid was thereafter determined using the method of Sutardi and Buckle (1955).

Estimation of tannic acid:

After defatting and extracting respectively with 5% ethyl ether and ethanol respectively, tannic acid was estimated using the method of Hoff and Singleton, (1977).

Estimation of hydrocyanic acid

Raw and autoclaved cowpea extracts were prepared by vigorously stirring 2g defatted sample of cowpea flour in 30ml of 0.1M orthophosphoric acid solution on a vortex for 3 mins. Total HCN contents of these samples were estimated by an autoclaved method using a Technicon AAII Autoanalyzer according to Rao and Hahn (1984).

RESULTS AND DISCUSSION

Trypsin inhibitor (TI):

TI contents of raw and autoclaved cowpea varieties presented in Table 2 reveal that IT 84E - 124 contained the lowest value of 10.88 TIU/mg protein while the highest value of 33.74 TIU/mg protein was obtained in IT81D-1137. The mean trypsin inhibitor activity for these varieties was 19.35 TIU/mg protein. No activity was observed in all the autoclaved cowpea varieties used in this study. They all recorded 100% loss in activity. Differences between varietal means were significant ($P < 0.001$). The widely held view that proteinase inhibitors are denatured by heat (Liener, 1962; Liener and Kakade, 1980; Aykroyd and Doughty, 1982) is shown to be true by the outcome of this study. However, Kunitz's discovery (1947) of a heat-labile TI in raw soyabean further lend more credence to this result in that all the TI activities were completely destroyed by autoclaving.

In addition, the claim that some trypsin inhibitors are resistant to heat treatment (Sohonie and Bhandankar, 1955; Ramshaw, 1982) was not observed in the cowpea varieties employed in this study.

Hemagglutinin (Lectin):

Table 3 shows specific hemagglutinating activity (SHA) expressed in Hemagglutinating Unit (HU)/mg protein of raw and autoclaved cowpea varieties. Percentage loss in SHA values in the raw samples ranged from 28.56 HU/mg protein in IT 84E - 124 to 76.57 HU/mg protein in IT 81D - 1137 whereas the overall average was 52.13 HU/mg protein.

In the autoclaved samples, no hemagglutinating activity was detected thus indicating a complete loss of lectin. However, differences between varietal means in the raw samples were significant ($P < 0.001$). It is evident from the result of this investigation that heat treatment was very effective in eliminating lectins from the cowpea varieties. This observation could be ascribed to the fact that lectin as a glycoprotein (Jaffe and Hanning, 1965) is easily denatured by

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adequate heat treatment. Effect of inadequate heat application was underlined by Bender (1983). He observed that kidney beans heated at 80°C for 20 mins. had increased hemagglutinating activity while beans heated at 100°C for 2 mins. lost all hemagglutinating activities.

raw and autoclaved samples. It is important to note that after autoclaving, there were appreciable losses in total HCN ranging from 52.90% in TVU 3000 to 74.36% in IT 81D - 1137 while the average percent loss was 61.92. These losses were significant ($P < 0.001$) between varietal means depicting varying

TABLE 2: TRYPSIN INHIBITOR ACTIVITIES IN RAW AND AUTOCLAVED COWPEA VARIETIES (TIU/mg PROTEIN)

Cowpea Varieties	Raw	Autoclave	% LOSS OF TI ACTIVITY
TVU 3000	13.27 c	Nil	100.00
IT 84E-1-108	12.17 c	Nil	100.00
IT 82D - 889	26.67b	Nil	100.00
IT 81D - 1137	33.74 a	Nil	100.00
IT 84E - 124	10.88 d	Nil	100.00
Mean	19.15	Nil	100.00
SE of means	±3.30	Nil	±0.00

Values are means of triplicate determination

a,b,c,d = Figures followed by different letters are significantly different ($P < 0.001$).

TABLE 3: SPECIFIC HEMAGGLUTINATING ACTIVITIES (HU/mg PROTEIN) WITH PERCENT LOSS IN ACTIVITIES AFTER AUTOCLAVING

Cowpea Varieties	Raw	Autoclaved	% Loss
TVU 3000	39.74d	Nil	100.00
IT 84E - 1-108	69.78 b	Nil	100.00
IT 82D-889	45.98 c	Nil	100.00
IT 81D - 1137	76.57 a	Nil	100.00
IT 84E - 124	28.56 e	Nil	100.00
Mean	52.13	Nil	100.00
SE of means	±6.44	Nil	±0.00

Values are means of triplicate determinations

a,b,c,d = Figures followed by different letters are significantly different ($P < 0.001$).

hydrocyanic acid:

Table 4 contains summary of HCN concentrations in raw and autoclaved samples. HCN contents in raw samples ranged from 1.55mg/100g DM in TVU 3000 to 3.90 mg/100 g DM in IT 81D- 1137 with an average of 2.85 mg/100g DM. Among the autoclaved samples, TVU 3000 had the lowest value of 0.73mg/100g DM while the highest value of 1.32mg/100g DM was recorded in IT 84E - 124 with an average of 1.03mg/100g DM. Mean values of varieties varied significantly ($P < 0.001$) in both

degrees in ability to release HCN and probably in the concentration of the appropriate beta-glucosidase enzyme that is responsible for the hydrolysis of the cyanogenic glycoside (Conn, 1973). The losses could also be attributed to the volatile nature of HCN because it has boiling point of 26°C (Viehoever, 1940).

Tannic acid:

In raw cowpea samples, IT 81D - 1137 contained the lowest concentration of tannic acid (0.21mg/g) while the highest value of

0.39mg/g was recorded in IT 84E - 124 and the average was 0.30mg/g (Table 5). Differences in varietal means were significant ($P < 0.001$). In the autoclaved samples, tannic acid levels varied from 0.17mg/g in IT 81D-1137 to 0.36mg/g in IT 84E-124. Varietal mean differences were also significant ($P < 0.001$).

Tannic acid lost after autoclaving varied between 5.41% in IT 82D-889 and 30.77% in TVU 3000. Averagely, 14.73% of tannic acid was lost. This result suggests that tannic acid is heat resistant. This may be due to the formation of linkages of tannic acid, like other phenolic compounds, with protein and other

TABLE 4: HYDROCYANIC ACID (mg/100gDM) CONTENTS WITH PERCENT LOSS AFTER AUTOCLAVING

Cowpea Varieties	Raw	Autoclaved	%Loss of HCN
TVU 3000	1.55	0.73c	52.90 c
IT 84E-1-108	3.36	1.13 b	66.37 b
IT 82D-889	2.48	0.97 b	60.89 b
IT 81D - 1137	3.90	1.00 b	74.36 a
IT 84E - 124	2.94	1.32 a	55.10 c
Mean	2.85	1.03	61.92
SE of means	± 0.28	± 0.07	± 2.76

Values are means of triplicate determinations

a,b,c, = Values followed by different letters are significantly different ($P < 0.001$).

TABLE 5: TANNIC ACID CONTENTS (mg/g DM) WITH PERCENT LOSS AFTER AUTOCLAVING

Cowpea Varieties	Raw	Autoclaved	% Loss of Tannic acid
TVU 3000	0.26 b	0.18 c	30.77 a
IT 84E-1-108	0.28 b	0.25 b	10.71 c
IT 82D - 889	0.37 a	0.35 a	5.41 d
IT 81D - 1137	0.21 c	0.17 c	19.05 b
IT 84E - 124	0.39 a	0.36 a	7.69 d
Mean	0.30	0.26	14.73
SE of means	± 0.02	± 0.03	± 2.96

Values are means of triplicate determinations.

a,b,c,d = Values followed by different letters are significantly different ($P < 0.001$).

TABLE 6: PHYTIC ACID CONTENTS (mg/100g DM) OF RAW AND AUTOCLAVED COWPEA VARIETIES WITH PERCENT LOSS AFTER AUTOCLAVING

Cowpea Varieties	Raw	Autoclaved	%Loss of Phytate
TVU 3000	543.42 a	420.54 a	22.61 b
IT 84E - 1 - 108	455.16 b	346.12 b	23.96 b
IT 82D - 889	422.27 c	337.47 b	20.08 c
IT 81D - 1137	432.66 c	314.97 c	27.20 a
IT 84E - 124	425.74 c	344.39 b	19.11 c
Mean	455.85	352.70	22.59
SE of means	± 16.01	± 12.63	± 1.02

Values are means of triplicate determinations.

Values followed by different letters are significantly different ($P < 0.001$)

macromolecule (Freudenberg, 1920). Also the intra - molecular force that exists within tannin structure would require much heat energy to overcome (Bate - Smith, 1973) thus reducing the rate of molecular thermo-disintegration thereby making elimination difficult.

Phytic acid and Phytic acid Phosphorus:

Phytic acid contents in raw cowpea samples ranged from 422.27mg/100g in IT 82D -889 to 543.42mg/100g in TVU 3000 with an average of 455.85mg/100g. Autoclaved varieties had values ranging from 314.97mg/100g in IT 81D-1137 to 420.54mg/100g in TVU 3000. The overall mean was 352.70mg/100g (Table 6). Varietal differences ($P < 0.001$) may not be unconnected with genetic variations. When compared with other legumes, cowpea seem to have higher concentration of phytic acid except soya bean. Phytic acid is present in most plant foodstuffs either as a phytate salt (Oberleas, 1973) or forming complex with protein (Anderson, 1985).

After autoclaving, loss of phytic acid ranged between 19.11% in IT 84E - 124 and 27.20% in IT 81D - 1137 with an average of 22.59. This observation depicts phytic acid as being heat-stable which may be due to a strong covalent linkage between the oxygen atoms and phosphate radicals within the phytate structure (De Boland *et al.*, 1975, O'Dell and De Boland, 1976). Phytic acid phosphorus in the raw cowpea varieties (Table 6) ranged from 0.119g/100g in IT 82D-889 to 0.153g/100g in TVU 3000 with an average of 0.128 g/100g. Values for phytic acid phosphorus expressed as percentage of total phosphorus in the raw samples ranged between 29.60 in IT 82D-889 and 33.15 in IT 81D-1137 while the average was 32.06.

Differences between means of the two variables were significant ($P < 0.001$). Because phytic acid chelates with mineral elements such as Ca, Mg, Zn, Cu, and Fe thereby reducing their bioavailability (Maga, 1982), it follows that the phosphorus content of phytic acid will be nutritionally unavailable even after autoclaving since phytic acid has been shown to be heat-stable.

In conclusion, having known the antinutritional factors that may limit nutrient utilisation in cowpea and the ability of these factors to resist heat treatment, it is now clear that trypsin inhibitor and lectin will not have any deleterious nutritional effect after cowpea has been heat-treated.

However, experiments on biological evaluation are in progress to determine the physiological effects of these antinutritional factors on cowpea utilisation by monogastric animals even after autoclaving.

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REFERENCES

- ANDERSON, P.A. (1985). Interactions between proteins and constitutes that affect protein quality. In: Finley, J.W. and Hopkins D.T. Eds., Digestibility and amino acid availability in cereals and oils seeds. Am. Assoc. of Cereal Chemists, St. Paul, Minnesota, 31 - 46.
- AYKROYD, W.R. AND DOUGHTY, J. (1982). Legumes in human nutrition, Food and Agric. Organisation (FAO), Rome, FAO Food and Nutrition Series No. 20.
- BATE - SMITH, E.C. (1973). Haemanalysis of tannins: The concept of relative astringency. *Phytochem.* 12: 907 - 912.
- BENDER, A.E. (1983). Hemagglutinins (Lectins) in beans. *Food Chem.* 11: 309 - 320.
- CONN, E.E. (1973). Cyanogenic glycosides: Their occurrence, biosynthesis and function. In Chronic cassava toxicity: Proceedings of an inter-disciplinary workshop, London, 29-30 January, 1973. IDRC. 55 - 63
- DE BOLAND, A.R., GORNER, C.B. and O'DELL, B.L. (1975). Identification and characterisation of phytates in cereal and oil seed products. *J. Agric. Food Chem.* 23: 1186 - 1189

- FREUDENBERG, K. (1920). Die chemie der Natürlichen gerbstoffe. Springer-Verlag, Berlin.
- HOFF, J.E. AND SINGLETON, K.I. (1977). A method for the determination of tannin. *J. Food Sci.* 42: 1566 - 1574.
- JAFFE, W.G. and HANNING, K. (1965). Fractionation of proteinase from Kidney beans (*Phaseolus vulgaris*). *Arch. Biochem. Biophys.* 109: 80-84.
- KAKADE, M.L., HOFFA, D.E. and LIENER, I.E. (1974): Contribution of trypsin inhibitors to the deleterious effects of unheated soybean feed to rats. *J. Nutr.* 103: 1772 - 1779
- KAKADE, M.L., SIMONS, N.R. and LIENER, I.E. (1969). An evaluation of natural versus synthetic substrates for measuring the antitryptic activity of soybean samples. *Cereal Chem.* 46: 518 - 526.
- KUNITZ, M. (1947). Crystalline soybean trypsin inhibitor. II. General properties. *J. Gen. Physiol.* 30: 291 - 310.
- LIENER, I.E. (1955). The photometric determination of the hemagglutinating activity of soyin and crude soybean extracts. *Arch. Biochem. Biophys* 54: 223 - 231.
- LIENER, I.E. (1962). Toxic factors in edible legumes and their elimination. *Am. J. Clin. Nutr.* 11: 281 - 284.
- LIENER, I.E. (1974). Phytohemagglutinins, their nutritional significance. *J. Agric. Food Chem.* 22: 17 - 22.
- LIENER, I.E. and KAKADE, M.L. (1980). Protease inhibitors, In Liener, I.E. (Ed) Toxic constituents of plant foodstuffs, 2nd Ed., Academic Press, New York.
- MAGA, J.A. (1982). Phytate. Its chemistry, occurrence, food interactions, nutritional significance and methods of analysis - Reviews. *J. Agric Food Chem.* 30: 1 - 9
- MONTGOMERY, R.D. (1980). Cyanogens. In Liener, I.E. ed., Toxic constituents of plant foodstuffs, 2nd ed. Academic Press, New York.
- OBERLEAS, D. (1973). Phytates. In: Toxicants occurring naturally in foods. National Academy of Science, Washington DC.
- O'DELL, B.L. and DE BOLAND, A. (1976). Complexation of phytate with proteins and cations in corn germ and oilseed meals. *J. Agric. Food Chem.* 24: 804-808
- PAMSHAW, J.A.M. (1982). In: Boulter, D., and PARTHIER, B. (eds) Encyclopedia of plant physiology. NS. 14A. Berlin, Springer - Verlag, 229 - 290.
- RAO, P.V. and HAHN, S.K. (1984). An automated enzymic assay for determining the cyanide content of cassava (*Manihot esculenta crantz*) and cassava products. *J. Sci Food Agric* 35: 426 - 436.
- SOHONIE, K. and BHANDANKAR, A.P. (1985). Trypsin inhibitor in Indian foodstuffs. I. Inhibitors in vegetables. *J. Sci. Ind. Res.* 138: 500 - 503.
- SUTARDI, and BUCKLE, K.A. (1985). Reduction in phytic acid levels in soybeans during tempeh production, storage and frying. *J. Food Sci.* 50: 260 - 261.
- VIEHOEVER, A. (1940). Edible and poisonous beans of the Lima type (*Phaseolus lunatus L.*). Comparative study including other similar beans. *Thailand Sci. Bull.* 2: 1-99.