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## Effect of De Cortication and Roasting on Trypsin Inhibitors and Tannin Contents of Cowpea (*Vigna unguiculata* L. Walp) Seeds

Nasara Gumaa Balail

Department of Animal Nutrition, Faculty of Agriculture, University of Sinnar, Abu Naama, Sudan

**Abstract:** This study was carried out to evaluate the nutritive value of cowpea seeds. Also to minimize the presence of some anti nutritional factors such as tannin and trypsin inhibitors. It is aimed to determined the best method by which we can reduce the anti nutritional factors. Samples of raw, decorticated and decorticated roasted cowpea seeds were subjected to proximate analysis. The results show that for dry matter (93.63, 94.33 and 95.9), for crude protein (29.18, 31.80 and 29.73), for ash (4.60, 4.0 and 5.52) for fiber (6.22, 3.75 and 4.32) for ether extract (2.30, 2.50 and 1.60) for nitrogen free extract (51.33, 52.28 and 54.73), respectively for raw, decorticated and decorticated roasted seeds. Tannin content percentages were determined using method. The results were (0.76, 0.02 and 0.005), respectively. Trypsin inhibitors were determined using enzymatic method the results were (1.68, 0.74 and 1.36), respectively for raw, decorticated and decorticated roasted cowpea seeds. It is concluded that chemical composition was varied between the treatments. Decorticated seeds gives high level of protein followed by the others. De cortication significantly reduced tannin content by 85%. Roasting significantly decreased trypsin inhibitors by 65%. Processing of cowpea seeds either mechanically or by heat, significantly improve their nutritional value.

**Key words:** Cowpea, de cortication, roasting, tannin, trypsin inhibitors

### INTRODUCTION

The cowpea (*Vigna unguiculata* L. Walp) belongs to the family leguminosae, other names commonly used include catjang, black-eyed bean or China pea (Taiwo, 1998), southern pea, clossus, or crowther peas (Uzogara and Ofuya, 1992). In the Sudan, it was known as lubia helo or white lubia.

There are many varieties of cowpeas that differ in nutrients content. They are a good source of protein and minerals. This variation also due to agricultural practices and geographical location (Kochhar *et al.*, 1988). Most of the nutrients are concentrated in the cotyledons as it makes up most of the seed weight. The proximate composition of cowpeas is shown as following (%): Crude protein range (18.3-35.0), crude fat (0.7-3.5), crude fiber (2.7-7.0), starch (31.5-48.0) and the ash (2.5-4.9) (Chavan *et al.*, 1989). Cowpeas include some antinutritional factors such as trypsin inhibitors, oligosaccharides and polyphenols (Chavan *et al.*, 1989). Trypsin inhibitor is known to inhibit the action of the enzyme trypsin. It has anegative effect on amino acid methionine liberation from protein. Generally it affect the digestion (Obloh *et al.*, 1998). Oligosaccharides are not digested by mono-gastric animals and they are thus fermented by microbes in the colon, which results in the production of flatus and other discomfort (Onyenekwe *et al.*, 2000).

Cowpea seeds contain anti-nutrients such as oligosaccharide phytate, condensed tannins, trypsin inhibitors and cystine inhibitors, their values are 5.85, 1.23, 0.9, 0.51 and 0.11, respectively in percentage as received (Pettersson *et al.*, 1997). These toxic constituents are found generally in several varieties of cowpeas, high levels of trypsin inhibitors and low hemagglutinating activity had been recorded (Aletor and Aladetimi, 1989).

The anti-nutritional factors of cowpeas can be reduced and nutritional quality improved by plant breeding de-hulling, heat treatment or supplementation of diets with enzymes.

Heat treatment such as cooking, autoclaving, steaming and roasting has been used to improve the nutritional value of cowpeas. Ologhobo and Fetuga (1984) conducted an experiment to reduce tannin content of cowpea seeds. They used different treatments; autoclaving, cooking, soaking and germination. They observed that tannic acid content decreased, the range were 0.33-0.69, 0.23-0.42, 0.37-0.69 and 0.29-0.56% for the above treatments, respectively, compared with the tannic acid content in raw seed which equal to 0.42-0.78%. Boiling and pressure cooking was destroyed tannin content and trypsin inhibitors of some legumes species. The varieties with the most tannin white cowpea seemed to be the best for feed efficiency. Plahar *et al.* (1997) observed that roasting decreased

tannin concentration of cowpea cultivars, they were 0.3-6.9 and 7.2-11.6 mg ceg<sup>-1</sup> flour from whole cowpea seeds and seed coats, respectively. Roasting is an effective method by which we can reduce the trypsin inhibitors and improve the nutritional value of cowpea seeds. However, imprvove the digestibility Roasting and pressure-cooking increased protein ratio values of the cowpeas by decreasing tannin and trypsin inhibitors activities. Some amino acids such as available lysine was not significantly affected by heating (Loayza and Bressani, 1988).

De-cortication of grain legumes seeds is an effective method for reducing tannin content that localized in the seed testa. Plahar *et al.* (1997) found that de-hulling removed the most tannin content of raw cowpeas seed. De-hulling of seeds after soaking for 1 h then soaking for 45 min and sun drying to 5% moisture, destroyed trypsin inhibitors activity almost entirely (Abbey and Nkang, 1988). The objective of this study is to investigate the effect of mechanical processing (de cortication) and heat treatment (roasting) on tannin and trypsin inhibitor content.

**MATERIALS AND METHODS**

The nutrients compositions of cowpea (*Vigna unguiculata*) seeds were carried out in Laboratory of Animal Nutrition, Faculty of Agriculture, University of Khartoum. Proximate analysis of seeds used in this experiment was shown in Table 1.

**Preparation of raw and processed cowpeas seeds:**

- Raw cowpeas seeds were milled using a local grinder
- Raw cowpeas seeds were decorticated mechanically, there after sieved so as to remove the seed coat leaving behind the smooth seed sample
- The de-corticated cowpeas seeds were roasted by using electrical furnace. Roasting process was carried out at temperature of 120°C for 30 min, the seeds were continually stirred until a characteristics slightly brown colored seeds was obtained. Roasting process were carried out after de-cortication

**Determination of tannin in raw and treated sample:**

Quantitative estimation of tannin for each sample was carried out using the modified vanillin HCl in methanol methods as described by Price *et al.* (1978). The specific reagent for the determination encompasses equal volumes of 1% vanillin in methanol (w/v) and 8% concentration

Table 1: Chemical analysis of raw and treated cowpeas seeds (%)

Treatment items (%)	Raw seeds	Decorticated seeds	Decorticated roasted
Dry matter	93.63	94.33	95.900
Protein	29.18	31.80	29.730
Ash	4.60	4.00	5.520
Fiber	6.22	3.75	4.320
Ether extract	2.30	2.50	1.600
Tannin (%)	0.76	0.02	0.005
Trypsin (mg g <sup>-1</sup> )	1.68	0.74	1.300
NEF	51.33	52.28	54.730

Analyzed by Lab of Animal Nutrition, Faculty of Agriculture, University of Ain Shams

HCl in methanol (v/v). These are tobe mixed just prior to use and were rejected whenever trace of the manipulation is in the sequence of 0.2 g of the ground sample placed into a 100 mL conical flask. Ten milliliters of 1% HCl in methanol (v/v) were added, shaken for 20 min. Using mechanical shaken and centrifuged at 2500 rpm. One mililiter of the supernatant was pipette into a test tube and 5 mL of vanillin HCl reagent were added. The optical density was read using colorimeter at 500 nm after 20 min incubation at 30°C, for zero setting colorimeter 1 mL of a blank (1% HCl in methanol (v/v) and 5 mL vanillin-HCl reagent in a test tube. The blank mixture was incubated at 30°C for 2 min.

**Calculation:**

$$QE (\%) = \frac{C \times 10 \times 100}{200}$$

Where:

- C = Concentration corresponding to optical density
- 10 = Volume of extract in mL
- 200 = Sample weigh in mg
- QE = Quantitative estimation

**Determiration of trypsin inhibitory activity:** The extract of the samples was obtained by shaking 4 g of the sample with 40 mL of 0.1 m phosphate buffer pH 7.5.

Buffer solution = (16 mL of 0.2 Na H<sub>2</sub>PO<sub>4</sub>)+84 mL Na HPO<sub>4</sub> (0.2 m) then diluted to 200 mL. Shaking the sample by using a mechanical shaker (shutted machine R%) for 3 h at room temperature. The extracts were then centrifuged at ambient temperature at 3000 rpm for 20 min.

The supernatants were diluted two times and then used for analysis.

For the essay of enzymatic activity, the casein substrate was used for determining trypsin inhibitor activity in the crude preparations (phosphate buffer extracts).

A 2% casein solution in phosphate buffer (0.1 M pH 7.5) was used as substrate while the enzyme used as trypsin (Beef pancreas), 5 mg mL<sup>-1</sup>. The incubation mixture consisted 0.5 mL trypsin. Two milliliters 2% casein, 0.9 mL phosphate buffer (0.1 M pH 7.5) 0.4 mL HCl solution (0.001 M) and 0.2 phosphate buffer extract. In all cases the total volume of incubation mixture were kept at 4 mL. Incubation was carried out at 37°C for 20 min, after which 60 mL of TCA solution was added to stop the reaction and corresponding blanks were run concurrently.

In this method one Trypsin Unit (TU) is arbitrarily defined as an increase of 0.01 absorbance unit at 280 nm in 20 min for 10 mL of reaction mixture under the conditions and the trypsin inhibitor activity as the number of trypsin units inhibited (Kumitz, 1947).

### RESULTS AND DISCUSSION

Chemical analysis, tannin content and trypsin inhibitors content of treated cowpeas seeds are given in Table 1. As a result of de cortication, tannin content decreased by 85%. Roasting decreased trypsin inhibitors by 56%. Dec-corticated roasted seeds contain 0.005% tannin.

Primary differences were noted in chemical and anti-nutritional factors contents of (*Vigna unguiculata*) cowpea seeds. Raw cowpea seeds contain (29.18%) crude protein. This legume contained substantial quantities of anti-nutritional factors. Therefore, processing must be done to reduce these toxicants. In the present study roasting at 200°C for 30 min reduced trypsin inhibitors and tannins contents, but left residual amount of these two ANFs. De-cortication resulting in high concentration of crude protein reduced the tannin contents and crude fiber. The results of the present study confirmed the well established view that de corticated legume seeds lead to high recovery of trypsin inhibitors activity in the cotyledons (Vanderpod, 1989).

Chemical composition of processed cowpea seeds either mechanically by de cortication or physically by dry heating (roasting) revealed that protein quantity and quality were improved. This attributed to the reduction of ANFs that contained in the cowpea seeds. This coincided with Andy (2006) finding who observed that processing of cowpea seeds by dry heating reduce trypsin inhibitors by 45%. This was in agreement with Simoongwe (1998) who reported that roasting legume can decrease the content of trypsin inhibitors. Dehulling resulting in high concentrations of crude protein and reduced the fiber contents in cowpea seeds. These results of the present study confirmed the well established view that de

cortication of legume seeds leads to high recovery of trypsin inhibitors activity and haemagglutinin in the cotyledons (Van Der Poel, 1990).

### CONCLUSION

It is concluded that de cortication significantly reduced tannin content by 85%. Roasting significantly decreased trypsin inhibitors by 65%. Processing of cowpea seeds either mechanically or by heat, significantly improve their nutritional value.

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