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Anti-Nutritional Factors of Green Leaves of *Cassia obtusifolia* and Kawal

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Abstract

Cassia obtusifolia (family leguminous) is a wild African plant found in wastelands in the rainy season. Its leaves can be fermented (named kawal) and is used by people from the eastern part of Chad and the western part of Sudan as meat replacer or meat extender. The role of kawal and the like is in providing the sauces which make these staples palatable. During famine years, kawal, a protein source, probably protected many children against kwashiorkor. Until a few years ago, kawal was little known to most Sudanese, for it was a product confined to the western provinces of the country, away from populated areas and centers of influence. Then as today, kawal was shunned by the elite who consider it unfit for modern social life because of its repugnant, fetid odor that lingers on the fingers for hours. The objectives of this study were to assess the effect of fermentation on anti-nutritional factors of *Cassia obtusifolia* leaves. The *in vitro* protein digestibility was significant (P<0.05) increased from 49.43 to 61.87%. It is recommended to use fermentation to decrease anti-nutritional factors of *Cassia obtusifolia*.

Keywords: Fermentation; Kawal; Anti-nutrutional; Phytic; Tannin

Introduction

The fermentation process that an African woman employed was behind the dramatic improvement in the protein value of the food [1]. The fermentation of meals of cereal and legumes knows to increases the protein content [2]. The international community of food scientists has, in the past decades, shown a deep interest in three areas of food science and technology. The first of these is the area of indigenous fermented foods, where a preponderance of literature has revealed interesting facts including a substantial enhancement of the food as a result microbial growth in it [3]. The second field of interest is the areas of solid substrate fermentation in which the substance to be fermented albeit wet, is not fluid [4]. The third area is that of leaf protein [5]. Scientists, in their relentless quest for new protein sources to help feed an ever-increasing world population, found that the plant leaf can be a truly commendable candidate. *Cassia obtusifolia* (family leguminous) is a wild African plant found in wastelands in the rainy season. Its leaves can be fermented (named kawal) and is used by people from the eastern part of Chad and the western part of Sudan as meat replacer or meat extender [6]. The role of kawal and the like is in providing the sauces which make these staples palatable. During famine years, kawal, a protein source, probably protected many children against kwashiorkor. Until a few years ago, kawal was little known to most Sudanese, for it was a product confined to the western provinces of the country, away from populated areas and centers of influence. Then as today, kawal was shunned by the elite who consider it unfit for modern social life because of its repugnant, fetid odor that lingers on the fingers for hours. The objectives of this study was to assess the effect of fermentation on the anti-nutritional factors of *Cassia obtusifolia*.

Material and Methods

Kawal preparation method

In kawal fermentation according to [6], the green leaves are first freed of all extraneous matter, such as leaves of other plants, pods and flowers of the kawal plant itself, caterpillars and insect-damaged leaves. This process of sorting out the kawal leaves is strictly observed and in fact this part of the preparation procedure is the most tedious step as it takes hours of painstaking work. Green flower buds and delicate young pods may, however, be processed with the green leaves. The unwashed, healthy green leaves, now clean from all adulteration are beaten in a mortar-and -pestle to give a green paste. Pounding is done in such a way that the leaves are crushed without releasing their juice. In the final paste can be seen partially crushed leaves, twigs, mid-ribs and petioles. Meanwhile, a pit is dug in the ground in a shaded cool place. And earthenware pot (Burma) is fitted into the pit, leaving only the neck of the container above ground. The green paste is now packed into the pot by hand. Next, green sorghum leaves are folded onto the surface of the leaf paste in the Burma so that it is completely covered. Washed, dry stones are then placed on top of the sorghum leaves to weight them down. The mouth of the pot is then covered with some metal tray or dish and the whole sealed off with mud to prevent insect from entering. Every 3-4 days the jar is opened, the now yellow and dry sorghum leaves removed and the Burma thoroughly hand-mixed and repacked, this time a little loose. Fresh sorghum leaves are folded on the surface of the past and weighted down as before the Burma covered and sealed off again. The paste is next molded into small. Irregular balls or flattish cakes which are then sun dried for 3-4 days. The duration of the fermentation is about 25 days for the supply of an average family. *Cassia obtusifolia* leaves and kawal were obtained in dry form after been sun dried and freed from foreign materials and powdered by hummer mill with same mesh size and was kept in clean bottles at room temperature for further use.

Anti-nutritional factors determination

Determination of phytic acid content: Phytic acid content was determined by the method described by Wheeler and Ferrel [7]. Two grams of dried sample were weighted in 125 ml conical flask. The sample was extracted with 50 ml of 3% trichloroacetic acid (TCA) for 3 hr with mechanical shaking. The supernatant was centrifuged for 5 min. ten milliliters aliquot of the supernatant was transferred to a 40 ml tube

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and 4 ml of $FeCl_3$ ($Fecl_3$ solution containing 2 mg Fe^{+3} ion/ml 3% TCA) were then added to the aliquot. The tube was heated in a boiling water bath for 45 min. One or two drops of 3% sodium sulphate (Na_2SO_4) in 3% TCA were added the tube was cooled and centrifuged for 10-15 min and the clear supernatant was decanted. The precipitate was washed by dispersing well in 25 ml 3% TCA, heated for 10-15 min in boiling water bath and then centrifuged again. Washing was repeated with distilled water , the washed precipitate was dispersed in few milliliters of distill water enriched with 3 ml of 1.5 N NaOH , and the volume completed to approximately 30 ml with distilled water . Heated on boiling water bath for 30 min and hot filtered using whatman No.2. The precipitate was washed with 60-70 ml hot water, and the washing was decanted. The precipitate from the filter paper was dissolved in 40 ml hot 3.2 N HNO_3 and placed in 100 ml volumetric flask. The paper was washed with hot distilled water and the washing was collected in the same flask then completed to volume. A 0.5 ml of aliquot was taken from the above solution and transferred into 10 ml volumetric flask. then 2 ml of 1.5 N KSCN(potassium) were added and completed to volume by water then immediately (with one min) read at 480 nm using (SP6 PyUnieam) spectrophotometer.

A standard curve of different Fe $(NO_3)^3$ concentrations was plotted to calculate the ferric ion concentration. The phytate phosphorus was calculated from the Iron concentration assuming 4:6 Iron to phosphorus molar ration.

$$\text{Phytate (mg/100 g)} = \frac{6/4\ A\times C\times 20\times 10\times 50\times 100}{1000\times S}$$

Where:

A = optical density

C = concentration corresponding to optical density

S = weight of sample

Determination of tannin content: Tannin content (TC) of *Cassia obtusifolia* leaves and kawal samples were estimated using modified vanillin-HCl in methanol as described by Price et al. [8]. About 0.2 g of ground sample was placed in 100 ml conical flask. 10 ml of 1% Hcl in methanol (v/v) were added, the contents were mechanical shaking for 20 min and centrifuged at 2500 rpm for 5 min. One ml of supernatant was pipettes into a test tube and 5 ml of vanillin-Hcl reagent (mixing equal volume of 8% concentrated Hcl in methanol and 1% vanillin in methanol) were added. The optical density was read using a colorimeter (Lab system Analyzer 9 filters, j, Mitra and Bros.Pvt .Ltd.) at 500 nm after 20 min incubation at 30°C, a blank sample was carried out with each run of samples. A standard curved was repeated expressing the result of tannic acid, i.e. amount of tannic (mg per ml) which gives color intensity equivalent to that given by tannin after correcting for blank.

Calculation:

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

Where:

C = concentration corresponding to optical density

10 = volume of extract in ml

200 = sample weight in mg

Total polyphenol (TP) determination: Polyphenolic of each sample was estimated using Prussian blue assay, as described by Price and Butter [9]. About 60 mg of ground sample was extracted with 3 ml methanol in a 50 ml conical flask, and then poured into a filter paper. The tube was quickly rinsed with additional 3 ml methanol and the content poured once into the filter paper. the filtrate was diluted to 50 ml with distilled water , mixed with 3 ml 0.1 M $Fecl_3$ in 0.1 N Hcl for 3 min, followed by the time addition of 3 ml 0.008 M $K_3Fe(CN)_6$. The absorption was read after 10 min at 720 nm on spectrophotometer (corning, 259).

Standard curve preparation: Tannic acid standard curve was prepared by dissolving 100 mg tannic acid in distilled water in a 0.1 liter volumetric flask and made up to mark . This spread stock solution of 100 ppm. Various standard concentrations (0, 2, 4, 6, 8 and 10) were repeated. The Prussian blue assay described above was then employed to the standard solution. The standard curve was obtained by plotting concentration against the corresponding absorbance reading, which gave linear relationship.

Calculation:

$$\text{Total polyphenol (\%)} = \frac{C\times 56\times 100}{60}$$

Where,

C = concentration corresponding to optical density

56 = total volume

60 = weight of sample in milligrams

Results and Discussion

Anti-nutritional factors of green leaves of *Cassia obtusifolia* and kawal is shown on Table 1. Fermentation was found to cause highly significant decrease ($p>0.05$) in phytic acid content. The phytic acid content was decreased from 649.13 to 340.92 mg/100 g. Generally fermentation is known to cause highly reduction in phytic acid content due to the low pH of fermented dough which considered to be optimum for the phytase activity. Fermentation was found to cause highly significant decrease ($p>0.05$) in tannin content. The tannin content was decreased from 2.39 to 2.24%. The values obtained in this study were in agreement with the value obtained by Babiker et al. [10] who reported that the tannin content of green leaves of *Cassia obtusifolia* 2.34%. But lower than the value obtained by Abdalla [11] who reported 2.50% for tannin content of green leaves of *Cassia Obtusifolia*. Fermentation was found to cause degradation of tannin content and this may be due to the action of enzymes. Fermentation was found to cause highly significant decrease ($p>0.05$) in total polyphenol content. The total polyphenol content was decreased from 4.77 to 3.80%. The total polyphenol of green leaves of *Cassia obtusifolia* in this study were in agreement with the value obtained by Ousman et al. [12] who was

sample	Tannin %	Phytic acid mg/100g	Polyphenol %
Cassia obtusifolia Leaves	2.39 (±0.012)[a]	649.13 (±7.137)[a]	4.77m (±0.252)[a]
Dry Kawal	2.24 (±0.021)[b]	340.92 (±5.952)[b]	3.80 (±0.200)[b]

- Each value in an average of three values expressed on dry weight basis.
- Values are means (± standard deviation).
- Means not sharing a common letter in a column are significant at $p \geq 0.05$ as assessed by Duncan's multiple range tests.

Table 1: Anti-nutritional factorof green leaves of *Cassia obtusifolia* and kawal (as dry matter).

reported 4.8%. Reduction in polyphenols may be due to activation of polyphenol oxidase [13].

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