Chemical composition, antinutrients and extractable minerals of Sicklepod (*Cassia obtusifolia*) leaves as influenced by fermentation and cooking

¹Nuha, M. O., ²Isam, A. M. A. and *,¹Elfadil, E. B.

¹Department of Food Science and Technology, Faculty of Agriculture, University of Khartoum, Khartoum North, P. O. Box 32, Shambat, Sudan ²United Graduate School of Agricultural Sciences, Tottori University, Tottori, Japan

Abstract: In Sudan Sicklepod (*Cassia obtusifolia*) leaves after fermentation and drying are commonly known as Kawal. The study was conducted to investigate changes in chemical composition, antinutritional factors and minerals content and extractability of two samples of Sicklepod leaves of different origin. The samples were fermented and dried into powder and then cooked in boiling water for a certain period of time (5 min). The dry matter, oil, fiber and carbohydrates contents were fluctuated during processing of both samples. However, the protein and ash contents increased after cooking of the samples. The total energy for both samples decreased. The antinutritional factors (tannin, phytate and total polyphenols) of the samples were significantly ($P \le 0.05$) decreased after cooking. Tannin content significantly ($P \le 0.05$) increased after fermentation but decreased after cooking. Ca and K were the major mineral constituents of raw leaves for both samples. Total major minerals were significantly ($P \le 0.05$) increased after processing. However, the trace minerals content was fluctuated. HCl-extractability of minerals for both samples was very low and fluctuated.

Keywords: Kawal, fermentation, cooking, antinutrients, chemical composition, minerals, extractability

Introduction

Leafy vegetables are well known sources of proteins, vitamins, minerals, dietary fiber and are low in carbohydrates and fats (Negi and Roy, 2001; Ganiyu, 2005). Leaves often contain toxic substances such as oxalic acid, nitrates, glycosides of hydrocyanic acid and alkaloids (Ganiyu, 2005). They also contain antinutritional factors such as tannins, oxalates (Falade et al., 2004), nitrates and polyphenols (Ousman et al., 2005). Sicklepod (Cassia obtusifolia) which belongs to leguminosae family is an annual, wild legume that has been described as an under shrub a herbaceous plant. In Sudan Sicklepod leaves are prepared by a solid state process of fermentation and used as an ingredient of sauces destined for consumption with porridge. It imparts a savory and a meaty flavour on the sauce (Dirar, 1993).

Kawal (fermented Sicklepod leaves) is used in relatively large quantities in the preparation of sauces as a meat substitute or meat extender by poor people and in small quantities as a spice by some urban rich people. It is used wildly by Fur who invented it and other neighbouring tribes (Dirar, 1993). The seeds of *Cassia obtusifolia* exert toxic effect on human skeletal muscles, kidney and liver. The green and dry leaves as well as the stem also contained toxins. The use of toxic plants as food after fermentation or heat treatment is known in Africa and other parts of the world (Ganiyu, 2005). Antinutritional factors such as enzyme inhibitors, hemagglutinin, flatulence factors, polyphenols, tannin and phytic acid inhibit the proteolytic activity of the digestive enzymes such as pepsin and trypsin as well as the availability of minerals (Deshpande and Cheryan, 1984).

Minerals from plant sources, particularly those from plant seeds are less bioaccessible than those from animal's sources due in part to phytic acid, tannins and fiber content (Moelijopawiro *et al.*, 1998). These antinutritional factors chelate dietary minerals in the gastrointestinal tract reducing bioaccessibility and bioavailability (Frolich, 1995). Polyphenols can form complexes with metal cations through carboxylic and hydroxylic groups and thus interfere with the intestinal absorption of essential minerals such as calcium (Valencia *et al.*, 1999).

Current evidence strongly supports a contribution of polyphenols to the prevention of cardiovascular

diseases (cancers) and osteoporosis and suggests a role in the prevention of neurodegenerative diseases and diabetes mellitus (Scalbert et al., 2005). Phytic acid is naturally occurring as phosphorylated carbohydrate found in plant and in almost all mammalian cells. It consists of a myo-inositol ring with six phosphate moieties and it chelates metal irons such as Ca, Mg, Zn, Mn, Cu and Fe to form insoluble complexes that are not readily absorbed (Graf and Eaton, 1993). Tannins also complex with enzymes of the digestive tract adversely affecting utilization of proteins and carbohydrates and resulting in reduced growth, feeding efficiency, metabolizable energy and bioavailability of amino acids (Onyango et al., 2005). Processing techniques such as soaking, cooking, germination and fermentation have been found to reduce significantly the levels of phytates and tannins by exogenous and endogenous enzymes formed during processing (Mosha and Svanberg, 1990). Sicklepod leaves was found to be rich in Ca, Mg and P as major minerals while Fe, Zn and Mn were determined at lower levels (Ousman et al., 2005). The objective of this study is to investigate the effect of fermentation and cooking of Sicklepod leaves on antinutritional factors contents and total and extractable minerals

Materials and Methods

Materials

Two different samples of dry Sicklepod (*Cassia obtusifolia* L) leaves were obtained from Algenina and Zalngy, Western States, Sudan. The leaves were cleaned from stems, seeds and foreign materials and then air dried. Leaves were ground to pass a 0.25 mm screen and stored at 0 °C until used. Unless otherwise stated all reagents used in this study were of reagent grade.

Methods

Fermentation of Sicklepod leaves

An earthenware jar was used in the fermentation process and prepared as follows: The interior of the jar is plastered with very sticky type of mud of the same kind used for pottery. The walls and the floor of the container are beaten to firmness using a stone. Then the interior is rubbed with a mucilaginous substance usually okra, but frequently, a wild plant called abadeib, which prevents the mixing of kawal with soil. The jar is buried in the ground up to the neck in a cool place (under a tree). Then the Sicklepod leaves were cleaned and pounded into paste without releasing the juice. The paste is placed in the earthenware jar and covered with sorghum leaves. 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 entering. Every 3 days the contents were mixed by hand and covered with new sorghum leaves. After 14 days, the strongly smelling black fermented paste is made into small balls and sun dried for 3-4 days, usually on a raised wooden platform called shukkaba. Then the balls were ground to pass a 0.25 mm screen and stored at 0° C until used.

Cooking of fermented Sicklepod leaves

The fermented leaves were cooked for 5 minutes in boiling water and dried in an oven at 50 °C for 16 h and then ground to pass a 0.25 mm screen and stored at 0°C until used.

Proximate composition determination

The proximate composition of raw and processed leaves was determined according to the AOAC (1984) methods.

Tannin content determination

Quantitative estimation of tannins was carried out using the modified vanillin–HCl method (Price *et al.*, 1978). A 200 mg sample was extracted using 10 mL 1% (v/v) concentrated HCl in methanol for 20 min in capped rotating test tubes. Vanillin reagent (0.5%, 5 mL) was added to the extract (1 mL) and the absorbance of the colour developed after 20 min at 30 °C was read at 500 nm. A standard curve was prepared expressing the results as catechin equivalents, i.e. amount of catechin (mg/ml) which gives a colour intensity equivalent to that given by tannins after correcting for blank. Then tannin content (%) was calculated according to the equation:

> Catechin equivalent (CE)% = C x Volume extracted (10 mL) x 100 Weight of sample (g)

Where C, concentration obtained from the standard curve (mg/mL).

Phytic acid content determination

Phytic acid content was determined according to the method described by Wheeler and Ferrel (1971) using 2.0 grams of dried sample. A standard curve of different Fe $(NO_3)_2$ concentrations was plotted to calculate the ferric ion concentration. Phytate phosphorus was calculated from the standard curve assuming 4:6 iron to phosphorus molar ratio.

Total polyphenols determination

Total polyphenols were determined by spectrophotometric method described by Price and Butler (1977). About 60 mg of the sample were shaken manually for 60 second with 3 ml of methanol in a test tube. The mixture was filtered, then the tube was guickly rinsed with additional 3 ml of methanol and the contents were poured at once into a funnel. The filtrate was mixed with 50 ml of water and analyzed within an hour. Three ml of 0.1M FeCl, in 0.1N HCl were added to 1 ml of filtrate, followed immediately by timed addition of 3 ml of 0.008M $K_{3}Fe(CN)_{6}$ The absorbance was read at 720nm after 10 min using spectrophotometer (Jenway 6306 uv/vis spectrophotometer). Tannic acid was used to prepare a standard curve following the above procedure.

Minerals composition

Minerals were extracted from the samples by the dry ashing method that described by Chapman and Pratt (1982). About 2.0 g of sample was aciddigested with diacid mixture (HNO3:HCIO4, 5:1, v/v) in a digestion chamber. The digested samples were dissolved in double-distilled water and filtered (Whatman No. 42). The filtrate was made to 50 mL with double-distilled water and was used for determination of total minerals. Calcium was determined by a titration method. Phosphorus was determined spectrophotometerically by using molybdovanadate method. All other minerals were determined by atomic absorption spectrophotometer (Perkin–Elmer 2380).

HCl extractability of minerals (in vitro bioavailability)

Minerals in the samples were extracted by the method described by Chauhan and Mahjan (1988). About 1.0 gm of the sample was shaken with 10 mL of 0.03 m HCl for 3 h at 37°C and then filtered. The clear extract obtained was oven-dried at 100°C and then acid-digested. The amount of the extractable minerals was determined by the methods described above. HCl extractability (%) was determined as follows:

Mineral extractability % =

Mineral extractable in 0.03 HCl (mg/100g) × 100 Total Mineral (mg/100g)

Statistical analysis

Each determination was carried out on three separate samples and analyzed in triplicate and figures were then averaged. Data were assessed by the analysis of variance (ANOVA) (Snedecor & Cochran, 1987). Duncan's multiple rang test was used to separate means. Significance was accepted at $P \le 0.05$.

Results and Discussion

Chemical composition of raw and processed Sicklepod leaves

Table 1 shows the proximate composition (%) of treated and untreated Sicklepod (Cassia obtusifolia) leaves from different origins. The dry matter, protein and fiber contents of the raw leaves from Algenina were lower than those from Zalngy. However, the oil, ash and carbohydrate contents from Algenina were higher than those from Zalngy. The energy (Kcal/100g) obtained from Algenina was higher than that from Zalngy. Fermentation of Algenina sample significantly ($P \le 0.05$) reduced the dry matter from 93.50 to 90.70% while that of Zalngy was slightly reduced from 93.93 to 92.50%. The reduction in dry matter of both samples is likely to be attributed to utilization of nutrients by microorganisms during fermentation as explained by Hong et al. (2005). Cooking of Algenina sample increased the level of the dry matter to 92.05% but still below the raw sample and significantly ($P \le 0.05$) increased the dry matter of Zalngy sample to 95.00% compared to the raw. Fermentation and cooking of Algenina sample was significantly ($P \le 0.05$) increased the protein content from 21.87 to 30.20% while that of Zalngy slightly decreased the protein content from 25.17 to 24.32%. The higher protein content of Sicklepod leaves after fermentation may be due to the decrease in carbon ratio of the total mass, resulting in redistribution of nutrients percentages. Microorganisms utilize carbohydrates as an energy source and produced carbon dioxide as a by-product. Such a process caused the nitrogen in the fermented slurry to be concentrated and thus the protein in the total mass increases as reported by Onyango et al. (2005). Fermentation and cooking of both samples reduced oil, carbohydrates and energy contents. The reduction in energy level after fermentation may be attributed to the reduction in oil and carbohydrates of the fermented dough. Fiber content of Algenina sample was increased after fermentation and cooking but that of Zalngy was significantly ($P \le 0.05$) decreased. For both samples fermentation and cooking significantly $(P \le 0.05)$ increased the ash content. The high level

| Samples | Dry matter | Protein | Oil | Fiber | Ash | Carbohydrate | Energy (K cal/100 o) |
|-------------|----------------------------|----------------------------|---------------------------|----------------------------|----------------------------|----------------------------|-----------------------------|
| Algenina | | | | | | | (<u>1</u> |
| Raw | $93.50 \pm (0.20)^{a}$ | $21.87 \pm (0.20)^{b}$ | $3.97 \pm (0.24)^{ m ab}$ | $18.72 \pm (0.75)^{\circ}$ | $12.53 \pm (0.03)^{\circ}$ | $36.41 \pm (1.02)^{a}$ | $268.85 \pm (1.23)^{a}$ |
| Fermented | $90.70 \pm (0.30)^{\rm b}$ | $30.20 \pm (0.20)^{a}$ | $4.12 \pm (0.47)^{a}$ | $19.29 \pm (1.55)^{\rm b}$ | $18.16 \pm (0.28)^{b}$ | $18.75 \pm (0.41)^{b}$ | 232.88± (3.22) ^b |
| Fermented & | $92.05 \pm (0.87)^{\circ}$ | $30.01 \pm (0.20)^{a}$ | $3.79 \pm (0.51)^{b}$ | $21.84 \pm (0.89)^{a}$ | $19.55 \pm (0.15)^{a}$ | $16.86 \pm (0.56)^{\circ}$ | $221.59 \pm (4.01)^{\circ}$ |
| Zalngy | | | | | | | |
| Raw | 93.93 ± (0.13)° | $25.17 \pm (0.13)^{a}$ | $3.62 \pm (0.02)^{a}$ | $24.18 \pm (1.22)^{a}$ | $12.08 \pm (0.33)^{\circ}$ | $28.88 \pm (0.73)^{a}$ | $248.78\pm(3.78)^{a}$ |
| fermented | $92.50 \pm (0.80)^{\circ}$ | $26.08 \pm (0.12)^{a}$ | $3.34 \pm (0.55)^{a}$ | $21.66 \pm (2.02)^{b}$ | $18.74 \pm (0.08)^{\rm b}$ | 22.68± (0.34)° | $225.10\pm(0.97)^{\circ}$ |
| Fermented & | $95.00 \pm (0.20)^{a}$ | $24.32 \pm (0.12)^{\rm b}$ | $2.95 \pm (0.34)^{b}$ | $21.80 \pm (2.05)^{\rm b}$ | $20.04 \pm (0.13)^{a}$ | $25.89 \pm (0.13)^{b}$ | $227.39\pm(2.75)^{b}$ |

| Table 1. |
|--------------------------------------|
| l. Proximate c |
| omposition |
| % |
| tion (%) of treated and untreated Si |
| k |
| epod (Cassia obtusifolia) |
| a) leaves of different origin |

Values are means \pm (SD) of three different samples. Values not sharing a common superscript in a column for each sample are significantly different at P \leq 0.05.

of ash in the fermented leaves even after cooking is likely to be due to trace contamination with clay from the walls of the earthenware container.

Antinutritional factors content of raw and processed Sicklepod leaves

Table 2 shows the antinutritional factors content of treated and untreated Sicklepod (Cassia obtusifolia) leaves of different origin. Tannin content of Algenina raw sample was found to be 453.53 mg/100g. Fermentation of Algenina sample significantly ($p \leq$ 0.05) increased tannin content to 1204.30 mg/100g. This result disagree with observation of Mohamed et al. (2007) who found that fermentation significantly decreased tannin content of pearl millet. The increment in tannin content after fermentation may be due to insolubility of condensed tannin of raw Sicklepod leaves in methanol during determination because it had tannins with higher molecular weight or tannin bound to fiber as described by Schofield et al. (2001). Moreover, hydrolysis of condensed tannin during fermentation and air drying of raw leaves may increase the level of tannin in the fermented dough. Cooking of fermented Algenina sample significantly ($p \le 0.05$) decreased tannin content to 1183.65 mg/100g compared to the feremtnted dough but still higher than that of the raw sample. Similar trend in tannin content during processing was observed for Zalngy sample. The reduction in tannin after cooking may due to heat degradation of tannin molecule as well as changes in chemical reactivity or the formation of insoluble complexes as explained by Alonos et al. (1998). Fermentation of Algenina sample significantly ($p \le 0.05$) reduced polyphenols content from 1402.75 to 981.26 mg/100g. Similar results of polyphenol reduction after fermentation was observed by Giami (2004) for fluted pumpkin seeds. Reduction in polyphenols after fermentation might be due to the activation of polyphenoloxidase during fermentation process (Dhankher and Chauhan, 1987). Cooking of the fermented dough significantly $(p \le 0.05)$ decreased total polyphenols for both samples. For Algenina sample cooking decreased total polyphenol to 890.01mg/100g whereas for Zalngy sample it decreased to 620.58mg/100g. The reduction in polyphenols after cooking might be due to the fact that phenols react with protein during cooking forming poorly extractable protein-phenolic complexes. Phytate content of Algenina raw sample was found to be 310.41mg/100g. Fermentation of the sample significantly ($p \le 0.05$) decreased phytate content to 196.14 mg/100g. Similar reduction level of phytic acid after fermentation was observed by Giami (2004), and Ugwu and Oryanye (2006) for fluted

pumpkin seed and breadfruit seeds, respectively. The loss in phytic acid during fermentation possibly due to the action of fermenting microorganisms which hydrolyze phytate into inositol and orthophosphate (Sandberg and Andlind, 2002). Cooking of the fermented dough significantly ($p \le 0.05$) decreased the phytate content of both samples. For Algenina phytate content decreased to 77.90 mg/100g while for Zalngy it decreased to 146.28 mg/100g. Similar trend in phytic acid reduction after cooking was reported by AbdelRahman et al. (2005) for fermented pearl millet flours. The apparent decrease in phytate content during cooking may be due to the formation of insoluble complexes between phytate and other components such as phytate-protein and phytatemineral complexes and accordingly the amount of free phytate was reduced (Kumar et al, 1978). Authors suggested that phytate reduction also can be carried out by changing the pH level of the media to avoid the optimum pH at which phytate complex with proteins and minerals.

Total and extractable major minerals of raw and processed Sicklepod leaves

The major minerals content and extractability of raw and processed Sicklepod leaves are shown in Table 3. The data obtained showed that Ca and K were the major mineral constituents while Na was the least constituents of raw leaves of both Algenina and Zalngy samples. Ca content of Algenina raw sample averaged 2933.96 mg/100g and out of this amount about 32.98% was extractable, while that of Zalngy averaged 3006.17 mg/100g and out of this amount about 28.20% could be extracted. The results showed that Algenina sample had lower Ca content and higher extractability compared to Zalngy. The result obtained for Ca content in this study was lower than that reported by Dirar et al. (1985) for Sicklepod leaves. The difference in Ca content between the samples may likely due to differences in soil type, season and location. The lower extractability of Ca for both samples may be due to the presence of high level of antinutritional factors such as tannin, oxalic and phytic acids (Fairwether-tait and Hurrel, 1996).

Fermentation of raw leaves of Algenina sample significantly ($p \le 0.05$) decreased Ca content to 2866 mg/100g and out of this amount about 73.77% was found to be extractable. However, for Zalngy fermentation significantly ($p \le 0.05$) increased Ca contents and extractability. The results obtained for Algenina disagree with that reported by Dirar *et al.* (1985) who stated that Ca content of Sicklepod leaves increased after fermentation. Fermentation significantly ($p \le 0.05$) increased Ca extractability

| | | | | Antinutritional factors | actors | | |
|---|---|---|--|---|---|---|--|
| Sampies | | Tannin | Increment (%) | Polyphenols | Reduction (%) | Phytate | Reduction (%) |
| Algenina | | | | | | | |
| Raw | 4 | $453.53 \pm (2.50)^{\circ}$ | I | $1402.75\pm(1.32)^{a}$ | I | $310.41 \pm (14.53)^{a}$ | I |
| Fermented | 1 | $1204.30 \pm (5.15)^{a}$ | 165 | $981.26 \pm (1.36)^{b}$ | 30 | $196.14 \pm (15.44)^{b}$ | 37 |
| Fermented & cooked | | $1183.65 \pm (6.79)^{b}$ | 161 | $890.01 \pm (1.34)^{\circ}$ | 37 | $77.90 \pm (0.00)^{\circ}$ | 75 |
| Zalngy | | | | | | | |
| Raw | | $505.91 \pm (4.06)^{\circ}$ | I | $1008.39 \pm (2.27)^{a}$ | I | $382.60 \pm (13.52)^{a}$ | I |
| Fermented | | $981.97 \pm (1.26)^{a}$ | 94 | $814.35 \pm (1.33)^{b}$ | 19 | $166.51 \pm (0.59)^{b}$ | 56 |
| Fermented & cooked | | $977.65 \pm (2.50)^{b}$ | 93 | $620.58 \pm (1.30)^{\circ}$ | 38 | $146.28\pm(0.87)^{\circ}$ | 62 |
| | al (mg/100g) a | nd extractable (%) sc | me major minerals (| Total (mg/100g) and extractable (%) some major minerals of treated and untreated Sicklepod (<i>Cassia obtusifolia</i>) leaves of different origin Samples | ed Sicklepod (<i>Cassia o</i> Samples | <i>btusifolia</i>) leaves of d | ifferent origin |
| al | al (mg/100g) a | nd extractable (%) sc | me major minerals (Algenina | of treated and untreated | Sicklepod (<i>Cassia o</i> nples | <i>btusifolia</i>) leaves of d Zalngy | ifferent origin |
| al | al (mg/100g) a | nd extractable (%) sc | me major minerals (Algenina Fermented | of treated and untreated Sar Fermented & cooked | Sicklepod (<i>Cassia o</i> nples Raw | <i>btusifolia</i>) leaves of d Zalngy Fermented | Fermented & cooke |
| al | al (mg/100g) a Total | nd extractable (%) sc | me major minerals Algenina Fermented $247.17 \pm (1.29)^{a}$ | of treated and untreated Sau Fermented & cooked $162.96 \pm (0.98)^{b}$ | Sicklepod (<i>Cassia c</i> nples Raw 133.08 ± (0.89)° | btusifolia) leaves of d Zalngy Fermented $175.67 \pm (0.09)^{a}$ | ifferent origin Fermented & cooke $151.32 \pm (2.40)^{b}$ |
| | al (mg/100g) a Total Extractable | nd extractable (%) sc | me major minerals (Algenina Fermented $247.17 \pm (1.29)^{a}$ $12.65 \pm (0.56)^{c}$ | of treated and untreated Satisfies Satisfies $162.96 \pm (0.98)^{\circ}$ $18.94 \pm (1.00)^{\circ}$ | Sicklepod (<i>Cassia c</i> nples Raw 133.08 \pm (0.89)° 29.81 \pm (0.92) ^a | <i>btusifolia</i>) leaves of d Zalngy Fermented $175.67 \pm (0.09)^{a}$ $17.69 \pm (0.14)^{c}$ | ifferent origin Fermented & cooke $151.32 \pm (2.40)^{b}$ $24.00 \pm (1.12)^{b}$ |
| | al (mg/100g) a Total Extractable Total | nd extractable (%) sc Raw 150.40 ± (4.73) ^c 31.98 ±(0.74) ^a 1924.97 ± (5.98) ^c | me major minerals (Algenina Fermented 247.17 \pm (1.29) a 12.65 \pm (0.56) c 2260.02 \pm (7.78) a | of treated and untreated Satisfies Satisfies | Sicklepod (<i>Cassia o</i> . nples Raw 133.08 \pm (0.89) ^c 29.81 \pm (0.92) ^a 1703.41 \pm (4.67) ^c | <i>btusifolia</i>) leaves of d Zalngy Fermented $175.67 \pm (0.09)^{a}$ $17.69 \pm (0.14)^{c}$ $2054.05 \pm (6.04)^{a}$ | ifferent origin Fermented & cookee 151.32 \pm (2.40) ^b 24.00 \pm (1.12) ^b 1894.58 \pm (3.60) ^b |
| | al (mg/100g) a Total Extractable Total Extractable | nd extractable (%) sc Raw 150.40 ± (4.73) ^c 31.98 ±(0.74) ^a 1924.97 ± (5.98) ^c 37.87 ± (1.03) ^c | me major minerals (Algenina Fermented $247.17 \pm (1.29)^{a}$ $12.65 \pm (0.56)^{c}$ $2260.02 \pm (7.78)^{a}$ $68.30 \pm (0.94)^{b}$ | of treated and untreated San Fermented & cooked $162.96 \pm (0.98)^{b}$ $18.94 \pm (1.00)^{b}$ $2009.78 \pm (1.05)^{b}$ $72.77 \pm (0.92)^{a}$ | Sicklepod (<i>Cassia c</i> nples Raw 133.08 \pm (0.89)° 29.81 \pm (0.92) ^a 1703.41 \pm (4.67) 42.59 \pm (1.16)° | btusifolia) leaves of d Zalngy Fermented $175.67 \pm (0.09)^{a}$ $17.69 \pm (0.14)^{c}$ $2054.05 \pm (6.04)^{a}$ $50.00 \pm (2.63)^{b}$ | lifferent origin Fermented & cookee 151.32 \pm (2.40) ^b 24.00 \pm (1.12) ^b 1894.58 \pm (3.60) ^b 54.17 \pm (1.39) ^a |
| Table 3. Tota Minerals Na K | al (mg/100g) a Total Extractable Total Extractable Total | nd extractable (%) sc Raw $150.40 \pm (4.73)^{\circ}$ $31.98 \pm (0.74)^{\circ}$ $1924.97 \pm (5.98)^{\circ}$ $37.87 \pm (1.03)^{\circ}$ $2933.96 \pm (7.65)^{\circ}$ | me major minerals (Algenina Fermented 247.17 \pm (1.29) ^a 12.65 \pm (0.56) ^c 2260.02 \pm (7.78) ^a 68.30 \pm (0.94) ^b 2866.59 \pm (3.34) ^b | of treated and untreated San Fermented & cooked $162.96 \pm (0.98)^{b}$ $18.94 \pm (1.00)^{b}$ $2009.78 \pm (1.05)^{b}$ $72.77 \pm (0.92)^{a}$ $2620.78 \pm (2.05)^{c}$ | Sicklepod (<i>Cassia c</i> nples Raw 133.08 \pm (0.89)° 29.81 \pm (0.92) ^a 1703.41 \pm (4.67) 42.59 \pm (1.16)° 3006.17 \pm (8.90) | btusifolia) leaves of d Zalngy Fermented $175.67 \pm (0.09)^{a}$ $17.69 \pm (0.14)^{c}$ $2054.05 \pm (6.04)^{a}$ $50.00 \pm (2.63)^{b}$ $5973.95 \pm (0.56)^{b}$ | lifferent origin Fermented & cookee 151.32 \pm (2.40) ^b 24.00 \pm (1.12) ^b 1894.58 \pm (3.60) ^b 54.17 \pm (1.39) ^a 5757.68 \pm (6.03) ^c |
| | al (mg/100g) a Total Extractable Total Extractable Total Extractable | nd extractable (%) sc Raw 150.40 \pm (4.73) ^c 31.98 \pm (0.74) ^a 1924.97 \pm (5.98) ^c 37.87 \pm (1.03) ^c 2933.96 \pm (7.65) ^a 32.98 \pm (0.59) ^c | me major minerals (Algenina Fermented $247.17 \pm (1.29)^{a}$ $12.65 \pm (0.56)^{c}$ $2260.02 \pm (7.78)^{a}$ $68.30 \pm (0.94)^{b}$ $2866.59 \pm (3.34)^{b}$ $73.77 \pm (2.65)^{b}$ | of treated and untreated San Fermented & cooked $162.96 \pm (0.98)^{b}$ $18.94 \pm (1.00)^{b}$ $2009.78 \pm (1.05)^{b}$ $72.77 \pm (0.92)^{a}$ $2620.78 \pm (2.05)^{c}$ $81.76 \pm (0.75)^{a}$ | Sicklepod (<i>Cassia o</i> . nples Raw 133.08 \pm (0.89) ^c 29.81 \pm (0.92) ^a 1703.41 \pm (4.67) ^c 42.59 \pm (1.16) ^c 3006.17 \pm (8.90) ^a 28.20 \pm (1.05) ^c | btusifolia) leaves of d Zalngy Fermented $175.67 \pm (0.09)^{a}$ $17.69 \pm (0.14)^{c}$ $2054.05 \pm (6.04)^{a}$ $50.00 \pm (2.63)^{b}$ $5973.95 \pm (0.56)^{b}$ $42.57 \pm (1.31)^{b}$ | Ifferent origin Fermented & cookee 151.32 \pm (2.40) ^b 24.00 \pm (1.12) ^b 1894.58 \pm (3.60) ^b 54.17 \pm (1.39) ^a 5757.68 \pm (6.03) ^c 44.66 \pm (0.99) ^a |
| | al (mg/100g) a Total Extractable Total Extractable Total Extractable Total | nd extractable (%) sc Raw 150.40 \pm (4.73) ^c 31.98 \pm (0.74) ^a 1924.97 \pm (5.98) ^c 37.87 \pm (1.03) ^c 2933.96 \pm (7.65) ^a 32.98 \pm (0.59) ^c 388.58 \pm (0.89) ^c | me major minerals (Algenina Fermented $247.17 \pm (1.29)^{a}$ $12.65 \pm (0.56)^{c}$ $2260.02 \pm (7.78)^{a}$ $68.30 \pm (0.94)^{b}$ $2866.59 \pm (3.34)^{b}$ $73.77 \pm (2.65)^{b}$ $594.60 \pm (3.50)^{b}$ | of treated and untreated Sat Fermented & cooked $162.96 \pm (0.98)^{b}$ $18.94 \pm (1.00)^{b}$ $2009.78 \pm (1.05)^{b}$ $72.77 \pm (0.92)^{a}$ $2620.78 \pm (2.05)^{c}$ $81.76 \pm (0.75)^{a}$ $700.27 \pm (0.98)^{a}$ | Sicklepod (<i>Cassia c</i> nples Raw 133.08 \pm (0.89) ^c 29.81 \pm (0.92) ^a 1703.41 \pm (4.67) 42.59 \pm (1.16) ^c 3006.17 \pm (8.90) 28.20 \pm (1.05) ^c 357.65 \pm (4.23) ^c | btusifolia) leaves of d Zalngy Fermented $175.67 \pm (0.09)^{a}$ $17.69 \pm (0.14)^{c}$ $2054.05 \pm (6.04)^{a}$ $50.00 \pm (2.63)^{b}$ $5973.95 \pm (0.56)^{b}$ $42.57 \pm (1.31)^{b}$ $626.50 \pm (0.45)^{a}$ | lifferent origin Fermented & cookee 151.32 \pm (2.40) ^b 24.00 \pm (1.12) ^b 1894.58 \pm (3.60) ^b 54.17 \pm (1.39) ^a 5757.68 \pm (6.03) ^c 44.66 \pm (0.99) ^a 595.05 \pm (5.30) ^b |
| | al (mg/100g) a Total Extractable Total Extractable Total Extractable Total Extractable | nd extractable (%) sc Raw 150.40 \pm (4.73) ^c 31.98 \pm (0.74) ^a 1924.97 \pm (5.98) ^c 37.87 \pm (1.03) ^c 2933.96 \pm (7.65) ^a 32.98 \pm (0.59) ^c 388.58 \pm (0.89) ^c 20.26 \pm (0.21) ^c | me major minerals (Algenina Fermented 247.17 \pm (1.29) ^a 12.65 \pm (0.56) ^c 2260.02 \pm (7.78) ^a 68.30 \pm (0.94) ^b 2866.59 \pm (3.34) ^b 73.77 \pm (2.65) ^b 594.60 \pm (3.50) ^b 39.47 \pm (1.04) ^a | of treated and untreated Satisfies Satisfies | Sicklepod (<i>Cassia c</i> nples Raw 133.08 \pm (0.89)° 29.81 \pm (0.92) ^a 1703.41 \pm (4.67) 42.59 \pm (1.16)° 3006.17 \pm (8.90) 28.20 \pm (1.05)° 357.65 \pm (4.23)° | btusifolia) leaves of d Zalngy Fermented $175.67 \pm (0.09)^{a}$ $17.69 \pm (0.14)^{c}$ $2054.05 \pm (6.04)^{a}$ $50.00 \pm (2.63)^{b}$ $5973.95 \pm (0.56)^{b}$ $42.57 \pm (1.31)^{b}$ $626.50 \pm (0.45)^{a}$ $33.96 \pm (0.22)^{b}$ | Ifferent origin Fermented & cooke 151.32 \pm (2.40) ^b 24.00 \pm (1.12) ^b 1894.58 \pm (3.60) ^b 54.17 \pm (1.39) ^a 5757.68 \pm (6.03) ^c 44.66 \pm (0.99) ^a 595.05 \pm (5.30) ^b 40.56 \pm (0.12) ^a |
| | al (mg/100g) a Total Extractable Total Extractable Total Extractable Total Extractable Total | nd extractable (%) sc Raw 150.40 \pm (4.73) ^c 31.98 \pm (0.74) ^a 1924.97 \pm (5.98) ^c 37.87 \pm (1.03) ^c 2933.96 \pm (7.65) ^a 32.98 \pm (0.59) ^c 388.58 \pm (0.21) ^c 380.22 \pm (1.71) ^c | me major minerals (Algenina Fermented $247.17 \pm (1.29)^{a}$ $12.65 \pm (0.56)^{c}$ $2260.02 \pm (7.78)^{a}$ $68.30 \pm (0.94)^{b}$ $2866.59 \pm (3.34)^{b}$ $73.77 \pm (2.65)^{b}$ $594.60 \pm (3.50)^{b}$ $39.47 \pm (1.04)^{a}$ $733.58 \pm (1.85)^{a}$ | of treated and untreated San Fermented & cooked $162.96 \pm (0.98)^{b}$ $18.94 \pm (1.00)^{b}$ $2009.78 \pm (1.05)^{b}$ $72.77 \pm (0.92)^{a}$ $2620.78 \pm (2.05)^{c}$ $81.76 \pm (0.75)^{a}$ $700.27 \pm (0.98)^{a}$ $33.45 \pm (0.43)^{b}$ $694.59 \pm (1.81)^{b}$ | Sicklepod (<i>Cassia c</i> nples Raw 133.08 \pm (0.89)° 29.81 \pm (0.92) ^a 1703.41 \pm (4.67) 42.59 \pm (1.16)° 3006.17 \pm (8.90) 28.20 \pm (1.05)° 28.20 \pm (1.05)° 25.7.65 \pm (4.23)° 22.75 \pm (0.31)° 414.63 \pm (1.71)° | $\begin{aligned} & btusifolia) \text{ leaves of d} \\ & & \text{Zalngy} \\ & & \text{Fermented} \\ & & 175.67 \pm (0.09)^{a} \\ & & 17.69 \pm (0.14)^{c} \\ & & 2054.05 \pm (6.04)^{a} \\ & & 50.00 \pm (2.63)^{b} \\ & & 5973.95 \pm (0.56)^{b} \\ & & 42.57 \pm (1.31)^{b} \\ & & 626.50 \pm (0.45)^{a} \\ & & 33.96 \pm (0.22)^{b} \\ & & 593.35 \pm (0.53)^{a} \end{aligned}$ | Ifferent origin Fermented & cooked $151.32 \pm (2.40)^{b}$ $24.00 \pm (1.12)^{b}$ $1894.58 \pm (3.60)^{b}$ $54.17 \pm (1.39)^{a}$ $5757.68 \pm (6.03)^{c}$ $44.66 \pm (0.99)^{a}$ $595.05 \pm (5.30)^{b}$ $40.56 \pm (0.12)^{a}$ $535.37 \pm (5.16)^{b}$ |

Table 2. Antinutritional factors content (mg/100g) of treated and untreated Sicklepod (Cassia obtusifolia) leaves of different origin

for Algenina sample and this could be due to the fact that fermentation reduces the antinational factors of the sample (Giami, 2004). However, tannin content significantly ($p \le 0.05$) increased after fermentation and this departure from an otherwise good correlation between tannin and minerals extractability. The explanation for this may lie on the chemical as well as the structure of tannin of both samples. Moreover, the increment in Ca extractability of the leaves after fermentation may also attributed to its concentration by microorganism as explained by AbedelHady (2005). Cooking of Algenina fermented dough significantly ($p \le 0.05$) decreased Ca content but significantly ($p \le 0.05$) increased Ca extractability to 81.76%. Cooking of Zalngy sample significantly ($p \le 0.05$) increased both Ca content and extractability. This finding agreed with AbedelHady et al. (2005) who found that cooking of fermented maize significantly ($p \le 0.05$) increased Ca content and extractability. Divalent cations, such as Ca are generally present in association with phytic acid and this may be responsible for lower extractability of Ca in raw samples. However, reduction in phytic acid as a result of fermentation and cooking may explain the significant increase in extractability of Ca and other minerals (Duhan et al., 2002). Moreover, it has been reported that a significant increase in Ca extractability after processing might be due to the reduction in phytic acid and oxalic acid (Fairwethertait and Hurrel, 1996).

The results obtained for K and Mg follow a trend similar to that obtained for Ca with few exceptions. Compared to the raw samples, P content from Algenina and Zalngy was significantly ($p \le 0.05$) increased after fermentation and cooking but the extractability was significantly ($p \le 0.05$) decreased. Dirar et al. (1985) found that fermentation of Sicklepod leaves increased P content. However, P content of both raw samples obtained in this study was higher than that obtained by Dirar et al. (1985). Fasasi et al. (2004) reported that P content of fermented breadfruit flour increased from 375.36 to 415.55 mg/100g. Fermentation of Algenina raw sample significantly ($p \le 0.05$) increased P content to 733.58 mg/100g and out of this amount about 15.17% was found to be extractable. The increment in P content after fermentation of the leaves may be due to synthesis and release of P by phytase enzyme during fermentation as explained by Fasasi et al. (2004). Cooking of both fermented samples significantly ($p \le p$ 0.05) decreased P content compared to the fermented dough but still significantly ($p \le 0.05$) higher than that of the raw samples. AbedelHady (2005) found that cooking decreased P content and extractability

for both maize and lentil and explained the reduction of P extractability after cooking to complixation of P with other food constituents. Nutrients interaction during processing is not a common phenomenon and even if occurred it has no nutritional implications because the element will be inactive when complexed with other constituents. Data obtained for Na follows a trend similar to that obtained for P.

Total and extractable trace minerals of raw and processed Sicklepod leaves

Table 4 shows the total (mg/100g) and extractable (%) some trace minerals of treated and untreated Sicklepod (Cassia obtusifolia) leaves of different origin. The data obtained in this study showed that Fe and Mn are the major mineral constituents of both samples while Cu and Zn are the least constituents. Fe content of Algenina raw sample averaged 32.42 mg/100g and out of this amount about 8.27% could be extracted, while that of Zalngy raw sample was 41.60 mg/100g with extractability of 6.25%. Algenina raw sample had lower Fe content and higher extractability compared to Zalngy sample. The iron content of both raw samples was lower than that obtained by Dirar et al. (1985) for Sicklepod leaves. Fe content was significantly ($p \le 0.05$) increased after fermentation of Algenina sample and it was 48.24 mg/100g and out of this amount about 9.05 could be extracted. Cooking of Algenina fermented dough significantly $(p \le 0.05)$ increased Fe content to 53.35 mg/100g with extractability of 6.68% and that of Zalngy was also significantly ($p \le 0.05$) increased to 60.69 mg/100g with extractability of 4.89%. The results obtained agreed with those of AbedelHady (2005) who reported that fermentation and cooking of maize flour increased Fe content and extractability. The lower extractability of Fe for all raw and processed leaves may be due to the presence of phytic acid as well as the high content of both polyphenols and tannins. Mn content of the raw leaves of Algenina was 11.85 mg/100g and out of this amount about 16.33% could be extracted. Mn content of Algenina was decreased after fermentation and it was 8.89 mg/100g with extractability of 12.22%. The reduction in Mn content after fermentation disagree with the finding of Dirar et al. (1985) who found that fermentation increased Mn content of Sicklepod leaves. Cooking of both Algenina and Zalngy fermented samples increased Mn content. For Algenina, Mn content increased to 9.06 mg/100g with extractability of 11.86% while for Zalngy it increased to 17.16 mg/100g with extractability of 10.87%. Cooking of Zalngy sample significantly ($p \le 0.05$) decreased Mn extractability. AbedelHady et al. (2005) reported that cooking of

| | 1 | | | Samples | S | | |
|-------------|-------------|----------------------------|--------------------------------|---------------------------|---------------------------------|--------------------------------|--------------------------------|
| Mineral | | | Algenina | | | Zalngy | |
| | | Raw | Fermented | Fermented & cooked | Raw | Fermented | Fermented & cooked |
| 5 | Total | $32.42 \pm (0.60)^{\circ}$ | $48.24 \pm (0.42)^{b}$ | $53.35 \pm (0.83)^{a}$ | $41.60 \pm (0.58)^{\circ}$ | $61.48 \pm (0.67)^{a}$ | $60.69 \pm (0.95)^{\rm b}$ |
| re | Extractable | $8.27 \pm (0.21)^{b}$ | $9.05 \pm (0.14)^{a}$ | $6.68 \pm (0.31)^{\circ}$ | $6.25 \pm (0.05)^{a}$ | $6.18 \pm (0.11)^{a}$ | $4.89 \pm (0.28)^{\mathrm{b}}$ |
| | Total | $11.85 \pm (0.80)^{a}$ | $8.89 \pm (0.21)^{b}$ | $9.06 \pm (0.36)^{b}$ | $6.14 \pm (0.28)^{\circ}$ | $14.79 \pm (0.47)^{b}$ | $17.16 \pm (0.08)^{a}$ |
| IVIN | Extractable | $16.33 \pm (0.10)^{a}$ | $12.22\pm(0.05)^{b}$ | $11.86 \pm (0.29)^{b}$ | $20.33 \pm (0.58)^{\mathrm{a}}$ | $11.97 \pm (2.63)^{b}$ | $10.87 \pm (0.34)^{b}$ |
| 5 | Total | $5.74 \pm (0.20)^{a}$ | $3.53 \pm (0.32)^{\mathrm{b}}$ | $3.11 \pm (0.19)^{b}$ | $4.82 \pm (0.43)^{a}$ | $5.03 \pm (0.34)^{\mathrm{a}}$ | $2.64 \pm (0.06)^{b}$ |
| Cu | Extractable | $16.03 \pm (0.71)^{\circ}$ | $43.53 \pm (1.03)^{b}$ | $48.32 \pm (1.15)^{a}$ | $15.13 \pm (0.36)^{b}$ | $15.10 \pm (0.24)^{b}$ | $27.09 \pm (0.25)^{a}$ |
| 7. | Total | $2.14 \pm (0.30)^{b}$ | $4.68 \pm (0.76)^{a}$ | $5.24 \pm (0.31)^{a}$ | $2.54 \pm (0.51)^{\rm b}$ | $6.92\pm(0.26)^{a}$ | $6.55 \pm (0.35)^{a}$ |
| Z 11 | Extractable | $20.28 \pm (0.42)^{a}$ | $11.23 \pm (0.06)^{b}$ | $9.84 \pm (0.21)^{\circ}$ | $21.25 \pm (0.31)^{a}$ | $7.23 \pm (0.02)^{b}$ | $6.81 \pm (0.14)^{b}$ |

| Table 4. Total (mg/100g) and extractable (%) some trace minerals of treated and untreated Sicklepod (<i>Cassia obtusifolia</i>) leaves of different origin | Table 4. |
|--|---|
| (Cassia obtusifolia) leaves of differe | Total (r |
| (Cassia obtusifolia) leaves of differe | ng/100g) |
| (Cassia obtusifolia) leaves of differe |) and extractable |
| (Cassia obtusifolia) leaves of differe | °(%) |
| (Cassia obtusifolia) leaves of differe |) some trace minerals of treated and untreated \$ |
| (Cassia obtusifolia) leaves of differe | Sicklepod |
| ere | l (Cassia ol |
| ere | otusifolia) |
| | ere |

Values are means \pm (SD) of three different samples. Values not sharing a common superscript in a row for each sample are significantly different at P \leq 0.05.

International Food Research Journal 17: 775-785

fermented maize flour had no significant change in Mn content and extractability. The reduction in Mn extractability after processing may be due to the fact that high amounts of Ca, P, fiber and phytate increased the requirement of Mn possibly via the formation of insoluble Mn complexes, resulting in a reduction of insoluble fraction available for absorption (Keen and Zidenberg-Cherr, 1990). Davis et al. (1992) mentioned that high amount of dietary Fe inhibit Mn absorption, possibly by competition for similar binding and absorption sites (Fairwether-tait and Hurrel, 1996). Other minerals (Cu and Zn) follow a trend similar to that obtained for Mn with few exceptions. As shown in Table 5 despite the higher content of mineral of raw and processed samples the extractability of most minerals is very low. When the extractable minerals of both samples before and after processing were compared to Britain daily

requirements only Ca of both samples was sufficient also the available Cu content of fermented and cooked Algenina dough was higher than daily requirements.

Conclusion

The observations about antinutrients and minerals extractability in the studied samples tend to suggest that fermentation greatly reduced the antinutrients level of both Algenina and Zalngy Sicklepod leaves and improved minerals availability. Cooking of the fermented samples had no great effects on antinutrients and minerals availability when compared to the raw samples. Therefore, both fermentation and cooking if applied will alleviate the severe problem of the antinutritional factors as well as the toxic substances of Sicklepod leaves.

Table5. Extractable (mg/100g) major and trace minerals compared to daily requirements of processed (Kawal) and unprocessed Sicklepod (*Cassia obtusifolia*) leaves of different origin

| | | Sample | | | | | | aily |
|----------|--------|-----------|-----------------------|--------|-----------|-----------------------|--------|--------|
| Minerals | | Algenina | | | Zalngy | | requir | ement* |
| Wincluis | Raw | Fermented | Fermented & cooked | Raw | Fermented | Fermented & cooked | Men | Women |
| Na | 46.49 | 31.26 | 30.86 | 39.67 | 31.08 | 36.32 | 1609.3 | 1609.3 |
| Κ | 728.90 | 1543.66 | 1462.43 | 725.57 | 1027.03 | 1026.32 | 3519 | 3519 |
| Ca | 967.65 | 2114.72 | 2142.47 | 751.28 | 2543.06 | 2571.34 | 700 | 700 |
| Mg | 78.73 | 234.67 | 234.27 | 81.36 | 212.78 | 241.38 | 294.95 | 264.93 |
| Р | 107.11 | 115.21 | 109.99 | 123.95 | 107.24 | 98.61 | 541.98 | 541.98 |
| Fe | 2.68 | 4.37 | 3.56 | 2.60 | 3.80 | 2.97 | 8.7 | 14.8 |
| Cu | 0.92 | 1.54 | 1.50 | 0.73 | 0.76 | 0.72 | 1.2 | 1.2 |
| Mn | 1.94 | 1.09 | 1.07 | 1.25 | 1.77 | 1.87 | 2-9* * | 2-9** |
| Zn | 0.43 | 0.53 | 0.51 | 0.54 | 0.50 | 0.45 | 9.5 | 7 |

* British Nutrition Foundation, London, Chapman and Hall (1995).

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