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PROXIMATE AND ANTI-NUTRIENT PROFILE CHANGES IN DIFFERENTLY PROCESSED TUBER FLOURS OF *ICACINA SENEGALENSIS* A. JUSS *(ICACINACEAE)* FROM SOUTHERN NIGERIA

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ABSTRACT

Obective: The proximate and anti-nutrient profile changes in differently processed tuber flours of *Icacina senegalensis* A. Juss *(Icacinaceae)* from Southern Nigeria were investigated. **Method:** Peeled, chopped and washed tubers were divided into four portions for different treatments: drying (RD), boiling / drying (BD), fermenting / drying (FD), and fermenting/ boiling / drying (FBD). Fermentation was by soaking in water for three days while drying was by oven at 40^{o}C for 48hrs. The dried samples were pulverized into respective flours and later subjected to proximate and anti-nutrient profiling using standard methods. **Results:** Fermentation caused significant improvement in crude protein content (%) of FD (3.64) and FBD (4.03) over RD (3.36) and BD (3.39); while boiling resulted in drastic reduction of crude fat (%) in BD (2.5) and FBD (1.00) when compared to RD (7.48) and FD (3.64). FBD had the least % crude fibre content (6.26) which was significantly lower ($p<0.05$) than the other treatment groups RD, BD, and FB (7.49-7.64). Even though the mean % carbohydrate content of BD, FD, and FBD (77, 70, and 79 respectively), were significantly higher ($p<0.05$) than that of RD (68), RD still retained significantly higher % energy (350) compared to the other samples (332-339). A combination of fermentation and boiling was the most efficient in reduction/elimination of the anti-nutrients – oxalate cyanate and phytate when compared to boiling and fermentation separately. **Conclusion**: A combination of fermentation and boiling improves protein and carbohydrate contents and is more efficient in eliminating anti-nutrients in *Icacina senegalensis* tuber.

KEYWORDS: *Icacina senegalensis*; Tuber flours; Proximate composition; Anti-nutrients; Boiling; Fermentation.

INTRODUCTION

Food can be described as a substance solid or liquid usually of plant or animal origin which when consumed helps the body perform its physiological functions. Staple foods which are basically carbohydrates furnish the energy requirement of the human body. They are typically inexpensive and should be readily available. As earlier noted by,[1] there is a search for new agents in the management of body mass and blood sugar level and prevention of degenerative disease. The plant *Icacina senegalensis* is known for its edible starchy root, which is usually eaten in times of famine when other tuber crops especially yam and cassava are in short supply; it has a good quantity of starch.[2,3] The root crop of *Icacina senegalensis* popularly called "false yam" is an indigenous drought-resistant root crop found in the wild and arid parts of West and central Africa including Nigeria.[4,5] All the parts of *Icacina senegalensis* are essential; its tubers, fruits and seeds are used as food[1] while the leaves serve several medicinal purposes.[6]

As is common with most tuberous plants (for example cassava), anti-nutritional factors have been reported to be present in the tuber of *Icacina senegalensis*.). Several processing methods including boiling[7] and soaking,[8] have been applied to eradicate the bitter principles present in the tuber. So far, the results from such attempts are not satisfactory in eliminating anti-nutritional factors thereby limiting the use of *Icacina senegalensis* tuber flour as a substitute source of dietary energy for humans and animals.[1] This study was therefore designed to assess the proximate and anti-nutritional changes following the processing of *Icacina senegalensis* tubers into flours by drying, boiling, fermenting, and the combination of fermenting and boiling.

MATERIALS AND METHODS

Collection and identification of plant material

Icacina senegalensis tuber used for this research work was obtained from Akpabuyo Local Government Area of

Cross River State. The tubers were identified in the Department of Botany, University of Calabar by a taxonomist who deposited it with herbarium specimen voucher number 0620.

Processing of *Icacina senegalensis* tuber flours

The harvested *Icacina senegalensis* tubers were peeled, washed, reduced into small sizes about (4-5cm) and then divided into two portions. The first portion was further divided into two parts; the first part was dried directly in an oven while the other part was boiled and thereafter boiled. The second portion was first fermented in water at room temperature for 3 days, strained and then divided into two parts; the first part was dried in an oven while the second was boiled and thereafter strained, and oven dried. All the boiling was in water (1:2, w/v) at 100^{o}C for 1 hour, while drying was with an oven (40^{o}C, 48 hours). All the four dried samples were pulverized using a hammer mill into respective flours; a) raw dried (RD), b) boiled dried (BD), fermented dried (FD), and fermented, boiled then dried (FBD). They were thereafter preserved in tightly capped labeled bottles for analyses. The flow chart for the processing of *Icacina senegalensis* tuber into flours is as shown in Fig.1.

Proximate assessment of the *Icacina senegalensis* tuber flours

Moisture, ash, crude fibre, crude lipid and protein were analyzed by standard methods.[9]

Determination of moisture content

Hundred grams of the sample was weighed in a crucible and dried to a constant weight at 150^{o}C for 24 hours. The crucible with its content was weighed in a desiccator. The loss in weight represented the moisture content.

$$\% \text{ moisture} = \frac{\text{Final (weight after drying)}}{\text{Initial sample weight}} \times \frac{100}{1}$$

Determination of ash content

Five grams of the dried sample were weighed in a crucible of known weight, ignited for 24 hours at a temperature of 55^{o}C until a grey ash was obtained. The crucible and its content were cooled in a desiccator and weighed. The weight of the ash was obtained as follows;

$$\frac{W_3 - W_1}{W_2 - W_1} \times \frac{100}{1}$$

Where,
W_1 = Weight of the empty crucible
W_2 = Weight of empty crucible + sample
W_3 = Weight empty crucible ignited + ignited sample

Determination of crude fibre content

Five grams of the sample was weighed and carefully transferred into a 250ml beaker. Water and H_2SO_4, were added and boiled for 30 minutes. The content was filtered and the residue was transferred into another beaker. Water and NaOH were added and allowed to boil gently for 30 minutes. The content was washed thoroughly with hot distilled water, and then rinsed once with 10% HCL, twice with ethanol and finally three times with petroleum ether. It was allowed to dry and into a crucible and allowed to dry overnight. It was then removed and cooled in a desiccator. The percentage crude fibre was calculated;

$$\frac{W_3 - W_1}{W_2 - W_1} \times \frac{100}{1}$$

Where,
W_1 = Weight of the empty beaker
W_2 = Weight of the empty beaker + sample
W_3 = Weight of the empty beaker + dry sample

Determination of crude lipid content

The sample (5g) was weighed into a porous thimble which was cover with clean white glass wool. Petroleum ether was poured into an extraction flask which was previously dried in an oven and weighed. Extraction was done for 5 hours. The thimble was removed carefully and the extraction flask placed in a bath so as to evaporate the petroleum ether and then dried in the oven at a temperature of 70^{o}C to completely free from the solvent and moisture. It was then cooled in a desiccator and reweighed.

The percentage crude lipid was calculated using the equation below:

$$\% \text{ of crude lipid content} = \frac{W_2 - W_3}{W_2 - W_1} \times \frac{100}{1}$$

Where,
W_1 = Weight of the empty round bottom flask
W_2 = Weight of the empty flask + sample
W_3 = Weight of the sample -dry sample

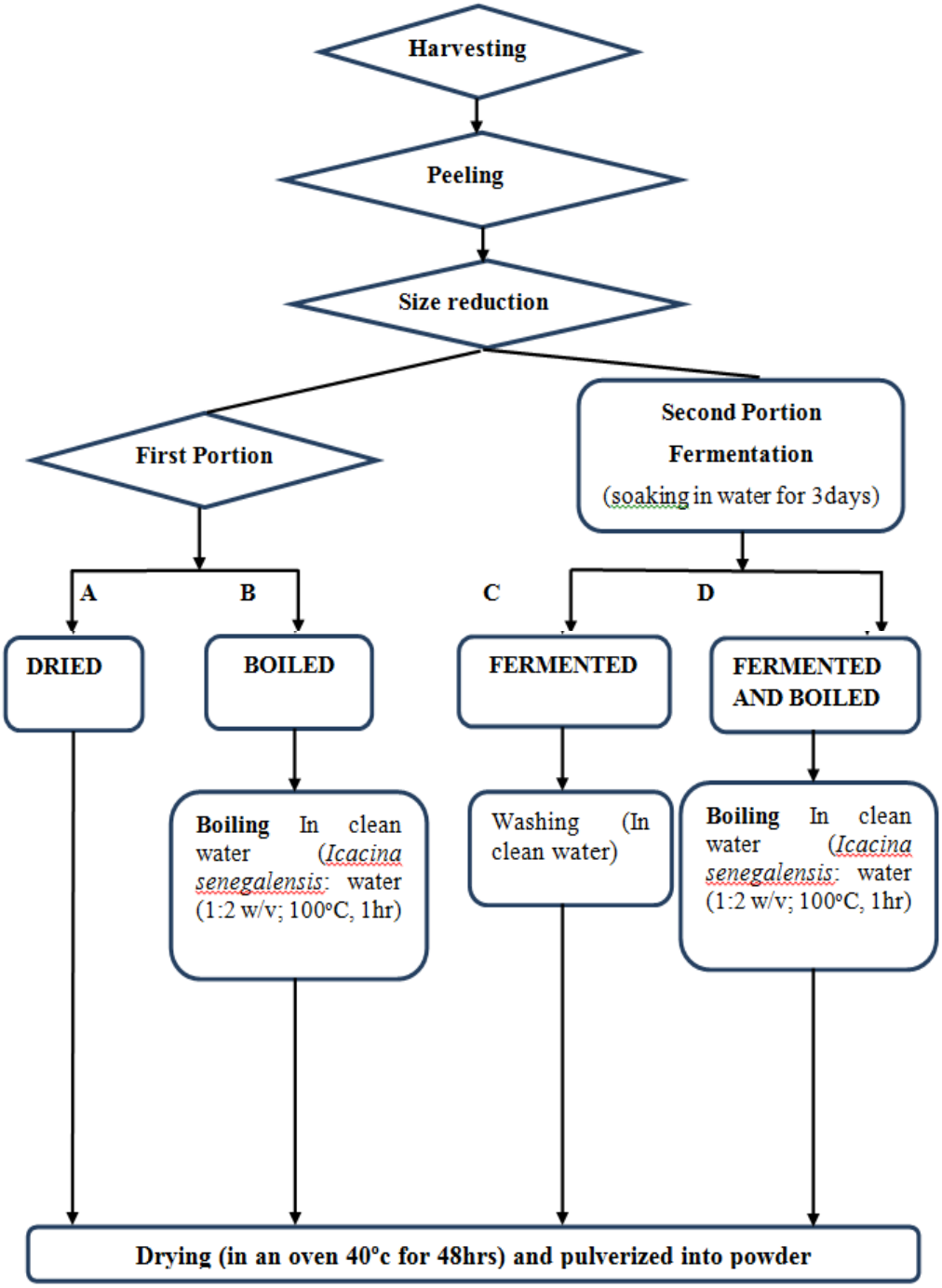

Fig. 1: Flow chart for the processing of *Icacina senegalensis* tuber into flours.

Determination of protein content

The ground sample (5g) was weighed into a standard Kjedahl digestion flask, and 30ml of concentrated sulphuric acid and 15g of Kjedahl catalyst (copper sulphate) added to the content of the flask with some anti-bumping chips. The digestion flask and its content were placed in a digestion rack and heated gently at first to prevent charring after which the temperature was raised to 90^{o}C and maintained for vigorous heating. The heating was done for about 8 hours until a clear blemish solution was obtained. The solution was allowed to cool and then diluted to 100ml with distilled water. Then 10ml of this solution was measured into a distillation flask and decomposed with 30ml of 40% sodium hydroxide solution. The ammonia liberated was distilled with 10ml of boric acid and a drop of double indicator. The solution was then titrated with 0.1M hydrochloric acid until a pale pink end point was reached. This was done three times for each digest and the percentage nitrogen (w/w) determined by approximate calculations.

$$\text{Percentage Nitrogen} = \frac{14 \times 0.1N\ HCL \times \text{titre value} \times 100}{\text{Weight of sample} \times 1000}$$

The crude protein was obtained by multiplying the percentage nitrogen content by the factor 6.25.

Crude protein = %N x 6.25

Determination of carbohydrate content

The total carbohydrate content (%) was determined by arithmetic difference[10] using the equation below:

Percentage CHO = 100-% (A+B+C+D+E)

Where A =Protein, B= Fat, C= Ash, D= Crude fibre and E= Moisture

Determination of anti-nutrients

Total soluble oxalate content

Oxalate was determined using the method as earlier described by of Sanchez-Alonso and Lachica.[11] One gram of the sample was placed in 250 cm^3 volumetric flask, followed by the addition of 190 ml of distilled water and 10 cm3 of 6M HCl. The content in the flask was warmed in a water bath at 90°C for 4 hour and the digested sample centrifuged at 2,000 rpm for 5 min. The supernatant was then diluted to 250 cm^3. Three 50 cm^3 aliquots of the supernatant were evaporated to 25 cm^3, and the brown precipitate was filtered and washed. The combined solution and washings were then titrated with concentrated ammonia solution in drops until the pink colour of methyl orange changed to yellow. The solution was then heated in a water bath to 90°C, and the oxalate precipitated with 5% $CaCl_2$ solution then allowed to stand overnight and then centrifuged at 2000rpm for 15 minutes. The precipitate was washed with hot 25% H_2SO_4, diluted to125ml with distilled water and titrated against 0.05 M $KMnO_4$.

Calculation: 1 ml 0.05 M KMnO4 = 2.2 mg Oxalate

Determination of hydrocyanide content

Cyanogenic glycoside was determined using alkaline picrate method.[12] In this method, 5.0 g of grounded sample was weighed and dissolved in 50 cm^3 distilled water. The cyanide extraction was allowed to stay overnight and then filtered to 1 ml of the sample filtrate and standard cyanide solution in test tubes, 4 ml of alkaline picrate solution (1 g of picrate and 5 g of Na_2CO_3 in 200 cm^3 distilled water) was added and incubated in water bath for 15 min. After color development, the absorbance was read at 490 nm against blank containing only 1 ml distilled water and 4cm^3 alkaline picrate solution. The cyanide content was calculated from the equation below.

Calculation: Cyanogenic glycoside (mg/100 g)

$$= \frac{C\ (mg)}{\text{Weight of sample}} \times 100$$

Where, C (mg) = concentration of cyanide content.

Determination of phytic acid content

Phytic acid was determined by the procedure of.[13] Two grams of the samples (dry and latex) were shaken in a small stoppered bottle with 100ml of 0.5m. HCl for 2 hours to extract the phytic acid. After this 40 ml of the filtered extract were neutralized in phenolphthalein with 10ml of NaOH added, then rendered slightly acidic with the HCl and made up to 50ml. Duplicate 20ml aliquots were treated in 50ml centrifuge tubes with 4ml $FeCl_3$ solution. The tubes were heated in boiling water bath for 15 minutes to flocculate the precipitate of ferric phytate. The precipitate was cooled, centrifuged at 2500 rpm for 15mins and the supernatant poured off. The precipitate was then stirred up with 2ml distilled water and heated in a boiling water bath for 15 minutes.

After this, 2ml of 2% NaOH was then added and the heating continued for another 15minutes. The solution contained the phytic acid as sodium phytate was filtered into kjeldhal digestion flask. The precipitated ferric hydroxide was washed with hot water and all the washings were added to the filtrate in the flask. Two ml of concentrated H_2SO_4 and 2ml of 65% tetrahydrochlorate ($HClO_4$) were added to the filtrate and the mixture was incinerated at 680°C very gently until it was completely digested. The digest was further heated strongly at 970°C for 1 hour to drive off any residual tetrahydrochlorate ($HClO_4$). Thereafter, 20ml of distilled water was added and the content of the flasks were neutralized in phenolphthaleins with 40% NaOH. The solution was made up to 100ml with distilled water. An aliquot of 5ml was taken and made up to 10ml with the blank solution. The blank solution was prepared by neutralizing 2ml conc. H_2SO_4 with 40% NaOH and made up to 100ml with distilled water. Five millilitres of aliquot were pipetted in a test tube, and 2ml of molybdate solution were added. Then, 1ml of sodium sulphate ($NaSO_4$) solution and 2 ml of hydroquinone solution was added. The solution was diluted to 10ml with distilled water. It was allowed to stand for about 30 minutes for the development of blue colour. The absorbance of the solution was read at 620nm using a UV spectrophotometer.

1g of digestion sample is made up to 20ml of digest

Concentration of element in the sample is equal to.

Absorbance x Standard Concentration x Dilution Factor (DF)

Absorbance of Standard 1 = Xmg/L

Therefore, 20ml of digest contains 20 x mg1000, where, 1g of sample contains 0.02 x mg of digest or 0.02 x mg of element per sample.

RESULTS

From the results of proximate analysis of *Icacina senegalensis* tuber flours as shown on Table.1, flour from fermented sample (FD) retained moisture more than the raw (RD), fermented/boiled (FBD), and boiled (BD) (FD>RD>FBD>BD), ranging from 5.55 to 11.39%. The crude protein content of the samples ranged from 3.36 to 4.03% with FBD having significantly higher value (followed by FD) than RD and BD. FBD also had a significant reduction in crude fibre (6.26%) while similar value for the other samples ranged from 7.49 to 7.64%. On the other hand boiling resulted in a drastic reduction

of crude fat thus leaving RD and FD with significantly higher levels than BD and FBD respectively. Dry matter (%) levels in BD and FBD (boiled samples) were significantly higher than those of RD and FD respectively.

Crude ash levels (%) in fermented samples (FD and FBD) were significantly higher than in RD and BD. Boiling released more carbohydrate from BD and FBD than RD and FD samples. A similar trend was found for energy where BD and FBD had higher values than FD but all three were significantly lower than RD.

Table 2 Shows the results of the anti-nutrient profile of *Icacina senegalensis* tuber flours. Boiling, fermenting and boiling after fermenting of *Icacina senegalensis* tuber flours resulted in a significant loss of the cyanide, oxalate and phytate levels in the respective processed tuber flours.

Table 1: Proximate composition of processed *Icacina senegalensis* tuber flours.

Parameters	Raw Dried (RD)	Boiled Dried (BD)	Fermented Dried (FD)	Fermented Boiled Dried (FBD)
Moisture %	10.78±0.090	5.55±0.144 [a,c,d]	11.39±0.666 [a,b,d]	6.61±0.805 [a,b,c]
Crude protein%	3.36±0.049	3.39±0.141 [c,d]	3.64±0.642 [a,b,d]	4.03±0.021 [a,b,c]
Crude fat %	7.48±0.24	2.50±0.10 [a,c,d]	3.73±0.04 [a,b,d]	1.00±0.00 [a,b,c]
Crude fibre%	7.64±0.12	`7.49±0.04 [a,d]	7.51±0.01 [a,d]	6.26±0.46 [a,b,c]
Dry matter %	89.11±0.18	94.33±0.88 [a,c]	88.41±0.44 [a.b,d]	93.27±0.91 [a,c]
Crude ash %	3.17±0.05	3.04±0.14 [a,c,d]	3.67±0.64 [a,b,d]	3.95±0.02 [b,c]
CHO %	68.27±0.71	76.66±0.13 [a,c,d]	70.5±0.53 [a,b,d]	79.30±0.75 [a,b,c]
Energy%	350.35±1.09	342.5±6.64 [a,c,d]	332.71±1.00 [a,b,d]	339.84±0.22 [a,b]

Values are expressed as mean ±SD. n=replicate of three (3) samples. a = significantly different from dried ($p < 0.05$), b = significantly different from boiled ($p < 0.05$), c = significantly different from fermented ($p < 0.05$) and d = significantly different from fermented and boiled ($p < 0.05$).

Table 2: Result of Anti-nutrient content of processed *Icacina senegalensis* tuber flours.

Parameters (mg/100g)	Raw Dried (RD)	Boiled Dried (BD)	Fermented Dried (FD)	Fermented Boiled Dried (FBD)
Oxalate	0.49±0.04	0.24±0.01 [a,c,d]	0.38±0.04 [a,b,d]	0.16±0.01 [a,b,c]
Cyanate	0.67±0.005	0.01±0.001 [a,c]	0.03±0.004 [a,b,d]	0.01±0.001 [a,c]
Phytate	0.70±0.012	0.35±0.032 [a]	0.31±0.234 [a]	0.26±0.029 [a]

Values are expressed as mean ±SD. n=replicate of three (3) samples. a = significantly different from dried ($p < 0.05$), b = significantly different from boiled ($p < 0.05$), c = significantly different from fermented ($p < 0.05$) and d = significantly different from fermented and boiled ($p < 0.05$).

DISCUSSION

The lower moisture levels reported in this work for the samples subjected to boiling (BD and FBD) indicate that the respective resulting flours will have longer shelve life as moisture content of any food substance is known to contribute to microbial spoilage.

The processing methods of fermentation and a combination of fermentation and boiling resulted in significant improvement in crude protein. Fermentation is reported to increase protein content in root crops like cassava.[14,15] This could be attributed to the softening of the fibrous tissue and microbial bio-conversion of carbohydrates and lignocelluloses into protein.[16] This process may also contribute to the significant reduction in crude fibre content of fermented/boiled sample obtained in this study. The concomitant increase in crude ash content also indicates that mineral nutrients will be more available during fermentation. The process of boiling released more carbohydrate and hence more energy despite the drastic loss of crude fat; thus making the processed product attractive for animal feed formulations.[8]

In this study boiling eliminated 50, 99, and 50 percent of oxalate, cyanate, and phytate respectively, from *Icacina senegalensis* tuber. Similar values for fermentation are 22, 96, and 56 percent respectively. But the combination of fermentation and boiling was more efficient with 67, 99, and 62 percent elimination of oxalate, cyanate, and phytate respectively.

Phytic acid and oxalate are anti-nutrients and potent inhibitors of mineral elements. Oxalates are regarded as an undesirable constituent of diets as they reduce assimilation of calcium and favour the formation of renal calcium. However, thermal processing is reported to degrade them in tubers.[17,19] The near total elimination of cyanide in this study is quite impressive. Earlier reports from other studies indicated that cyanide levels can be reduced by 50% by boiling[20,21] and as low as 70-95 percent after soaking in water for 3 days.[22] Passive diffusion of anti-nutrients during soaking and fermentation and subsequent decantation of the medium ensures safety.[23,25] Adanlawo and Ajibade[26] reported >60mg of cyanide as toxic dose for human consumption. Consumption of improperly processed cyanogenic plants is capable of causing chronic and acute health problems:

neurological, respiratory, cardiovascular and thyroid debilities.[25,27]

CONCLUSION

A combination of fermentation and boiling improves protein and carbohydrate contents and is more efficient in eliminating anti-nutrients in *Icacina senegalensis* Tuber. This method is akin to the processing and preparation of cassava foofoo whose safety margin is yet to be refuted.[28]

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Conflict of Interest and Source of Funding Statement

The authors declare that there are no conflicts of interest whatsoever. The research was privately funded.

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