Effect of Processing Method on the Antinutrient Content of *Tacca leontopetaloides* (L.) Kuntze Flour

Collinlaw J. Ndouyang¹, Nicolas Y. Njintang²*, Balaam Facho³, Joel Scher⁴ and Carl M. F. Mbofung¹

¹ENSAI, University of Ngaoundere, P.O. Box 455, Ngaoundere, Cameroon.
²Faculty of Sciences, University of Ngaoundere, P.O. Box 454, Ngaoundere, Cameroon.
³Faculties of Exact Sciences and Appliquees, University of N'Djamena, B.P. 1027 N'Djamena, Chad.
⁴Biomolecular Engineering Laboratory (LiBio), ENSAIA- University of Lorraine2, Avenue de la Forêt de Haye, B.P.172, 54500 Vandœuvre-lès-Nancy, France.

ABSTRACT

**Aims:** This study was aimed at evaluating the effect of the method of processing on the antinutrient content of flours made from *Tacca leontopetaloides* (*Tacca*) tubers.

**Study Design:** Two experimental designs - the randomized block and the doehlert designs - were used in the study.

**Place and Duration of Study:** The study was carried out in the Laboratoire d'ingenierie et Biomolecules of the University of Lorraine, France between September 2011 and May 2012.

**Methodology:** Fresh tacca tubers were peeled, sliced and subjected to six different soaking and boiling conditions: unprocessed, 36 h soaking- 4h boiling, 72h soaking, 4h boiling, 4h boiling-36 h soaking, 4h acid boiling, 4h alkaline boiling. Following the doehlert design, 9 treatments were obtained within the experimental domain of soaking (0 to 48h) and boiling time (3 to 5 h).

*Corresponding author: E-mail: njintang@yahoo.fr,*
Results: The results revealed that untreated tacca flour contained high levels of antinutrients: total oxalates 870 mg/100g; soluble oxalates 399.7 mg/100g; phytates 458.0 mg/100g; cyanides 1.59 g/100g; saponins 4081.2 mg/100gDM; total polyphenols 419.3 mg/100g; total tannins 355.2 mg/100g; flavonoids 23.5 mg/100g and alkaloids 803.9 mg/100g. Soaking or boiling alone induced only limited reductions (up to 50% in most cases) in the antinutrients content of the flours. While boiling compared to one step soaking generally had a much higher impact on the reduction in antinutrients, a significant difference was observed amongst boiling solutions. In fact while boiling in water had a limited effect on the elimination of oxalate and saponins, boiling in alkaline was less efficient in the removal of alkaloids. Boiling followed by soaking was more effective in reducing antinutrients compared to boiling or soaking alone. Experiments carried out to optimize the treatment conditions showed 25 and 4-5 hours as the respective optimum conditions for soaking and boiling of the tubers to obtain flour of low antinutrient content.

Conclusion: Soaking and/or boiling in solution leads to more than 50% reduction in the antinutrients contents of tacca. The most efficient treatment condition resulting in 90% reduction in all antinutrients consists of double soaking for periods of 36h each. The residual antinutrients are below non toxic levels, and might play positive roles in metabolism, but this is yet to be investigated.

Keywords: Tacca leontopetaloides tubers; soaking/boiling treatments; antinutrients.

1. INTRODUCTION

In Sub-Saharan Africa, the consumption of non-conventional plant foods during periods of food shortages or famines has been documented as very common [1]. In Chad, one of such plant foods usually processed and consumed is a tuber obtained from the plant *T. leontopetaloides* (Taccaceae) or tacca. The traditional method of processing the tuber as food for consumption is not only very laborious and time consuming but uses a lot of water consuming; it involves pulping the tuber followed by several cycles of soaking and washing of the pulp so as to reduce its bitter taste and as such its content in toxic substances [2]. Some of these substances have been reported to include taccalin [3], oxalates [4] and toxic saponins [5]. As of now, little or no attention has been paid to improving on the traditional method of processing this tuber. More specifically, the interaction between soaking and eventual cooking has not been investigated. In addition to this, the level of common plant antinutrients such as phytic acid, tannins and phenolic acids expected to be present in these tubers has not been determined in *T. leontopetaloides*.

In addition to the context in which these tubers are often processed for food, the need for large amounts of water in areas where water supply is often a problem, the risk of toxicity to consumers resulting from improper processing could be very high. In view of the above, the present study was carried out to bridge our knowledge gap on the effects of different processing methods on the anti-nutritional profile of the resulting product. The final objective here being to improve on the traditional method of processing to reduce the anti-nutrient content of the food so processed from *T. leontopetaloides* tubers.

2. MATERIALS AND METHODS

2.1 Pretreatments of *T. leontopetaloides* Tubers and Production of Flour

Fresh *T. leontopetaloides* samples were harvested from the wild in the locality of Binder in Western Mayo-Kebbi Region, Chad and subjected separately to two different processing protocols: the first being a traditional household process as described by Ukpabi et al. [3] while the second involved the optimization of the soaking and boiling conditions. In either case, the changes in the anti-nutrients content of *T. leontopetaloides* during processing were monitored.

2.1.1 Effects of household processing

In the first design, tubers were pretreated following the scheme shown in Fig. 1. Known weights (100-150g) of sliced tubers were subject to the boiling and soaking regime in 5 L aqueous, alkaline or acid solution. In this process the acid solution consisted of *Hibiscus sabdariffa* leaves juice (10 g in 1 L of water) while alkaline solution was obtained by solubilizing 4g of natron in 1 L of water. After all the pretreatments, the slices were...
dried at 50°C for 18 h, and then stored at 4°C for antinutrients analyses. Besides these treatments, two other flours derived from untreated slices and two-steps consecutive soaking slices were also produced. For the unprocessed *T. leontopetaloides* flour, the tubers were peeled, cleaned, pulped and dried for 24 h in an electrical convection dryer set at 50°C. In the procedure of production of consecutive soaking flour, the tubers were chopped in a Blender machine (Japan), mixed with water (1:3 w/v) for 30 min, left for 60 min for the suspension to settle discarding the water. This process was repeated twice and which the resulting precipitate presumably free of toxic substances spread out on an aluminum foil and allowed to dry in the same conditions as above.

### 2.1.2 Optimization of soaking and cooking conditions

In order to determine the optimum soaking and boiling times necessary to reduce the phytate and saponins contents of the processed tacca, effects of varying soaking (0 to 48 h) and boiling (3 to 5 h) times were studied following a Doehlert [6] experimental design Table 1. The randomized conditions were generated by the statgraphic software.

The experimental design consisted of 9 points, and according to the optimizing design methodology, a second order polynomial equation was assumed for the variation of phytates and saponins (y) as follows:

$$y = a_0 + a_1x_1 + a_2x_2 + a_{12}x_1x_2 + a_{11}x_1^2 + a_{22}x_2^2$$

---

**Fig. 1. Soaking and boiling treatments protocols of *Tacca leontopetaloides* tubers**

*Treatments are shown in italic*

**Table 1. Experimental design of optimization in coded and true variables**

| Test no | Coded variables | True variables |
|---------|-----------------|----------------|
|         | Soaking time $x_1$ | Cooking time $x_2$ | Soaking time (h) | Cooking time (h) |
| 1       | 0               | 0              | 24              | 4                |
| 2       | 1               | 0              | 48              | 4                |
| 3       | 0.5             | 0.866          | 36              | 5                |
| 4       | 0               | 0.866          | 24              | 5                |
| 5       | -0.5            | 0.866          | 12              | 5                |
| 6       | -1              | 0              | 0               | 4                |
| 7       | -0.5            | -0.866         | 12              | 3                |
| 8       | 0               | -0.866         | 24              | 3                |
| 9       | 0.5             | -0.866         | 36              | 3                |
In this equation coefficients $a_i$ indicate the factors effects, and $x_1$ represents the soaking time (hours) and $x_2$ the cooking time (hours).

### 2.2 Determination of the Antinutrient levels in *T. leontopetaloides* Flour Samples

#### 2.2.1 Total phenolic compounds

The total phenolic compounds were quantified in methanolic extract using the folin ciocalteu reagent following the method earlier described by Nguimbou et al. [7]. The results are expressed in gallic acid equivalent (GAE).

#### 2.2.2 Flavonoids

The level of flavonoids in the samples was determined following the method described by Siddiq et al. [8].

#### 2.2.3 Total, condensed and hydrolysable tannins

The determination of total tannins level in *T. leontopetaloides* flours was based on the ability of tannins to combine and precipitate with polyvinyl polypyrrolidone (PVPP) as recently described [9]. Condensed tannins level expressed as proanthocyanidins equivalent was determined according to Makkar et al. [10]. Condensed tannins were extracted by stirring 200 mg flour sample in 10 mL 70% acetone. The mixture was agitated for 2 hours at 200 rpm using a temperature controlled water batch agitator (Polytest 20) set at 23-25°C followed by a centrifugation at 3000 rpm for 20 min. For the spectrophotometric determination, suitable aliquots (0.1 to 0.5 mL) of the extracts were put in test tubes and the volume adjusted to 0.5 mL with 70% aqueous acetone. Butanol-HCl reagent (3.0 mL) and ferric reagent (0.1 mL) were then added with thorough mixing, and the glass tube covered with a glass marble before being transferred in a boiling water bath for 60 min. After cooling the tubes, absorbance were recorded at 550 nm. For each sample, a blank composed of 0.5 mL extract, 3 mL n-butanol without HCl, and 0.1 mL ferric reagent was used. The condensed tannins (g/100g dry matter) level was expressed as leucocyanidin equivalent/100g dry matter. The level of hydrolysable tannins was determined as the difference between the total tannins and condensed tannins contents.

#### 2.2.4 Total and soluble oxalates

The total and soluble oxalates were quantified according to the method of Day and Underwood [11]. For total oxalates content determination, the test sample (0.5g) was mixed with 40 mL of 3N H$_2$SO$_4$, agitated at 200 rpm for 60 min before centrifuging at 3000 rpm for 20 min. The supernatant was collected and completed to 75 mL with distilled water. For the soluble oxalates extraction, the sample (0.5g) was treated with water in a boiling water bath for 60 min and the oxalate extracted following similar procedure for total oxalates. Each of the solutions so treated was acidified with 3N sulfuric acid and titrated to a 30 sec end point violet color using 0.01 N KMnO$_4$ solution in a water bath set at 80-90°C. The oxalates contents in the samples were calculated and expressed in mg/100g dry matter.

#### 2.2.5 Phytates

The phytate content was determined according to the method of Vaintraub and Lapteva as modified by Gao et al. [12]. Essentially, the time of extraction was prolonged to 16 hours instead of 1 hour; while the temperature of centrifugation was reduced to 10°C, with the introduction of a cleaning stage aimed at improving the phytic acid extraction. Thus, a sample of 0.5 g was introduced into a centrifuge tube containing 10 mL of 2.4% HCl and shaken for 16 hours at 200 rpm (Polytest 20 shaker), followed by centrifuging at 3000 rpm for 20 min at 10°C. The supernatant was recovered and a pinch (approximately 1g) of NaCl was added, followed by agitation at 200 rpm for 20 min to dissolve NaCl before being placed at 4°C during 60 min for a decantation. The mixtures were centrifuged at 3000 rpm at 10°C for 20 min, and clear supernatants, hereafter referred to as the NaCl-treated supernatant, were collected for color development. This treatment precipitated matrix components that could interfere with the colorimetric reaction. 250 µL of the clarified supernatant was mixed with 2750 µL distilled water in a test tube, and 3 mL of the extract thus obtained was added to 1 mL of Wade reagent (FeCl$_3$.6H$_2$O 0.03% + acid sulfosalicylic 0.30%), the tube was agitated and centrifuged at 3000 rpm at 10°C during 10 min. The optical density (OD) was read at 500 nm against a blank made up of distilled water at the place of sample extract. Phytic acid solution concentrations from 0 to 40 µg/mL were used as standards.
2.2.6 Total cyanides
The total cyanides were extracted by distillation followed by a colorimetric determination following the method described by Makkar et al. [10]. Essentially, 4 g of flour sample were introduced into a flask in which 125 mL of distilled water and 2.5 mL of chloroform were added, and the lot stirred up to obtain a homogeneous mixture. The flask was then connected to the distillation system, and the distillate collected in a flask containing 5 mL of 2% KOH for trapping the HCN. The volume of the distillate was adjusted to 25 mL with water, and the cyanide content quantified based on its reaction with picrate in hot and alkaline medium to form a red complex which absorbs at a maximum wavelength of 520 nm. In this respect, 4 mL of distillate was added to 4 mL of the alkaline picrate in a tube and heated in a boiling water bath during 5 min to allow the development of the color. The intensity of colour was read at 520 nm against the blank made up with water. The cyanides content was expressed as mg equivalent HCN per 100 g dry matter.

2.2.7 Saponins
Saponins were quantified according to the method of Makkar et al. [10]. Essentially, 1.5 g of finely crushed and defatted sample was added to 30 mL of 80% aqueous ethanol and mixed for 16 hours, followed by centrifugation at 3000 rpm during 20 min. The resulting supernatant was collected and the residue resuspended in 80% aqueous ethanol and treated in the same manner as before. The two supernatants were combined and filtered using a coffee filter paper in order to withdraw the possible particles being able to float on the surface. Then, the ethanol was evaporated at 42-45°C in Rotavapor, the aqueous phase was centrifuged to withdraw non-water-soluble materials before being transferred in a separating funnel for decantation and to undergo two extractions with equal volume to trap the pigments. The precise final volume of each extract was noted. The calibration curve was carried out with a saponin standard (Quillaja bark, Sigma, USA). The absorbance was read against a blank at 544 nm on a visible spectrophotometer UV (UV/VIS SP8001 Spectrophotometer, Axiom, Germany). The results were expressed in mg saponins/100g dry matter.

2.3 Statistical Analysis
Means and standard deviations (±SD) were calculated from 6 individual values. One way analysis of variance was tested to detect the effect of treatment on the antinutrients. Optimization was done according to the statistic of response surface methodology: analysis of variance and polynomial modeling. The significance of an effect was observed for p<.05, and the Duncan multiple test range was used to compare two means. The link between residual levels of antinutrients in the flour was analyzed using the Spearman rank correlation. The software Statgraphics Plus 5.0 was used for the statistical analysis. Principal component analysis and Pearson correlation were done using the Statbox 6.4 statistical software (Paris, France).

3. RESULTS AND DISCUSSION
3.1 Effect of Household Processing Method on the Level of Antinutrient Factors in T. leontopetaloides Flour
The antinutrients contents of T. leontopetaloides flour as affected by household treatments are presented in Tables 2a & 2b. Generally the antinutrients levels in flour samples resulting from the soaking and boiling conditions were lower compared to the levels in the untreated sample. Significant reductions were observed in the level of antinutrients in the flours. These results clearly demonstrated that soaking and/or boiling have an important effect on the antinutrients of the final product.

Table 2a. Effect of the soaking and boiling conditions on the antinutrients content (mg/100g DM) of Tacca leontopetaloides flour

| Treatments                   | Total oxalates | Soluble oxalates | Phytates | Cyanides | Saponins |
|------------------------------|----------------|------------------|----------|----------|----------|
| two steps soaked             | 16.2±1.6a      | 14.0±1.6a        | 55.7±3.5a| 0.16±0.03a| 67.9±6.19a|
| Untreated-flour              | 870±161b       | 400±163b         | 458±185a | 1.59±0.02a| 4081±237b |
| Soaked boiled                | 312±153c       | 286±173c         | 65.5±3.4a| 0.49±0.12a| 226±877a  |
| Boiled                      | 374±189e       | 354±177c         | 193±13a  | 1.02±0.13b| 961±444b  |
| Boiled-soaked               | 318±177a       | 305±186b         | 68.1±6.8a| 0.08±0.04bc| 982±106b  |
| Acid-boiled                 | 808±193f       | 297±165b         | 164±27a  | 0.29±0.06bc| 1690±191b |
| Alkaline-boiled             | 509±177g       | 18.3±1.76a       | 28.4±9.40a| Nd       | 1685±77b  |
| One-step-soaked             | 354±186b       | 296±166b         | 351±21a  | 1.11±0.05d| 2308±955a |

Mean ± sd= Mean value ± standard deviation of error of means of six experiments. The means in the same column followed with different letters are significantly different at p<.01. Nd = not detected
Table 2b. Effect of the soaking and boiling conditions on the antinutrient content (mg/100g.DM) of Tacca leontopetaloides flour

| Treatments            | Total polyphenols | Total tannins | Condensed tannins | Flavonoids | Alkaloids |
|-----------------------|-------------------|---------------|-------------------|------------|-----------|
| Two step soaking      | 9.1±1.3a          | 6.3±0.6a      | 0.55±0.10abc      | 0.38±0.15a | 44.3±8.1a |
| Untreated flour       | 419±14d           | 355±7ab       | 1.83±0.23a        | 23.5±0.62e | 804±12f   |
| Soaked boiled         | 105±2b            | 5.9±1.9a      | 0.10±0.00a        | Nd         | 392±25c   |
| Boiled                | 18±17.8c          | 95±3e         | 0.15±0.03a        | 5.6±1.08b  | 473±49d   |
| Boiled soaked         | 84±7c             | 9.0±0.5ab     | 1.02±0.15a        | Nd         | 105±4a    |
| Acid boiled           | 164±7d            | 104±12a       | 0.36±0.09a        | 13.1±0.3c  | 364±92c   |
| Alkaline boiled       | 247±1e            | 104±16b       | 0.73±0.05bcd      | 5.67±0.38b | 825±156d  |
| One step boiled       | 242±22g           | 121±29ac      | 0.91±0.16de       | 16.2±0.16d | 453±12g   |

**Mean ± sd** = Mean values± standard deviation of error of means of six experiments. The means in the same column followed with different letters are significantly different at p<.01. Nd = not detected

### 3.1.1 Total and soluble oxalates

The total oxalates contents of processed *T. leontopetaloides* were 311±15 mg/100g (soaked boiled), 374±18 mg/100g (boiled) and 318±17 mg/100g (boiled soaked), values which fall in the range (294-694 mg/100g) reported for taro corm, one of the richest tubers in oxalates [13]. The treatments generally applied to *T. leontopetaloides* tubers significantly (p<0.05) influenced the oxalates content. The most effective treatment was that involving the multiple soaking which was observed to lead to a 95% reduction in oxalates content. A mean of 40% reduction in total oxalates was achieved when the slices were boiled either in acid, aqueous or alkaline solutions. Similar decrease was observed upon one step soaking. Generally soaking was shown to be effective in total oxalate reduction. As expected, leaching of oxalates in soaking or boiling solution led to significant (p<0.05) reduction in oxalates. As demonstrated in recent studies [13], soluble oxalates represented the leached fraction of total oxalate. However the soluble oxalates in this work did not followed this trend as the level in flours resulting from either boiling or soaking still very high. This may result from hydrolysis of calcium oxalate, the most important form of insoluble oxalate in food systems. In the same vein, Noonan and savage [14] in their studies found that boiling can reduce the soluble oxalates contents of a food if the boiling water is discarded. The presence of oxalates in food poses the risk for hyperoxaluria which often results in the formation of calcium oxalate stone. Thorough multiple steps soaking seemed to be effective in reducing oxalates in *T. leontopetaloides*. The additional importance of this cannot be overemphasized since oxalates have been reported to form complexes with calcium ions Ca$^{2+}$ and other minerals such as iron in food systems leading to a reduction in their bioavailability [30]. However oxalates contents in all flours were lower than the harmful value of 0.78%.

### 3.1.2 Phytates

Phytates are common antinutrients in plant foods which complexe Ca, Mg, Fe and Zn and decrease their bioavailability. *T. leontopetaloides* tubers were observed to be less concentrated in phytates than commonly reported for cereals grains [15] and leaves [16] with range values of 500 to 6000 mg/100g, and 100-600 mg/100g respectively. Meanwhile, boiling and soaking processes significantly (p<0.05) reduced the phytates content in *T. leontopetaloides* flour. The highest reduction was observed for multiple steps soaking (88% reduction) while the lowest was observed for one step soaking (23% reduction). Aloys and Zhou [17] had also reported that soaking tends to reduce phytates levels through the mechanism of solubilisation in water. In addition the levels of phytates in our *T. leontopetaloides* samples were comparable to values reported for commonly consumed tubers [18]. In this respect varieties of yam and taro dasheen were reported to possess intermediate values (290 mg/100g), while highest value was reported in wild cocoyam (1010 mg/100g) and lowest in pumpkin (35.8 mg/100g) and breadfruit (59.1 mg/100g).

### 3.1.3 Cyanides

Cyanides are harmful substances generally associated with acute intoxication, tropical ataxic neuropathy, paralytic disease with slow onset, abrupt onset of permanent spastic paralysis of both legs, iodine deficiency disorders [10]. In addition cyanides toxicity has been mainly associated to cassava ingestion [17]. The
present study revealed that *T. leontopetaloides* has a cyanide content of 1.6 mg/100g, value 4 times lower than that of unfermented cassava 8.7 g/100g reported by Aloys and Zhou [17].

Treatments, particularly multiple steps soaking and boiling followed by soaking significantly (p<.05) reduced the cyanides content perhaps through a mechanism of solubilisation. Theoretically, cyanides appear in food in two forms: free cyanides and cyanogenetic glycosides. While free cyanide is easily removed by solubilisation, the glycoside form is generally removed by enzymatic hydrolysis. In this respect endogenous or bacterial-induced linamarase has been shown to play an important role in the reduction of cyanides in cassava [17].

Whatever the treatment, the levels of total cyanides in the *T. leontopetaloides* samples were lower compared to the lethal dose 5 mg/kg [10] and the FAO recommended level (10 mg/kg) in food.

### 3.1.4 Saponins

Saponins were observed to be the most abundant antinutrient in *T. leontopetaloides* with mean values as high as 4 g/100g of dry matter. Saponins are glycosidic compounds composed of a steroid (C-27) or triterpenoid (C-30) nucleus with one or more carbohydrate branches [39]. Most saponins are bitter in taste and toxic substances [5]. Concentrations of 3 to 7% are represented as powerful poisons while at 1% they are known as inoffensive; at 1.5% some biological activities on damaged mucous membranes are observed. Saponins in *T. leontopetaloides* have been identified as taccalin (3, 5, 7, 4'-tetracydroxy-flavylium-3-xyloside), a steroidal saponin endowed with a molluscicidal activity and responsible for the bitterness of the tuber [3,5]. Saponins are highly soluble component as shown by its significant reduction during soaking or boiling. In fact up to 41% reduction in saponin level was observed following one step soaking, while a two-steps soaking let to 94% saponins reduction. Soaking followed by boiling and vice versa induced a mean reduction of 82%.

### 3.1.5 Phenolic compounds, tannins and flavonoids

Phenolic compounds including tannins are the most common phytochemicals often found in some plant groups. The beneficial or harmful/toxic effects of plants are mainly attributed to the polyphenols depending on the amount ingested by the animals. The tannins are polyphenols characterized by their aptitude to complex with macromolecules such as proteins or polysaccharides and reduce their bioavailability. *T. leontopetaloides* tubers possess remarkable levels of polyphenols and flavonoids evaluated to 419 mg/100g DM and 23.5 mg/100g DM, respectively. The total polyphenols values were higher than those commonly reported for tubers such as potato (18.3mg/100g). Some yam varieties (16.0 mg/100g) have the highest level while yellow yam and breadfruit (3.4 mg/100g) have the lowest [18].

Our results showed that tannins and particularly hydrolyzed tannins were the most common (85%) phenols in *Tacc*. The different processing methods let to significant (p<.05) reduction in the level of total polyphenols, flavonoids and tannins. In particular two steps soaking led to a 98% reduction in total polyphenols, flavonoids and tannins. Boiling as a processing step only let to 56 and 73% reduction in total polyphenols and tannins, respectively. On the whole the levels of polyphenols in treated or untreated *T. leontopetaloides* flours were generally found to be below 2.7 g/100g, which have been reported to have no harmful effect on animals [15].

### 3.1.6 Alkaloids

Next to saponin, alkaloids were observed to be the most representative antinutrients in *T. leontopetaloides*. The total alkaloids content in *T. leontopetaloides* was 804 mg/100g DM. Different processing methods let to significant reduction ranging from 40% reduction in boiling and one step soaking to 90% for two-steps soaking or boiling-24h soaking treatments. Alkaloids generally include those basic substances which contain one or more nitrogen atoms, usually in combination as part of a cyclic system. Although some biological activities are attributed to some secondary metabolites, alkaloids are often toxic to humans, and many have dramatic physiological and neurological activities [10]. Two alkaloids have been identified in *Tacc* tubers and their activity on isolated cells in mitosis demonstrated their stabilizing effect on microtubules in interphase and an initiating activity of apoptosis [19]. The most studied alkaloid in *tacc* sp. is berberine which has several activities against the parasitic infections of the body and the intestine, the infections of ocular trachoma [20]. From a
physiological point of view, berberine affects lipidemia, cholesterolemia, triglyceridemia and glycemia [20]. In the opposite, berberine causes an inhibition of breathing in mitochondria, decreases the calcic load capacity through the induction of the transition from the mitochondrial permeability [21].

3.2 Use of Response Surface Methodology for the Optimization of the Processing Conditions of Tacca

Optimization curves showing the effect of the soaking and cooking times on the phytates and saponins content of Tacca flour are presented in Figs. 2 and 3. As earlier observed, soaking and cooking induced significant reductions in the antinutrients content of T. leontopetaloides slices. The changing trends in the phytates and saponins contents were similar and influenced by the interaction of processing variables. Irrespective of the cooking time, the change in soaking time was accompanied by significant linear reduction in phytates. On the other hand effect of cooking tended to vary depending on the soaking time. Soaking for short time did not significantly influenced phytates concentrations. Cooking for beyond 4 to 5 hrs was accompanied by a drop in phytates level. After long soaking period (20 to 40 hrs), the level of phytates in the slices was close to zero while cooking had no significant effect. This observation suggests that soaking time is the major processing variable that contributes to the reduction in phytates concentrations. Concerning saponins levels, soaking for 20 hours produced a drop from 1200 to 800 mg/100g. Beyond this soaking period an increase (up to 1400 mg/100g) was observed in the saponin content. This change in saponins levels during soaking may be as a result of simple diffusion mechanism which governs the exchange between the slices and soaking solution. Generally cooking following soaking did not have significant effect on the saponins content of slices. From the above observations, it appeared that soaking was the most important factor responsible for the decrease in antinutrient content of tacca during processing. The superposed contour plot showing the effect of soaking and cooking times on the phytates and saponins is presented in Fig. 4. From Fig 4 the optimal condition for soaking and cooking can be visualized as 25 hours soaking time and 4-5 hours boiling time.

3.3 Principal Component Analysis of the Change in Antinutrients during Soaking and Boiling in Households

An overview of the general change in antinutrients during processing is presented in Fig. 5. This figure resulted from a principal component analysis (PCA) with the different treatments representing individuals and the antinutrients as variables. The analysis is based on the correlation between the variables, from which virtual axes linearly correlated to existing variables are generated. The first step in the PCA analysis is the identification of the number of significant axes, called principal components (PC) [22]. It is generally believed that principal components with eigen value higher or equal to 1 are significant. Based on this, 2 principal components, PC1 and PC2 were revealed following the execution of PCA of the data Table 3.

It can be seen that all the variables are close together thus highlighting positive correlation between them as shown in Table 4. For that reason, all the variables were positively related to the principal component axis 1 (PC1) which explained 72% variations of antinutrients in T. leontopetaloides. Flours that are on the right side of PC1 possessed high levels of antinutrients while those at the left side possessed low levels. In this respect untreated flour was on the opposite side of two steps soaked flour. This was expected since two-steps-soaking has been identified as the most prominent treatment for the reduction of all the antinutrients in this study. Nearby two-steps-soaking are soaked-boiled and boiled-soaked flours which are superposed on the PC1xPC2 graph. This result suggested that boiling the slices before soaking reduced antinutrients in the same manner like soaking before boiling. However the reduction was less efficient than double soaking particularly for the soluble oxalates and cyanide levels. The difference between the different boiling solutions (water, acid and alkaline) on the PC2 axis was mostly linked to the variables soluble oxalates (40% variation), followed by alkaloids (representing 22% variation) and cyanides (16.5% variation). While soluble oxalates and cyanides had positive contribution to PC2, alkaloids had negative contribution. In this respect, flours with high level in alkaloids were positioned on the negative side of the PC2 axis, while those with high levels of soluble oxalates and cyanides were featured on the positive side of the PC2 axis. In this
organization, water-boiled sample situated at the top of the PC2 axis has high soluble oxalate and cyanides level but low alkaloids level, while alkaline boiled flour at the opposite has high alkaloids content and low oxalates and cyanides. Not far from water-boiled flour is one-step-soaked flour which not only possessed the characteristics of water boiled, but also contained several other antinutrients. Acid-boiled flour featured in the middle of both groups characterized relative medium values of all the variables. However the PC2 axis represented only 12% of variance. Based on the above observation the alkaline cooking of

| Table 3. Effect of the soaking and boiling conditions on the antinutrients content (mg/100g.DM) of Tacca leontopetaloides |
|---|---|---|---|---|---|---|---|
| PC1 | PC2 | PC3 | PC4 | PC5 | PC6 | PC7 |
| Eigen value | 7.20 | 1.19 | 0.76 | 0.52 | 0.26 | 0.07 | 0.00 |
| % variance | 71.96 | 11.89 | 7.64 | 5.19 | 2.58 | 0.72 | 0.03 |
| % cumulated | 71.96 | 83.84 | 91.48 | 96.67 | 99.25 | 99.97 | 100.00 |

Fig. 2. Effect of soaking and cooking times on the phytates level in Tacca leontopetaloides flour
The surface plot equation was as followed: phytate content (mg phytates/100 g.DM=12.82-0.21*x1-4.27*x2-0.01*x1*x2+0.004*x1^2+0.57*x2^2 where x1 and x2 are respectively soaking and cooking times

Fig. 3. Effect of soaking and cooking times on the saponins level in Tacca leontopetaloides flour
The surface plot equation was as followed: Saponins content (mg /100 g.DM)=6302.12-56.47*x1-2500.5*x2+2.34*x1*x2 +1.21*x1^2 +292.42*x2^2; where x1 and x2 are respectively soaking and cooking times

| Table 4. Spearman rank correlation matrix between the residual antinutrients levels in Tacca leontopetaloides flours |
|---|---|---|---|---|---|---|---|---|
| TO | SO | Phy | Cya | Sap | TPC | Totan | Cotan | Flav |
| TO | 1 | | | | | | | |
| SO | 0.50 | 1 | | | | | | |
| Phy | 0.55 | 0.65 | 1 | | | | | |
| Cya | 0.41 | 0.69 | 0.93 | 1 | | | | |
| Sap | 0.79 | 0.45 | 0.85 | 0.69 | 1 | | | |
| TPC | 0.78 | 0.43 | 0.79 | 0.72 | 0.94 | 1 | | |
| Totan | 0.77 | 0.44 | 0.85 | 0.77 | 0.95 | 0.94 | 1 | |
| Cotan | 0.44 | 0.22 | 0.64 | 0.46 | 0.81 | 0.67 | 0.74 | 1 |
| Flav | 0.77 | 0.48 | 0.92 | 0.76 | 0.94 | 0.87 | 0.91 | 0.64 | 1 |
| Alk | 0.66 | 0.13 | 0.45 | 0.46 | 0.70 | 0.88 | 0.74 | 0.37 | 0.61 |

TO=total oxalates; SO=soluble oxalates, Phy=phytates, Cya=cyanides, Sap=saponins, TPC=total phenolic Compounds, Totan=total tannins, cotan=condensed tannins, Flav=Flavonoids, Alk=alkaloids. Coefficient values in bold are significant at P<.05
The different flours are represented by blue points
4. CONCLUSION

*Tacca leontopetaloides* tubers contain appreciable amounts of antinutrients especially saponins and oxalates. Generally soaking and/or boiling in solution lead to more than half reduction in all the antinutrients. The most efficient processing method is that involving two-steps repeated soaking for 36h each. This tends to lead to a 90% reduction in all antinutrients. The next effective treatment is that in volving boiling for 4 h followed by 36h soaking or vice versa, boiling in either water, alkaline or acid solutions, ended with 36h soaking. Boiling in different solutions produces flours of varying residual cyanides, alkaloids and saponins levels. While boiling in water eliminates most of the alkaloids and less oxalates and saponins, a reverse observation was observed with boiling in alkaline medium. The residual secondary metabolites in *T. leontopetaloides* flour may be of some physiological and health importance, but this needs to be investigated.

COMPETING INTERESTS

Authors declared that no competing interests exist.

REFERENCES

1. Garine I. Bush food in Muzey Masa and Northern Cameroon. Mega-Chad, CNRS, Paris. 2002:13.
2. Ruffo CK, Birnie A, Tengnäs B. Edible wild plants of Tanzania. Regional Land Management Unit, Technical book. 2002:27:642-643.
3. Ukpabi UJ, Ukenye E, Olojede AO. Raw-material potentials of Nigerian wild polysynian arrowroot (*Tacca leontopetaloides*) tubers and starch. J Food Technol. 2009;7(4):135-138.
4. Ndouyang CJ, Ejoh AR, Aboubakar, Facho B, Njintang YN, Mohammadou BA, Mbofung CMF. Valeur nutritionnelle de *Tacca leontopetaloides* (L.) Kuntze, tubercule non conventionnel. Revue Genie industriel. 2009;3:24-32. French
5. Abdel-Aziz A, Brain K, Bashir AK. Screening of Sudanese plants for molluscicidal activity and identification of leaves of *Tacca leontopetaloides* (L.) O. Ktze (Taccaceae) as a potential new exploitable resource. Phytother Res. 1990;4(2):62-65.
6. Doehlert DH. Uniform Shell Designs. J Royal Statistical Soc. 1970;19:231-239.
7. Nguimbou RM, Njintang NY, Makhlouf H, Gaiani C, Scher J, Mbofung CMF. Effect of cross-section differences and drying temperature on the physicochemical, functional and antioxidant properties of giant taro flour. Food Bioprocess Tech. 2013;6:1809-1819.
8. Siddiq M, Rav R, Harte JB, Donal KD. Physical and functional characteristics of selected dry bean (*Phaseolus vulgaris* (L.)) flours. LWT-Food Sci Technol. 2010;43:232-237.
9. Ngatchic TJM, Njintang NY, Oben JE, Mbofung CMF. Protein quality and antigrowth effect of protein isolate of Mucuna (*Mucuna pruriens*) and Canavalia (*Canavalia ensiformis*) seeds. School Acad J Biosci. 2013;1(5):183-191.
10. Makkar HPS, Siddhuraju P, Becker K. Plant secondary metabolites. Methods Mol Biol. 2007;393:67-111.
11. Day RA, Underwood AL. Quantitative analysis. 5th ed. Prentic-Hall publication; 1986.
12. Gao Y, Shang C, Saghai MA, Biyashev RM, Grabau EA, Kwanyuen P, Burton JW, Buss GR. A modified colorimetric method for phytic acid analysis in soybean. Crop Sci. 2007;47:1797-1803.
13. Savage GP, Martensson L, Sedcole JR. Composition of oxalates in baked taro (*Colocasia esculenta* var. Schott) leaves cooked alone or with additions of cow’s milk or coconut milk. J Food Comp Anal. 2009;22:83-86.
14. Noonan SC, Savage GP. Oxalic acid and its effects on humans. Asia Pacific J Clin Nutr. 1999;8:64-74.
15. Gupta K, Barat GK, Wagle DS, Chawla HKL. Nutrient contents and antinutritional factors in conventional and non-conventional leafy vegetables. Food Chem. 1989;31:105-116.
16. Mohammed AM, Wolf W, Spie WEL. Physical, morphological and chemical characteristics, oil recovery and fatty acid composition of *Balanites aegyptiaca* Del. Kernels. Plant Food Hum Nut. 2002;57:179-189.
17. Aloys N, Zhou HM. Comparative study on nutrient and anti-nutrient changes in Ikivunde and Inyange, two Burundian traditionally processed cassava products. J Sci Food Agric. 2006;86:1878-1886.
18. Dilworth L, Brown K, Wright R, Oliver M, Hall S, Asemota H. Antioxidants, minerals and bioactive compounds in tropical staples. Afr J Food Sci Technol. 2012;3(4):90-98.

19. Tinley TL, Randall-Hlubek DA, Lea RM, Jackson EM, Cessac JW, Quada JC, Hemscheidt TK, Mooberry SL. Taccalonolides E and A: Plant-derived steroids with microtubule stabilizing activity. Cancer Res. 2003;63:3211-3220.

20. Wongbutdee J. Physiological effects of Berberine. Thai Pharmaceut Health Sci J. 2009;4(1):78-83.

21. Pereira CV, Machado NG, Oliveira PJ. Mechanisms of berberine (Natural Yellow 18)–induced mitochondrial dysfunction: Interaction with the Adenine Nucleotide Transl Toxicol Sci. 2008;105(2):408-417.

22. Abdou Bouba A, NJintang YN, Scher J, Mbofung CMF. Phenolic compounds and radical scavenging potential of twenty Cameroonian spices. Agric Biol J North Am. 2010;1(3):213-224.

23. White WLB, McMahon JM, Sayre RT. Regulation of cyanogenesis in cassava. Acta Hortic. 1994;375:69-77.

© 2015 Ndouyang et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?id=760&id=5&aid=6613