



Effects of Different Processing Methods on Anti-Nutritional Factors of Cassava (*Manihot esculenta crantz*)

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Abstract

The effects of different treatments/methods (sun drying, cabinet drying, blanching, boiling, roasting, and fermentation) on the anti-nutrient (cyanogen, tannin, oxalate, and phytate) contents of harvested raw cassava tubers (collected from landrace of Jhapa, Nepal) were examined.

The mean value of cyanogen, tannin, oxalate, and phytate content in raw cassava were found to be 86.29, 29.81, 12.3, and 23.75 mg/kg respectively. Maximum reduction of anti-nutrients: cyanogen (65.5%), tannin (94.57%), oxalate (80.49%), and phytate (99.99%) were found when cassava was fermented for 21 days after it has been cooked for 10 min. Sun-drying was the second most effective method in terms of anti-nutrients removal. Boiling of cassava for 5 min reduced the cyanogen and the other anti-nutrients significantly ($p < 0.05$). The reduction of cyanogen by roasting for 5 min was not significantly different ($p > 0.05$) than roasting for 4 min. The reduction of anti-nutrients by other techniques employed was less as compared to that of fermentation, and hence we conclude that fermentation is the most effective method for cassava processings.

Keywords

Anti-nutritional factors; Cassava; Cyanogenic glycoside

Introduction

Root and tuber crops are used as a staple food in most countries. Cassava root is mainly a starchy food that is very rich in carbohydrates, a major source of energy. But these are toxic when eaten raw, because of the various anti-nutritional factors present in them [1].

Anti-nutritional factors are chemical compounds synthesized in natural food and/or feedstuffs by the normal metabolism of the species and by different mechanisms (for example inactivation of some nutrients, diminution of the digestive process, or metabolic utilization of food/feed) which exerts effect contrary to optimum nutrition [2]. Some of these anti-nutrients are deleterious to health. To reduce the effect of these anti-nutrients, proper processing before consumption is necessary. Cooking improves digestibility, promote palatability, improves keeping quality, and also makes root crops safer to eat [3], ultimately reducing the anti-nutrients to the safer level to eat.

In spite of the fact that, cassava has the significant potential for being staple crop, scientific validation of traditional processing methods in terms of food quality and safety has not been attempted. Although a number of treatment methods like drying, blanching, boiling, roasting and fermentation are used for the lowering its anti-nutrients and toxicity, the information on comparative effectiveness of these methods are still the subject matter of research. The aim of this intensive research is to provide the documentation about the processing methods those are effective in reducing these factors that may significantly contribute in reducing health risk that are associated with consumption of cassava.

Materials and Methods

This study was conducted on a single variety of Cassava i.e. *Manihot esculenta crantz*, collected from the landrace of Jhapa, Nepal. For fermentation of cassava, Murcha, A local product used as a source of yeast, were brought from the local manufacturers of Dharan.

Processing methods of cassava

The processing was performed at the laboratory of the Central Campus of Technology (CCT) in Dharan, Nepal. The different processing methods employed were:

Blanching: Fresh cassava was peeled manually with a kitchen knife, washed, cut into a cylindrical shape, and blanched in the water of temperature about $88 \pm 1^\circ\text{C}$ for 3 min in an open pan.

Boiling: For boiling, the tubers were peeled, washed, cut into a cylindrical shape, and put into boiling 100 ml water, with a lid. The samples were taken for analysis with every 1 min interval.

Roasting: The fresh cassava roots were peeled, washed, and roasted up to 5 min, the analysis of the samples were done in a 1 min interval.

Fermentation: The alcoholic fermentation of cassava was performed after the peeled and washed cassava cooked for 10 min and cooled till $28 \pm 2^\circ\text{C}$. 5 gm murcha were activated and inoculated to the gelatinized cassava, and fermentation was carried on for 21 days at room temperature.

Sun-drying: The fresh cassava roots were peeled, washed, cut into pieces of 10 mm uniform size and sun-dried for 15 consecutive days, until moisture content declined to 11%.

Cabinet drying: The fresh cassava roots were peeled, washed, cut into pieces of 10 mm uniform size, and dried in a hot air oven at 55°C for 3 h.

Analytical methods for the determination of antinutrients

Determination of oxalate: Powdered sample 5 gm was taken in a 250 ml volumetric flask with 60 ml distilled water, 11 ml (6 N HCl) was added and boiled for 15 min and cooled. The volume was made up to 100 ml with distilled water. The solution was left overnight, the next day the solution was centrifuged and 25 ml supernatant was taken in which 5 ml tungstophosphoric acid added, the solution left for 4 h and centrifuged. Again the supernatant was taken in a beaker in which ammonium hydroxide was added dropwise to maintain a

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pH of 4-4.5. Then 5 ml acetate buffer was added and the solution left overnight.

The solution was centrifuged and the supernatant was discarded. The precipitate was washed with 20 ml distilled water further centrifuged again discarding the supernatant. To the precipitate 5 ml sulphuric acid (1:9) was added and the solutions transferred in a conical flask with distilled water. The whole solution was boiled in a water bath and titrated hot with standardized KMnO_4 until the pink color persisted for 30 s. The calculation was done as:

1 ml of 0.002 M KMnO_4 = 0.45 mg anhydrous oxalic acid.

Determination of phytate: Phytate was determined using the method with some modifications. 5 g of the ground sample was soaked in 100 ml of 2% HCl for 5 h and filtered. To 25 ml of the filtered, 5 ml 0.3% ammonium thiocyanate solution was added. The mixture was then titrated with Iron (III) chloride solution until a brownish-yellow color that persisted for 5 min was obtained [4].

Determination of tannin: Tannin was quantitatively determined according to the manual of food quality control, following the colorimetric determination technique. Colorimetric estimation of tannins is based on the measurement of the blue color formed by the reduction of phosphotungstomolybdic acid by tannin-like compounds in alkaline condition. The measurement is done at 760 nm [5].

Determination of cyanide content: HCN content in cassava roots was determined by an improved version for determination of the free and potential cyanide based on autolysis of cassava roots in which endogenous linamarase enzyme was used to hydrolyze linamarin and release CN-free cyanide determination is based on the colorimetric reaction of the picrate solution [6,7].

5 gm of prepared sample was taken in clean mortar and pestle. Then extraction was carried out in 50 ml distilled water by grinding it homogeneously for 1 min to 2 min. The extract was filtered through a moderately retentive filter paper whatman No. 42. The filtrate was taken and 5 ml of this aliquot was transferred to a clean test tube followed by the addition of 10 ml of alkaline picrate and 5 ml of distilled water. This was a sample solution. Similarly, a blank was made with each set of samples containing 0 ml of sample filtrate, 10 ml of alkaline picrate, and 5 ml of distilled water. The test tubes containing sample and color reagents were incubated for 15 minutes in a water bath at 37°C. Then, 15 μl of sulfuric acid was added to stop the reaction and increase the stability of reading. All the sample and blank were shaken well and the absorbance was immediately read at 535 nm [7].

HCN content (ppm or mg/kg) = $(Y \times 50 \times 1000) / (\text{aliquot taken} \times \text{sample weight taken})$

Where, $Y = 0.2476x + 0.009$ ($R^2 = 0.9536$)

x = absorbance

Statistical analysis

For all chemical analyses, triplicates of the same sample were used and their mean value was used for statistical analysis for the determination of each anti nutrient. Mean values with standard deviation were computed. Data on boiling and roasting were subjected to Analysis of Variance (ANOVA) and considered a 95% confidence level using statistical software GenStat (Twelfth Edition developed by VSN International Limited).

Results and Discussion

Distribution of anti-nutrients in raw cassava tuber

Cyanogen, tannin, oxalate, and phytate content of a fresh raw sample of *Manihot esculenta* crantz was determined and is shown in Table 1. The mean values of cyanogen, tannin, oxalate, and phytate were 86.29 mg/kg, 29.81 mg/kg, 12.3 mg/kg, 23.75 mg/kg respectively.

Sundaresan et al. have classified cassava roots based on cyanogen content: Sweet varieties, having cyanide concentration <100 mg HCN equivalents/kg Fresh Weight (FW), bitter varieties, ranging from 100 mg to 450 mg HCN equivalents/kg FW, and very bitter varieties having concentration >450 mg HCN equivalents/kg FW [8]. The above-presented data is supported by Guédé et al. who worked on cassava tuber and observed that fresh tuber contains 230 mg/kg (WB) cyanide content in fresh cassava tuber. Based on these references, our analytical cassava tuber can be assumed of the sweet variety [9].

Likewise, tannins and oxalate content of raw tuber are found near to the range given by Omosuli and Haritha et al. But the phytate content was found less than that explained in the literature [10,11]. This may be due to the degradation of phytic acid by inherent phytase upon storage on low temperature or it may be due to differences in the variety of cassava [12].

Effects of drying

The sun-dried (15 days, 11% m.c.) and the cabinet dried (55°C for 3 h) samples of cassava were analyzed to study the reduction pattern of anti-nutrient.

Cyanide was found to reduce to 65.84 mg/kg and 37.29 mg/kg from 86.29 mg/kg in raw (i.e. 23.69% and 56.79% reduction) in the cabinet and sun-drying respectively. The result obtained agrees with Nambisan, who reported lower cyanide retention during sun-drying than in oven drying because the temperatures remain well below 55°C [13]. These temperatures are optimal for linamarase activity resulting in better cyanogen degradation. Similarly, the tannin, oxalate, and phytate were found to reduce in a similar manner. The cabinet drying of cassava chips showed 26.14 mg/kg, 7.72 mg/kg and 13.93 mg/kg (i.e. 12.31%, 37.2% and 41.34% reduction) respectively for tannin, oxalate, and phytate, while sun-dried cassava chips showed the readings 1.726 mg/kg, 2.58 mg/kg and 4.34 mg/kg (i.e. 94.2%, 79% and 81.73% reduction) respectively.

The changes in cyanogen, tannin, oxalate, and phytate in sun-dried and cabinet dried cassava are shown in Figure 1.

Effects of blanching

Cyanide was found to reduce to 67.9 mg/kg from 86.29 mg/kg in raw (i.e. 21.31% reduction) when blanched for 3 min. The tannins, oxalate, and phytate were found to reduce to 27.36 mg/kg, 6.44 mg/kg, and 8.15 mg/kg (i.e. 8.22%, 47.64%, and 65.68% respectively). For the cyanogen reduction, the result obtained were similar to those

Table 1: Cyanogen, tannin, oxalate and phytate content of raw cassava tuber (mg/kg).

Anti-nutrients	Values (mg/kg)
Cyanogen	86.29 ± 0.78
Tannin	29.81 ± 0.148
Oxalate	12.3 ± 1.782
Phytate	23.75 ± 2.086

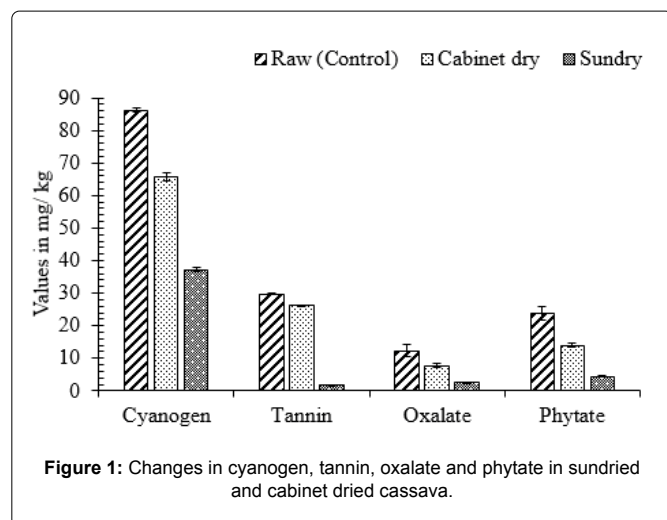


Figure 1: Changes in cyanogen, tannin, oxalate and phytate in sundried and cabinet dried cassava.

obtained by Udensi et al. which says, residual cyanide was found more in blanched samples but improved the functional properties of the flour [14].

The changes on cyanogen, tannin, oxalate, and phytate content in blanched cassava is shown in Figure 2.

Effects of boiling

For studying the effects of boiling, cassava samples were taken individually for boiling for 1 min to 5 min respectively. After boiling, the samples were analyzed to study the reduction pattern of anti-nutrients.

Cyanide was found to reduce to 71.7 mg/kg, 64.99 mg/kg, 59.79 mg/kg, 57.47 mg/kg and 54.72 mg/kg (i.e. 16.98%, 24.68%, 30.71%, 33.93% and 36.59% reduction) when boiled for 1 min, 2 min, 3 min, 4 min and 5 min respectively. Similarly, the tannins were found to reduce to 26.33 mg/kg, 22.93 mg/kg, 19.98 mg/kg, 17.20 mg/kg, and 15.05 mg/kg from initial 29.80 mg/kg on 1 min, 2 min, 3 min, 4 min, and 5 min boiling respectively, while oxalate found to reduce to 10.89 mg/kg, 8.46 mg/kg, 7.19 mg/kg, 6.65 mg/kg and 4.81 mg/kg from initial 12.3 mg/kg. The phytate was reduced from initial 23.75 mg/kg to 20.39 mg/kg, 16.55 mg/kg, 11.86 mg/kg, 7.87 mg/kg, and 4.35 mg/kg on boiling 1 min, 2 min, 3 min, 4 min, and 5 min respectively.

Apart from the first two minutes, further boiling reduced the cyanogen to the safer level, indicating, greater the boiling time, the larger is the reduction of cyanogen. But, increasing in boiling time alters the textural property of cassava, making it gelatinized and undesirable.

The effects of boiling on cyanogen, tannin, oxalate, and phytate content of cassava are shown in Figure 3.

Effects of roasting

For studying the effects of roasting, the peeled cassava pieces were treated directly in the fire for 1 min, 2 min, 3 min, 4 min, and 5 min. After roasting, samples were analyzed to study the reduction pattern of anti-nutrients.

Cyanide was found to reduce to 68.47 mg/kg, 56.64 mg/kg, 49.73 mg/kg, 46.18 mg/kg and 45.58 mg/kg (i.e. 20.65%, 34.34%, 42.37%,

46.48% and 47.18% reduction) when roasted for 1 min, 2 min, 3 min, 4 min and 5 min respectively. Similarly, the tannins were found to reduce to 24.50 mg/kg, 23.13 mg/kg, 22.11 mg/kg, 21.42 mg/kg, and 20.95 mg/kg from 29.80 mg/kg initial on roasting for 1 min, 2 min, 3 min, 4 min, and 5 minutes respectively which is not a significant reduction.

Similarly, Oxalate was found to reduce to 10.16 mg/kg, 8.75 mg/kg, 8.22 mg/kg, 7.74 mg/kg, and 7.69 mg/kg from initial of 12.3 mg/kg in raw tuber on roasting for 1 min, 2 min, 3 min, 4 min and 5 minute respectively. The phytate reduction on roasting was sharp on 1st min of roasting i.e. to 13.77 mg/kg and on further roasting for 2, 3, 4 and 5 min showed a reduction to 10.97 mg/kg, 9.6 mg/kg, 8.93 mg/kg, and 8.68 mg/kg respectively.

This shows the considerable reduction of anti-nutrients on roasting and it can be predicted that further reduction could be achieved if cassava is roasted for a longer time. However, a longer roasting period enhances the burnt texture on the surface and may impair the sensory attributes as well. The reduction pattern of cyanogen, tannin, oxalate, and phytate on roasted cassava is shown in Figure 4.

Effect of fermentation

For studying the effects of fermentation of cassava by murcha, cassava samples were taken and boiled for 10 min and let fermentation

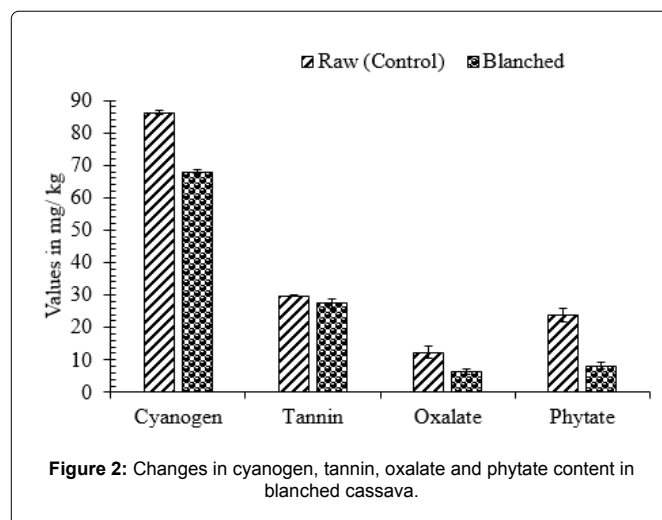


Figure 2: Changes in cyanogen, tannin, oxalate and phytate content in blanched cassava.

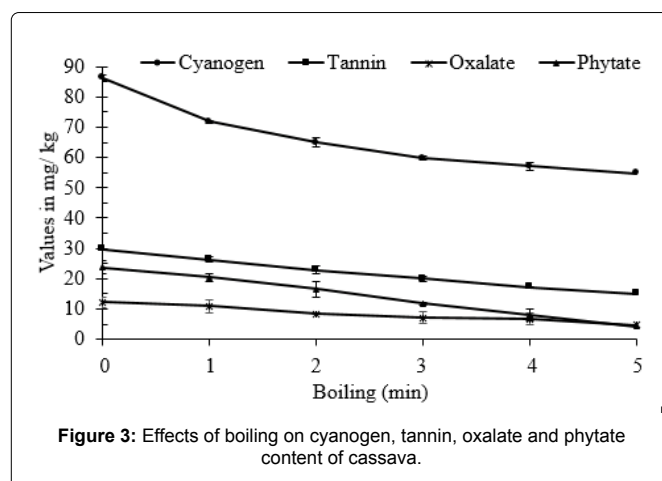


Figure 3: Effects of boiling on cyanogen, tannin, oxalate and phytate content of cassava.

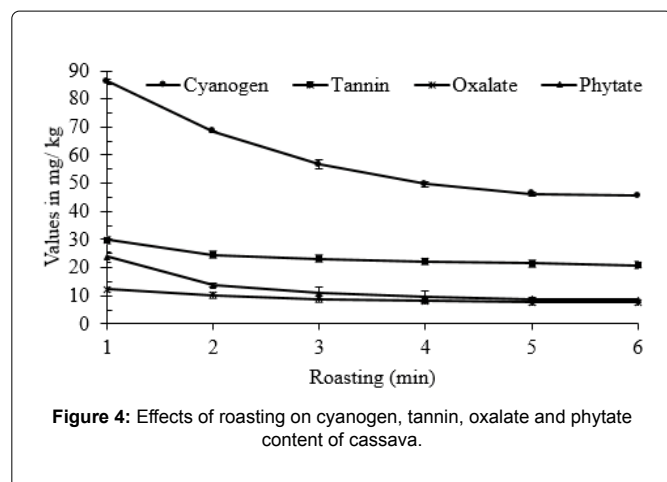


Figure 4: Effects of roasting on cyanogen, tannin, oxalate and phytate content of cassava.

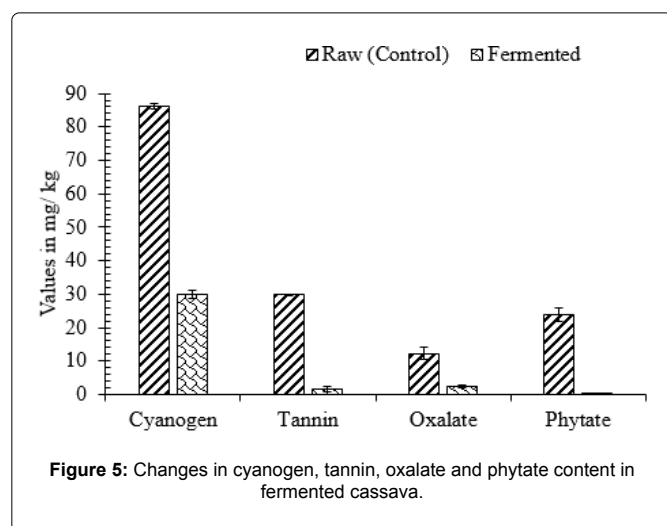


Figure 5: Changes in cyanogen, tannin, oxalate and phytate content in fermented cassava.

for 21 days at room temperature. After fermentation is complete, the samples were analyzed to study the reduction pattern of anti-nutrients.

Cyanide was found to reduce to 51.91 mg/kg on boiling for 10 minutes and to 29.80 mg/kg (i.e. 65.5% overall reduction) on alcoholic fermentation of cassava on the 21st day. The reduction of cyanogen content was below than that explained by Khadka which explains, a sheer drop of 90.8% in cyanogen contents of cassava during the overall process of jand preparation including both boiling and fermentation [15]. This incompatible reduction may be influenced by the lower temperature during fermentation, directly relating to the efficiency of murcha.

The tannins, oxalate, and phytate were found 1.62 mg/kg, 2.4 mg/kg, and 0.01 mg/kg i.e. 94.57%, 80.49%, and 99.99% of reduction, respectively. The phytate on the end of fermentation was noted around nil. The changes in cyanogen, tannins, oxalate, and phytate content in fermented cassava is shown in Figure 5.

Conclusion

In this study, cassava tuber was processed with several treatments and the variations in the reduction of anti-nutrients were analyzed in the lab. Within the scope of the present work following conclusions can be drawn. The mean value of cyanogen, tannin, oxalate, and

phytate content in raw cassava were found to be 86.29 mg/kg, 29.81 mg/kg, 12.3 mg/kg, and 23.75 mg/kg respectively on a dry basis. Maximum reduction of anti-nutrients such as cyanogen (65.5%), tannin (94.57%), oxalate (80.49%), and phytate (99.99%) was found when cassava was cooked for 10 min followed by fermentation for 21 days. In the case of boiling, the boiling of cassava for 1 and 2 min was insufficient to bring cyanogen less than lethal dose, while further boiling reduced significantly ($p < 0.05$), the cyanogen and other anti-nutrients. In the case of roasting, roasting for 1 min was not sufficient to reduce cyanogen. The 5 min roasting was not significantly different ($p > 0.05$) than roasting for 4 min. Every processing methods (excluding cabinet drying, blanching, roasting for 1 min, and boiling for 1 and 2 min) were effective in reducing the anti-nutrients to a safe level.

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Conflicts of Interest

We do not have any conflict of interest for the particular research.

Ethical Statement

This study does not involve any human or animal testing.

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