Cyanogenic Glycosides Retention in Juiced and Blanched Leafy Vegetables Commonly Consumed in South-Western Nigeria

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Authors’ contributions

This work was carried out in collaboration between all authors. Author CBA designed the study, wrote the protocol. Author AKA managed the laboratory work. Author KTO collected and identified the species of the plants while author BAS search the literature did the write up and supervised the project. All authors read and approved the final manuscript.

ABSTRACT

Vegetable is one of the rich sources of phytonutrients and it is part of the dietary components of inhabitants of south-western Nigeria. Despite its nutritional and health benefits, it is not without other factors that are detrimental to health such as anti-nutrients like cyanogenic glycosides. Comparative evaluation of the effect of juicing and blanching on cyanogenic glycoside was investigated in ten (10) leafy vegetables commonly consumed in southwest Nigeria. Varying cyanogenic glycoside content was noted among the leafy vegetables. Blanching significant reduced cyanogenic glycoside in virtually all the vegetables except Talinum triangulare which retained about 70% of its cyanogenic...
glycosides contents after 5 minutes exposure to boiling water. Also, reduction was observed in juicing but to a lesser magnitude. Thus, blanching is more effective in reducing cyanogenic glycoside when compared with juicing; hence further treatment may be required for *Talinum triangulare* because of its resistance to blanching in reducing its cyanogenic glycoside.

Keywords: Blanching; cyanogenic glycoside; juicing; leafy vegetables.

1. INTRODUCTION

It is a well known fact that vegetables, especially the leafy one such as *Amaranthus* spp., *Talinum triangulare*, *Corchorus olitorius*, *Telfairia occidentalis* and *Vernonia amygdalina* are rich sources of essential nutrients [1,2]. They occupy major portion of human and animal diets. Although, nutritional benefits abound in leafy vegetables, withal they secrete and bioaccumulate phytotoxins such as saponin, nitrates, phytates, oxalates, tannins and cyanogenic glycosides and may be accompanied by several health problems at high concentrations [2,3]. For instance ingestion of high concentration of cyanogenic glycoside leads to respiratory poisoning [4]. This is due to the ability of cyanogenic glycosides to release HCN by enzymatic hydrolysis which may cause cyanide poisoning [5]. Thus, anti-nutritional factors including cyanogenic glycosides, are increasingly recognized as significant items of the diet of humans and animals [6] which affect the overall nutritional value of foods, feeds and vegetables alike [7]. Cyanogen levels vary widely with cultivar, climatic conditions, plant part and degree of processing. Thus, their levels for some plant materials consumed by humans are *Manihot esculenta* root (15-1000 mg HCN/kg), *Sorghum vulgare* leaves (750-790 mg HCN/kg), *Prunus armeniaca* Kernel (89-2170 mg HCN/kg) and juice (2.2 mg HCN/kg), *Prunus spp.* juice (4.6 mg HCN/kg) [8].

In order to improve the nutritional quality, palatability, texture, reduce toxic agents and even preserve leafy vegetables several processing methods have been adopted by human from the time past and in recent time [9]. These methods include salting, sun drying, boiling, blanching, oven-drying, microwaving, crushing, grinding, to mention but few [10,11]. Reviews have shown that all of these processing methods alter cyanogenic glycosides level in leafy vegetables in varying manner and lenght [11,12]. Similarly, many of these processing methods have shown deteriorative effects on nutritional qualities of vegetables. One of the prominent leafy vegetables processing methods recently adopted is juicing; employed to preserve and concentrate beneficial components of the plants [11,12]. Similarly, blanching – brief exposure of leafy vegetables to heat at about 100°C has been documented to show lesser deteriorative ability on vegetables [13]. Unlike most other conventional and high-tech methods, blanching and especially juicing methods are sparingly investigated on cyanogenic glycosides constituents of leafy vegetables found in southwestern Nigeria [14]. Therefore this study is set to investigate juicing and blanching effect on cyanogenic glycoside level in ten selected vegetables commonly consumed in southwestern Nigeria.

2. MATERIALS AND METHODS

2.1 Plant Materials and Preparation of Extracts

Ten fresh leafy vegetables were sourced from major markets in Ago-ibowo, Ikenne and Sagamu, in Ogun State and Ketu in Lagos state, Nigeria. The weight of the samples ranged between 1 to 5 kg. The identification and authenticity of the plants were done by a botanist at the Olabisi Onabanjo University, Ago-Iwoye, Ogun state, Nigeria. The vegetables were destalked and rinsed with distilled water.

The lists of the vegetable with their Yoruba local names in parenthesis includes: *Amaranthus* spp. (Ebiden), *Crassocephalum rubens* (Eboito), *Talinum triangulare* (Efo Gbure), *Amaranthus viridis* (Efo tete), *Ipomia batatas* (Ewe Odunkun), *Manihot esculenta* (Ewe Paki), *Corchorus olitorius* (Ewedu), *Piper guineense* (Uziza), *Senecio biafrae* (Worowo) and *Launea taraxacifolia* (Yanrin).

2.2 Sample Preparation

The vegetables were destalked to remove the inedible part; afterwards samples of each specimen (from each market) were mixed together and divided into four replicates. Each group was further grouped into two subgroups: fresh and blanched.
2.3 Juicing
Juicing was achieved by using master chef high speed blender. The juice and pulp extracts were separated by using fine mesh.

2.4 Blanching
A method of Podşedek, [12] was used. Two (200 g) of each vegetables was immersed in 500 ml of hot water at 100°C and it was allowed to stay for five minutes. The vegetables were removed and then drained before analysis.

2.5 Quantitative Analysis of Cyanogenic Glycoside
Cyanogenic glycosides was analysed by alkaline picrate method of Sarkiyaki [15]. Five hundred milligram of the sample was added to 20 ml of 1N HCl and was boiled for 4 hours. After cooling it was filtered and 50 ml of petroleum ether was added to the filtrate. The mixture was allowed to evaporate at 60°C. 5 ml of acetone-ethanol was added to the residue and 0.4 ml of each sample was taken into different corresponding labelled test tubes. Six milliliter of ferrous sulphate reagent was added into them followed by 2 ml of concentrated H2SO4. It was thoroughly mixed after 10 minutes and the absorbance was taken at 490 nm.

2.6 Moisture Content
The moisture content of 10 g of each sample was determined. This was done by taking 10 g of each sample from each replicate (4 samples) into a 200 ml crucible and then it was dried in oven at a temperature of 105°C for 24 hour.

2.7 Statistical Analysis
Differences between groups were determined by One-way analysis of variance (ANOVA), and difference between mean values were checked using Duncan Multiple Range Test (DMRT) at p<0.05 level of significant. Graphical representation of values are mean ± standard error of mean (SEM) and were analyzed using Statistical Package for Social Sciences (SPSS, 16.0) software for windows.

3. RESULTS AND DISCUSSION
Health complications associated with long-term consumption of small amounts of cyanide are well documented to induce severe health problems such as tropical neuropathy [16], glucose intolerance, konzo (spastic paraparesis) [17,18]. This complication may provoke goiter and cretinism [19] if consumed with low iodine diet. Similarly, cyanogenic glycosides chronic toxicity is increasingly well documented in cassava (cyanogenic glycoside rich plant) consuming regions [20,21]. Although, some reviews have shown that cyanogenic glycoside consumption in minute amounts may be beneficial as may help to reduce the population of microflora of the GIT and inhibits cancer growth. However, prolong assimilation of this phytochemicals may be lethal.

This study revealed variation in cyanogenic glycoside level in different leafy vegetables. Although, no significant difference (p>0.05) was observed among six of the ten leafy vegetables investigated (Amaranthus spp., Crassocephalum rubens, Ipomia batatas, Manihot esculenta, Senecio biafrea and Launea taraxacifolia). Similarly, no significant difference (p>0.05) was noted among Talium triangulare, Manihot esculenta and Piper guineense. Also, Amaranthus viridis was not significantly different (p>0.05) from Corchorus oliterius.

Cyanogenic glycoside level in juiced Amaranthus spp., Talinum triangulare, Amaranthus viridis, Corchorus oliterius, Piper guineense and Launea taraxacifolia was significantly (p<0.05) lower when compared with their corresponding leafy vegetables. Cyanogenic glycosides content in the remaining four leafy vegetables was not different from their corresponding fresh leafy vegetables. Cyanogenic glycosides retained in juiced extract of each vegetable varies. While Manihot esculenta and Senecio biafrea had more cyanogenic glycoside than their corresponding fresh, over ninety percent (90%) was retained in Crassocephalum rubens and Ipomoea batatas. Amaranthus spp., Talinum triangulare and Piper guineense retained over 70% of the cyanogenic glycosides present in their corresponding fresh leafy vegetables. Cyanogenic glycoside content retained in Amaranthus viridis and Corchorus oliterus was between 50% and 70% of total cyanogenic glycosides present in the fresh leafy vegetables. Juiced Launea taraxacifolia lost the highest cyanogenic glycosides (more than 70%) when compared with to other juiced vegetables.

Vegetable pulp is the residue left after extracting juiced portion of vegetable. Cyanogenic glycoside retained in the pulp portions of
vegetables investigated was significantly (p<0.05) reduced when compared with their corresponding fresh samples. About 70% of cyanogenic glycoside present in *Manihot esculenta* fresh vegetables was retained in its pulp while, others had less than 50% of their initial cyanogenic glycoside retained in their respective pulp portions. In addition, the least cyanogenic glycoside retained was noted in *Amaranthus viridis*, with less than 10% retention level.

Blanching, a processing method that involve brief exposure of vegetables to water up to 100°C significantly reduced cyanogenic glycosides content in all the leafy vegetables investigated in this study. The cyanogenic glycosides present in blanched leafy vegetables ranged between 220 mg/100 g dry weight in *Talinum triangulare* and 80 mg/100 g dry weight in *Manihot esculenta*. An evaluation of percentage retention of cyanogenic glycoside showed that *Talinum triangulare* had the highest with about 60% while, the least percentage retention was recorded in *Senecio biafrae* which has below 20% cyanogenic glycosides retention level. More so, no significant difference was noticed in percentage retention level of most of the vegetables like *Amaranthus spp.*, *Ipomoea batatas*, *Piper guineense* and *Secenio biafrae*.

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**Fig. 1.** Comparative cyanogenic glycosides level of fresh and juiced leafy vegetables

**Fig. 2.** Comparative cyanogenic glycosides level of fresh and pulp extract leafy vegetables
A comparative evaluation of cyanogenic glycoside estimated in this study revealed that cyanogenic glycoside present in all the fresh leafy vegetables though vary nonetheless, were above World Health Organisation (WHO) recommended daily intake [22]. If one consumes 100 g dry of such vegetables (raw), Evidences have shown that several factors such as abiotic factors like temperature, soil components, weather condition [23]; and biotic agents including pest, herbivores, microbial infestation among others, [24] affects cyanogenic glycosides concentration in plants. Also, developmental or maturing stage (endogenous) as well as ecological (exogenous) factors [25] determines cyanogenic glycosides level. Similarly, environmental challenges also play vital role in the secretion and accumulation of cyanogenic glycoside [26]. Furthermore, cyanogenic glycoside concentration may be a subject of species variation as well as soil composition [27].

In comparison, blanching seems to be the most effective method of reducing cyanogenic glycoside content in leafy vegetables, as cyanogenic glycosides in most of the leafy vegetables were below 25% of the cyanogenic glycosides present in fresh leaves. This could be due to hydrolytic potential of heat on cyanogenic glycosides that largely dependent on many factors such as the nature and duration, temperature, texture of the leaf and concentration [28]. Though, juicing did not concentrate cyanogenic glycosides however; it retains more when compared to blanched vegetables. It tends to extract more cyanogenic glycosides into the juice portion with lesser extent remaining in the pulp.

4. CONCLUSION

It could be concluded that blanching of most of the leafy vegetables commonly consumed in Southwest Nigeria may be one of the efficient ways of reducing cyanogenic glycoside. However, effect of blanching on cyanogenic glycosides content in Talinum triangulare seem to be inefficient as more than 70% of it is retained after blanching. Thus, there may be need to extend boiling period of Talinum triangulare in order to remove substantial amount of cyanogenic glycoside from it. The use of vegetable juice especially when the level of cyanogenic glycosides is high in such fresh vegetable should be taken with caution as the percentage retention is relatively high in most of the vegetables juice. Thus, blanching is more efficient in reducing cyanogenic glycosides than juicing.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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