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Increase in Tannin Content of Some Selected Nigerian Vegetables during Blanching and Juicing

Bamidele Adewale Salau[1*], Kuburat Temitope Odufuwa[2], Olumide D. Olukanni[1], Adeleke K. Atunnise[2] and Gbenga G. Daramola[1]

[1]Department of chemical sciences, College of Natural Sciences, Redeemer's University, Km. 46 Lagos/Ibadan Expressway, P.M.B. 3005, Redemption City, Mowe, Ogun State, Nigeria.
[2]Department of Biochemistry, Faculty of Basic Medical Sciences Obafemi Awolowo College of Health Sciences, Olabisi Onabanjo University, Ogun State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author BAS designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author KTO managed the literature searches, analyses of the study performed the spectroscopy analysis and authors GGD and AKA managed the experimental process and author ODO identified the species of plant. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study was set to investigate the content of tannin in fresh, blanched vegetables and juiced extracts from the commonly consumed vegetables in South-Western Nigeria.
Place and Duration of Study: Chemical science department, Redeemers University, Nigeria. Research work was done between January and March, 2013.
Methodology: The vegetables were obtained from four different major markets in South West, Nigeria and grouped into three: fresh, blanched, and juiced, using standard laboratory procedures to analyze tannin content.
Results: The results from fresh vegetables showed that *Amaranthus viridis* has the highest tannin level (30.20±1.05 mg/100g dry weight) while lowest was in *Veronia amygdalina* (1.02±0.02

**Corresponding author: Email: delesalau@gmail.com, dele62@yahoo.co.uk;*

mg/100g dry weight). Also, in blanched vegetables *Amaranthus viridis* has the highest tannin level (46.81 mg/100g dry weight) and lowest was found in *Corchorus oliterus* (5.05 mg/100g dry weight). In the juiced extract however, *Launea taraxacifolia* has highest tannin level (735.77mg/100g dry weight) while lowest was recorded in *Celosia argentea* (67.18 mg/100g dry weight).
Conclusion: It can be concluded from this study that juicing and blanching substantially increase tannin content in most vegetables.

Keywords: Vegetables; tannin; juicing; blanched.

1. INTRODUCTION

Rich vegetable diets are the front runners of reduced rates of cancer, coronary heart disease, ageing and other metabolic disorders [1,2], and they are usually consumed to supplement the nutrient requirement of food due to their vitamins and minerals content and as such a cheap source of vitamins, minerals and other phytochemicals.

Plants produce secondary metabolites such as flavonoids, isoflavones, terpenes, glucosinolates and tannins which are available in diets and have been reported to have antioxidant and anti-carcinogenic properties [1]. Tannins are widely distributed in the plant kingdom, and are concentrated in large amounts in some sites or parts of plants in response to environmental influences [3].

Studies have shown diverse effect of tannins on biological systems due to their metal ion chelating and protein precipitation potentials, and as biological antioxidants [4]. In the gut of mammals, tannins induce astringency by binding to the proteins of the mucous membranes and saliva, [5] and gives a sharp unpleasant taste, as a result, it discourage herbivores from eating such plants [6]. The bitter and acrid taste of tannin is good measure of defensive mechanism in plants. The indigestible property of tannin-bond protein is explored as good preservative for leather production [7] also the aggressive lytic enzymes released by microorganism to initiate infection in plants are inactivated when tannins bound to them.

Pharmacologically, tannins are used as antibiotics; due to their antioxidant properties, they are good anti-mutagenic, anti-enzymatic, anti-carcinogenic as well as remedial agent of digestive disorders [8,9]. They speed up clotting time and reduce serum lipids, which in turn reduce cardiovascular risk and other metabolic disorders [10]. In addition to the bactericidal and biological activity, tannins are beneficial to human health in small doses by improving the immune response against microbial infection [11] but toxic at high doses [12].

Complications associated with consumption of high tannin containing foods are well documented. Tannins act as anti-nutrient by negatively altering absorption of essential amino acids and trace elements; hence it causes anemia, growth retardation, hypoglycemia as well as diarrhea. Also tannins have been implicated with decolouration of teeth, liver necrosis, kidney impairment and bowel irritation [5].

Studies have shown variation in tannin content as well as other phytochemicals occasioned by processing methods [13-15].

Processing of vegetables before consumption is a common practice in South-Western Nigeria. Such processing methods include steaming, drying, fermentation, salting, blanching, juicing, conventional cooking as well as microwave-cooking [16]. Recently, juicing of vegetables is spreading among the South Western populace with a view of concentrating the nutrients and phytochemicals which is either use for therapeutic purpose or nutritional supplements.

During processing, the quality such as palatability, nutrient, bioavailability as well as remediative abilities of vegetables is altered [17]. Various processing methods may display various effects on the nature and level of tannin in vegetables. High temperature for example, has been reported to increase the antioxidant capacity tannins in juice extract of certain vegetables [18].

Blanching has also been used to improve the efficiency of protein digestion and animal health under grazing, hence producing more sustainable grazing systems. However, the effect produced varies and also depend on the concentration and structure of the tannin [19].

Juicing as a means of concentrating phytochemicals and nutrients in fruits and vegetables has various downsides ranging from susceptibility to harmful microorganisms, toxin and high concentration of phytochemicals [20,21].

In view of the above, this study was set to investigate the content of tannin in fresh, blanched vegetables and also juiced extracts from the commonly consumed vegetables in South-Western Nigeria.

2. MATERIAL AND METHODS

2.1 Sample Collection

Eleven different vegetables used for this research work were sourced from major markets in Ago-iwoye, Ikenne and Sagamu, in Ogun state and Ketu in Lagos state, Nigeria in the month of January, 2013. The weight of the samples ranged between 1 to 5 kg; identified at the herbarium of the plant science and Zoology department, Olabisi Onabanjo University.

2.1.1 Sample preparation

The vegetables were destalked to remove the inedible part; afterwards samples of each specimen (two from each market) were mixed together and divided into four. Each group was further grouped into three subgroups: Fresh, juiced and blanched.

2.1.2 Blanching

This process was done by putting 200g of vegetables in 500 ml of boiled water and it was allowed to stay for five minutes. The vegetables were removed and then drained before analysis.

2.1.3 Juicing

This was done by using master chef juice extractor (model no: mc-J2101). The juice and pulp were collected separately and the pulp was discarded.

2.2 Sample Analysis

Quantitative analysis of tannin, Five hundred milligram (0.5 g) of the sample was added to 20 ml of 1NHCl and was boiled for 4 hours. After cooling it was filtered and 50 ml of petroleum ether was added to the filtrate then ether layer was evaporated. 5 ml of acetone ethanol was added to the residue. 0.4 ml of each was taken into 3 different test tubes. 6ml of ferrous sulphate reagent was added into them followed by 2 ml of conc. H_2SO_4. It was thoroughly mixed after 10 minutes and the absorbance was taken at 490 nm [22].

2.2.1 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 hours.

2.3 Statistical Analysis

The experimental design was completely randomized. Data were analyzed using the Statistical Package for Social Sciences (SPSS) 16. Significant different between the data was determined at $p< 0.05$ using Duncan multiple range test.

3. RESULTS AND DISCUSSION

3.1 RESULTS

Table 1 revealed the tannin content in fresh extract of vegetables, highest tannin content was observed in *Amaranthus viridis* (30.20±1.05 mg/100 g dry weight) which is significantly higher ($p<0.05$) than other vegetables. The decreased order of tannin was observed for *Amaranthus viridis*, *Manihot esculentus*, *Telfeiria occidentalis*, *Amaranthus* spp., *Piper guineesis*, *Celosia argentia*, *Launea taraxacifolia*, *Talinum triangulare*, *Colocasia esculenta* and *Veronia amygdalina* (1.02±0.02 mg/100g dry weight) respectively. However, no significant ($p>0.05$) difference was observed between *Talinum triangulare* (4.05±0.28 mg/100g dry weight), *Colocasia esculenta* (3.17±0.26 mg/100g dry weight) and *Corchorus oliterus* (3.99±0.19 mg/100 g dry weight); *Amaranthus* spp (11.32±0.48 mg/100 g dry weight), *Celosia argentia* (16.86±2.47 mg/100 g dry weight) and *Piper guineesis* (9.12±0.57 mg/100 g dry weight) but a significant difference ($p<0.05$) was observed between *Veronia amygdalina* (1.02±0.02 mg/100g dry weight), *Launea taraxacifolia* (6.32±0.18 mg/100 g dry weight), *Telfeiria occidentalis* (16.07±0.38 mg/100 g dry weight), *Manihot esculentus* (20.36±0.84 mg/100 g dry weight) and *Amaranthus viridis* (46.81±6.15 mg/100 g dry weight).

In Table 2, the blanched vegetables; *Amaranthus viridis* (46.81±6.15 mg/100 g dry weight) contains the highest tannin; and a decreasing order of tannin was observed in *Amaranthus viridis* to *Amaranthus* spp, *Talinum triangulare*, *Telferia occidentalis*, *Manihot esculentus*, *Celosia argentea*, *Piper guineesis*, *Launea taraxacifolia*, *Colocasia esculenta*, *Veronia amygdalina* (7.59±0.50 mg/100g dry weight) respectively. However, no significant difference (p>0.05) was observed between *Amaranthus* spp (25.04±4.42 mg/100g dry weight), *Launea taraxacifolia* (15.56±1.68 mg/100g dry weight), *Talinum triangulare* (20.92±3.31 mg/100g dry weight), *Colocasia esculenta* (14.86±0.93 mg/100g dry weight), *Celosia argentea* (16.86±2.47 mg/100g dry weight), *Veronia amygdalina* (7.59±0.50 mg/100g dry weight), *Manihot esculentus* (18.68±2.57 mg/100g dry weight) and *Telfeiria occidentals*, but *Amaranthus viridis* was significantly different (p<0.05) from other vegetables.

Tannin content of juice extracts of *Launea taraxacifolia* in Table 3 was observed to be the highest, while a decrease order was from *Launea taraxacifolia* (735.77±4.41 mg/100g dry weight), *Manihot esculentus*, *Piper guineesis*, *Amaranthus viridis*, *Telfeiria occidentalis*, *Veronia amygdalina*, *Talinum triangulare*, *Amaranthus spp*, *Colocasia esculenta* to *Celosia argentea* (67.18±0.49 mg/100g dry weight). No significant (p>0.05) difference was observed between *Amaranthus spp* (87.31±8.43 mg/100g dry weight) and *Talinum triangulare* (111.43±6.95 mg/100g dry weight) and *Corchorus oliterus* (74.43±12.67 mg/100g dry weight); *Telfeiria occidentalis* (234.42±29.40 mg/100g dry weight), *Piper guineesis* and *Amaranthus viridis* (247.88±19.73 mg/100g dry weight), but there were significant (p<0.05) difference between *Launea taraxacifolia* (735.77±4.41 mg/100g dry weight), *Manihot esculentus* (334.92±19.77 mg/100g dry weight), *Veronia amygdalina* (147.00±3.58 mg/100g dry weight) and *Colocasia esculenta* (11.87±0.78 mg/100g dry weight).

Table 4 revealed comparative effects of two processing methods on the tannin contents in vegetables. Both methods increased tannin content in all the vegetables investigated except for *Manihot esculenta* which was reduced in juice extract. In comparison, juicing increased tannin contents in all the vegetables except for *Colocasia esculenta* which was lower.

4. DISCUSSION

The distribution and variation in tannin content across the plant kingdom are well established [23]. Also, their levels in plant parts are based on many factors including genetic [24], environmental stress and pollution [25,26]. Hence, they are secreted in plants mainly for self-defense, protection and treatment. Though, the beneficial effect of tannin on human health for preventive and curative purposes are well established, also consumption of large amount of tannin has been reported to have adverse effect on health which ranges from diarrhea to liver and kidney damage.

Table 1. Tannin content of fresh leafy vegetable

Botanical names	English/ local names	Tannin contents (mg/100g dry wt.)	Moisture contents
Amaranthus spp.	Joy weed/ Ebiden	11.32±0.48^{d}	85.20±0.14^{d,e}
Launea taraxacifolia	Wild lettuce/ Yanrin	6.32±0.18^{c}	87.55±0.67^{f}
Talinum triangulare	Water leaf / Gbure	4.05±0.28^{b}	84.58±0.03^{c,d,e}
Colocasia esculenta	Cocoyam leaf/Ewe koko	3.17±0.26^{b}	82.53±0.58^{b}
Celosia argentia	Lagos spinach/ Soko funfun	8.43±0.15^{d}	82.50±0.00^{b}
Veronia amygdalina	Bitter leaf / Ewuro	1.02±0.02^{a}	44.10±0.00^{a}
Manihot esculentus	Cassava leaf	20.36±0.84^{f}	85.08±1.33^{d,e}
Telfeiria occidentalis	Fluted Pumpkin leaf/ Ugwu	16.07±0.38^{e}	85.60±0.40^{e}
Piper guineesis	West African black pepper/ Uziza	9.12±0.57^{d}	85.33±0.65^{d,e}
Amaranthus viridis	Green amaranth / Tete abalaye	30.20±1.05^{g}	83.53±0.08^{b}
Corchorus oliterus	Long-fruited vegetable/ Ewedu	3.99±0.19^{b}	83.23±0.74^{b}

Results presented are mean ± SEM (n = 4); values in the same column with the same superscript are not significantly different from each other (P > 0.05)

Table 2. Tannin content of blanched leafy vegetable

Botanical names	English/ local names	Tannin content (mg/100g dry wt.)	Moisture contents
Amaranthus spp.	Joy weed/ Ebiden	25.04±4.42^{d}	86.10±0.50^{c}
Launea taraxacifolia	Wild lettuce/ Yanrin	15.56±1.68^{b,c}	89.63±0.03^{d}
Talinum triangulare	Water leaf / Gbure	20.92±3.31^{c,d}	94.18±0.23^{e}
Colocasia esculenta	Cocoyam leaf / Ewe koko	14.86±0.93^{b,c}	85.88±0.69^{c}
Celosia argentea	Lagos spinach/ Soko funfun	16.86±2.47^{c,d}	87.63±0.27^{c,d}
Veronia amygdalina	Bitter leaf / Ewuro	7.59±0.50^{a,b}	80.58±1.32^{b}
Manihot esculentus	Cassava leaf	18.68±2.57^{c,d}	78.13±0.61^{a}
Telferia occidentalis	Fluted Pumpkin leaf/ Ugwu	20.20±0.91^{c,d}	87.28±0.60^{c,d}
Piper guineesis	West African black pepper/ Uziza	16.47±0.79^{c,d}	87.78±0.35^{c,d}
Amaranthus viridis	Green amaranth / Tete abalaye	46.81±6.15^{e}	87.65±1.76^{c,d}
Corchorus oliterus	Long-fruited vegetable/ Ewedu	5.05±1.27^{a}	87.03±0.41^{c}

Results presented are mean ± SEM (n = 4); values in the same column with the same superscript are not significantly different from each other (P > 0.05)

Table 3. Tannin content of leafy vegetable juice extract

Botanical names	English/ Local names	Tannin contents (mg/100g dry wt.)	Moisture contents
Amaranthus spp.	Joy weed/ Ebiden	87.31±8.43^{c}	95.23±0.46^{b,c}
Launea taraxacifolia	Wild lettuce/ Yanrin	735.77±4.41^{g}	97.73±0.63^{d}
Talinum triangulare	Water leaf / Gbure	111.43±6.95^{c}	94.03±0.33^{b,c}
Colocasia esculenta	Cocoyam leaf / Ewe koko	11.87±0.78^{a}	86.68±0.66^{a}
Celosia argentea	Lagos spinach/ Soko funfun	67.18±0.49^{b}	88.16±0.65^{a}
Veronia amygdalina	Bitter leaf / Ewuro	147.00±3.58^{d}	95.00±0.00^{b,c}
Manihot esculentus	Cassava leaf	334.92±19.77^{f}	95.20±1.34^{b,c}
Telfeiria occidentalis	Fluted Pumpkin leaf/ Ugwu	234.42±29.40^{e}	95.55±0.45^{c}
Piper guineesis	West African black pepper/ Uziza	261.07±36.69^{e}	95.08±0.52^{b,c}
Amaranthus viridis	Green amaranth / Tete abalaye	247.88±19.73^{e}	93.35±0.09^{b}
Corchorus oliterus	Long-fruited vegetable/ Ewedu	74.43±12.67^{b,c}	93.50±0.79^{b}

Results presented are mean ± SEM (n = 4); values in the same column with the same superscript are not significantly different from each other (P > 0.05)

Table 4. Percentage difference of Tannin from fresh leafy vegetable

Botanical names	English/ local names	Percentage difference in blanched (%)	Percentage difference in Juice (%)
Amaranthus spp.	Joy weed/ Ebiden	121.20	671.29
Launea taraxacifolia	Wild lettuce/ Yanrin	146.20	11541.93
Talinum triangulare	Water leaf / Gbure	416.54	2651.36
Colocasia esculenta	Cocoyam leaf / Ewe koko	368.77	274.45
Celosia argentea	Lagos spinach/ Soko funfun	100.00	696.92
Veronia amygdalina	Bitter leaf / Ewuro	644.12	14311.76
Manihot esculentus	Cassava leaf	(8.25)	1544.99
Telfeiria occidentalis	Fluted Pumpkin leaf/ Ugwu	25.70	1358.74
Piper guineesis	West African black pepper/ Uziza	80.59	2762.61
Amaranthus viridis	Green amaranth / Tete abalaye	55.00	720.79
Corchorus oliterus	Long-fruited vegetable/ Ewedu	26.57	1765.41

Values in brackets are negatives (-)

This study revealed various concentrations of tannin in different vegetables and the effect of processing methods on tannin contents in leafy vegetables. Tannin content in fresh leafy vegetables ranged between 1.02±0.02mg/100g dry weight in *Veronia amygdalina* and 30.20±1.05 mg/100g dry weight in *Amaranthus viridis*. Variation exists in tannin content of different vegetables [27], this could be linked to various factors which include genetic [24,28], and environmental pollution [25]. Also variation in tannin level has been reported to be due to its use as defensive mechanism which makes plant tissues non-palatable and indigestible to animals thereby making them to run away from feeding on it [6]. Furthermore, reports have suggested that certain plants have varying contents of tannin before and after harvest indicating environmental effect on tannin content [29].

Blanching alters various components of vegetables which may either decrease in case of minerals or increase as in case of protein in *Manihot esculenta* [30]. As observed in this study, tannin level in all the vegetables ranged between 5.05±1.27 mg/100g dry weight in *Corchorus oliterus* and 46.81±6.15 mg/100g dry weight in *Amaranthus viridis*. Comparing the level of tannin in fresh vegetables in to their corresponding blanched vegetables, the tannin level in blanched was significantly higher than the fresh. The increase observed in tannin ranged from 25% in *Piper guineesis* to about 650% in *Veronia amygdalina*, this is in consonance with various studies which showed the elevating effect of heat on tannin content in vegetables [31,32]. Though, blanched *Manihot esculenta* on the other hand, displayed about 8.30% decrease in tannin content. The possible reason for the increase could the resultant effect of high temperature on hydrogen bonding which exists between tannin complexes thereby increasing the level of free tannins [33] as it has been shown that most of the tannins present in fresh vegetables were in bound state with other compounds like protein, carbohydrates and other molecules [32].

The nature of complexes with other compounds is well documented, which could be interaction via covalent or electrovalent bonds, hydrophobic interaction, or hydrogen bonding [3].

The decrease observed in blanched *Manihot esculenta* is in line with the report of Oh et al. [34] who found that the complex formation of tannin with gelatin increased with increasing temperature and ionic strength, indicating hydrophobic interaction that may lead to increased tannins in bound state.

Advocacy for juicing vegetable, which is one of the means of concentrating the active ingredients that is being widely practiced and used in increasing nutrient intake as well as therapeutic purposes [5]. However; previous studies showed that while juicing increases nutrient contents, other phytochemicals such as saponin and alkaloids which its high intake may be injurious to health are also concentrated [35,36].

The tannin level in Table 3 revealed that juice extract of *Colocasia esculenta* 11.87±0.78 mg/100g dry weight was the lowest while the highest was *Amaranthus* spp. 87.31±8.43 mg/100g dry weight. Juice extract of each of the vegetables contain about ten folds high tannin level when compared with their blanched counterparts. As observed in Table 4, juicing increased tannin contents in all the vegetables when compared with corresponding blanched except for *Colocasia esculenta* that was the reverse. A plausible reason for this increase may be attributed to the removal of other components of plants' tissue thereby reducing the dry mass in turn, consequently increasing the concentration of tannins. Also, tannins interaction with other compounds such as protein is pH dependent; with the highest level of precipitation of tannins occurring near the isoelectric point of the protein. Therefore high tannin level in juice extract could be ascribed to the change pH which occurred due to the destruction and loss of cell contents that accompany juicing hence, making the precipitation process of tannins irreversible [11,37].

5. CONCLUSION

This study revealed the variation of tannin content in various vegetables and effect of two processing method. Blanching, which is an established practice of processing vegetables increased tannin level in the vegetables investigated except in *Manihot esculenta*. Also, Juicing which is a means of concentrating nutrients could greatly increase the tannin content in juice extracts of vegetables beyond desirable level, hence may lead to various complications associated with high consumption tannins.

Though, tannin may be useful as antibiotics, antioxidants and anti-cancer agents however, because of its anti-nutrients nature, several health disorders are linked to high consumption of tannin containing food. This study suggests that blanching considerably increased tannin while juicing outrageously increased it in most of the vegetables investigated. Therefore, caution must be exercised in using vegetable juices especially *Piper guineesis*, *Manihot esculenta* and *Launaea taraxacifolia,* by people that are vulnerable to tannin toxicity; such cases like anemia, protein malnutrition, pregnant women and other people with marginal level of nutrients and high requirement for protein and minerals which high tannin level can bind. The study would be useful to Nutritionists, Dieticians, Food Scientists and other health practitioners interested in tannin and vegetables as well.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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