PROFESSIONAL PAPER

Effect of postharvest UV-C irradiation as physical elicitor on anti-nutritional factor, B-vitamins and mineral profile of *Clerodendrum volubile* leaves

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Abstract

The effect of postharvest UV-C irradiation on the antinutrients (phytate, saponin, tannin), B-vitamins, and mineral profile of Clerodendrum volubile leaves was evaluated. The leaves were cleaned, detached, divided into two parts, one part was irradiated with UV-C lamp at wavelength 210 nm, average dose of 2.217 J/m² for 20 minutes while the other was left untreated, the two were stored for 24 hours at room temperature. The phytate, saponin, tannin, B vitamins and mineral content of the leaves were analysed after irradiation and storage using standard methods. Mineral and molar ratios were calculated. UV-C irradiation substantially decreased the phytate contents from 18.12 to 9.02 mg/g but increased the tannin contents from 0.68 to 1.42 g/100g and saponin contents from 1.44 to 1.52 g/100g. The phytate and saponin contents reduced in storage but the tannin content increased. The B vitamins reduced after UV-C irradiation and further in storage with the exception of pyridoxine (B₆) which increased significantly. The minerals P, Mn, Na, Ca and Mg content increased while K decreased with UV-C irradiation and no effect on the Fe content of white butterfly C. volubile leaves. After UV – C irradiation the mineral ratios Ca : P, Na : K, Ca : Mg, Ca : K and the miliequivalent ratio K : (Ca + Mg) were still within the required standard for their availability for absorption. [Ca][Phytate]/[Zn] molar ratios (0.0003 – 0.0006 mol/kg) were below 0.5 mol/kg the critical value. Irradiating food just like other food processing methods will depend on what the food has to contribute to the total diet.

Keywords: C volubile, antinutrient, B vitamin, mineral, UV-C irradiation, mineral ratio

Introduction

Leafy vegetables play crucial role in reducing the effect of food insecurity in many parts of the world. They contribute to a great extent to the intake of proteins, minerals, vitamins, fibre and health-supportive phytochemicals such as flavonoids, saponins, terpenoids, and lignans. This has made vegetables an important factor in the diet of man (Solanke and Awonorin, 2002, Oomah and Mazza, 2000). The nutritional and health-promoting benefits of vegetables tend to decrease over time as a result of the perishable nature of vegetables (Olaiya *et al.*, 2016).

Clerodendrum voluble White butterfly is a member of the family *Verbenaceae*, a climbing shrub, commonly grown in deciduous forests across Africa (Burkill 1985). It is known as'Eweta', 'Dagba' or 'Marugbojiyatan' in Ikale, Apoi and Ilaje land of Ondo State, also known as 'Obnettete' in Itsekiri and Urhobo land of Niger-Delta, Nigeria (Erukainure *et al.*, 2010). It is used in the management of arthritis, rheumatism, dropsy, swellings, oedema, and gout also as an anti-abortifacient and sedative (Burkill 1985). A lot has been done on *C. volubile*, the nutritional and phytochemical qualities, *in-vitro* antioxidant activity have been reported (Erukainure *et al.*, 2011, Ogunwa *et al.*, 2016). The evaluation has been carried out and reported on the phytochemicals,

antioxidant, anti-inflammatory and antihyperlipidemic potentials of C. volubile (Adetuyi et al., 2018) Adefegha and Oboh, 2016 in their study of C. volubile leaf extract reported the inhibitory properties of the leaf extract on enzymes related to non-insulin dependent diabetes mellitus and hypertension. Microbial spoilage has always been the bane of fresh fruits and vegetable. It can be prevented by surface treatments which has to be gentle so as to keep the integrity and the freshness of fruits and vegetables. The processing techniques of ultraviolet C (UV-C) light treatment has proved to be effective as the gentle surface treatment to reduce microbial loads of pathogens on fresh fruits and vegetables (Turtoi 2013). UV-light irradiation treatment holds considerable promise for shelf-life extension of fresh fruits and vegetables (Ribeiro et al., 2012). The non-ionized UV-light irradiation is simple, economical and more reliable in comparison with other ionized irradiation (Lu et al., 1991). UV-C at low doses has beneficial effect on vegetables in a phenomenon known as hormesis. UV-C irradiation can induce specific secondary metabolites biosynthesis in fresh fruits with concomitant antioxidant properties. Postharvest UV-C treatment induced accumulation of phytoalexins as a defense mechanism which in turn triggers the accumulation of phytochemicals like the total phenolics, carotenoids

and vitamin C thereby causing the improvement in the nutritional status of the fruits (Cisneros-Zevallos 2003, Alothman *et al.*, 2009). UV-C irradiation as reported by Pongprasert *et al.*, (2011) caused delay in deterioration processes, ripening and senescence of tropical fruits. Treatment of fresh fruits and vegetables with ultraviolet (UV-C) radiation has been described by Olaiya et al, (2016) as a new approach for the enhancement of antioxidant activity of vegetables.

It has been previously reported by Adetuyi *et al.*, (2018), that postharvest UV-C radiation of *C volubile* leaves for 20 mins had consistently increased the antioxidant capacity of *C volubile* leaves extracts and that UV-C treatments may be a useful non-chemical way for the enhancement of the antioxidant post-harvest quality of the vegetable (Adetuyi *et al.*, 2018). Since micronutrient and some vitamins are influenced by irradiation (Harder *et al.*, 2016). The aim of this study is to evaluate the effect of postharvest UV-C irradiation on minerals, antinutrients, B vitamins and mineral bioavailability of *C volubile* leaves in the process of enhancing the antioxidant post-harvest quality of *C volubile* vegetable through postharvest UV-C treatment.

Materials and methods

Sample collection

Fresh leaves of *C volubile* were harvested from a farm in Okitipupa, Ondo State, Nigeria, identified and authenticated at the herbarium of Biological Sciences Department, OSUSTECH. Edible portion of the leaves were separated, rinsed to remove dirt. Samples were divided into two groups: group 1 was treated with UV-C irradiation for 20 min and group 2 was without irradiation as control.

Radiation Treatment

The UV-C lamps were stabilized on for 15 min before treatment. Samples were placed on a rectangular polypropylene tray in the radiation chamber. Radiation was done under controlled condition at room temperature with UV radiation dose of 2.217 J/m² on the average and wavelength of UV-C lamp of 210 nm as described previously by Adetuyi *et al.*, (2018).

Storage Method

The treated and untreated samples were stored for 24h at controlled condition stimulating Nigeria local market storage condition for vegetables. They were placed inside a plastic basin; sprinkled with water, at 6 pm, placed inside polypropylene bag and left outside at room temperature of 27 ± 1 °C (Adetuyi and Ogundahunsi, 2010).

Sample preparation

The average serving size 50 g of the treated as well as untreated stored samples (50 g) were separated, washed, completely drained, chopped, air dried and analysed for minerals, antinutrients, B vitamins and mineral bioavailability. All analysis was carried out in triplicate.

Phytate determination

Spectrophotometric method for phytic acid determination from Vaintraub and Lapteva (1988) was adopted. The 0.5 g of both prepared samples were taken, 10 mL of 0.2 N HCl added and left to stand for 1 hour at room temperature, it was centrifuged at $3000 \times g$ for 30 mins. The clear supernatant (3 mL) and wade reagent (2mL) were added together, homogenized and centrifuged at $3000 \times g$ for 10 min. Absorbance was measured at 500 nm with a UV spectrophotometer (JENWAY 6305, Barloworld Scientific Ltd., Dunmow, Essex, UK). Phytate amount was calculated from determined phytic acid.

Saponin determination

The spectrophotometric method of Brunner (1994) was used for Saponin determination. 2 g of the ground sample was taken into 100ml of Isobutyl alcohol and mixed using laboratory shaker for 5 h. The mixture was filtered into 20 ml of 40% saturated solution of MgCO₃ and filtered again with Whatman No 1 filter paper to get a clean colourless solution. Then 1ml of the colourless solution and 2 ml of 5% FeCl₃ solution were added together in a 50 ml volumetric flask, made up to the mark with distilled water, allowed to stand for 30 min for colour development. Absorbance measured against the blank at 380 nm.

Tannin determination

The method of Makkar and Goodchild (1996) was adopted for tannin determination. 250 mg of the sample from which pigments and fat have been removed with diethyl ether containing 1% acetic acid, was taken into 10 mL of 70% aqueous acetone for extraction for 2 h at 30°C using water-bath. The total polyphenols (expressed as tannin) was determined using Folin ciocalteau reagent and 2.5 mL Na₂CO₃ solution. Absorbance was measured at 725 nm.

Minerals determination

The official method of AOAC (2005) was used for the mineral Zn, Fe, Mn, Ca and Mg determination. The minerals were determined on aliquots of the solutions of the ash by established atomic absorption spectrophotometry method using atomic absorption spectrophotometer (model 372) (Perkin Elmer, 1982). The Na and K contents were determined by Flame photometry with NaCl and KCl as standards, Vanado-molybdate method for P. The mineral ratio of Ca:P, Na:K, Ca:Mg, Ca:K, Fe:Zn and the milliequivalent ratio of [K:(Ca + Mg)] of the samples were calculated according to Adeyeye *et al.*, (2012).

Molar ratio of antinutrients to minerals determination

The method of Norhaizan and Nor Faizadatul Ain (2009) was used for the calculation of the molar ratios of the phytate to Ca, Zn and Fe. The method of Ferguson *et al.* (1988) was used for the calculation of Phy : Zn, Ca : Phy and [Ca] [phy]/ [Zn] molar ratios [Phytate = 660, Fe = 56, Zn = 65.40, Ca = 40].

Vitamins determination

The method of Harold *et al.*, (1987) was used for Pyridoxine (Vitamin B_6) determination while AOAC (2005) spectrophotometric method was adopted for the determination of Thiamine (Vitamin B_1) and Riboflavin (Vitamin B_2).

For Pyridoxine (Vitamin B_6): Two gram (2 g) of the sample, 5 ml of 2 N acetic acid, 5 ml of dichloroethane and 90 ml of distilled water were taken into 250 ml flask, placed in water bath for 20 min, cooled, and centrifuged; the first 10 ml of the aliquot was discarded. Standards were

prepared. The absorbance of the standards and the samples were read at 575 nm.

For Thiamine (Vitamin B_1): 0.5 g of the sample, 30 ml dichloroethane, 30 ml HCl (1:1) and 50 ml NH₄OH solution were added together. It was filtered with Whatman no1 filter paper. The absorbance was read at 415 nm.

For Riboflavin (Vitamin B_2): 1 g of the sample, 50 ml of 50% methanol and 50 ml of 17% NaCO₃ were added. It was filtered with Whatman no1 filter paper. The absorbance was read at 415 nm.

Result and discussion

Antinutrient Content

UV-C irradiation significantly decreased the phytate content of the vegetable, but increased the tannin and saponin contents of the vegetable (Table 1). Phytate content decreased by approximately 50%, while saponin and tannin contents increased by approximately 109% and 6%, respectively. The decrease in phytate could be attributed to the action of free radicals generated during irradiation which ultimately resulted to low inositol and inositol phosphates in the vegetable (De Boland, et al., 1975). Furthermore, the reduction may also be due to cleavage in the structure of the phytic acid itself by the irradiation (Duodu, et al., 1999). Bhat et al. (2007), also reported reduction in the phytate content of irradiated leafy vegetables. Antinutrients adversely affect the bioavailability and utilization of nutrients, which may result in neurological disorders and sometimes death (Olagunju et al., 2018). Our findings that UV - C irradiation caused an increase in the tannin content of the vegetable is in agreement with the report of Bhat et al., (2007) and Mohammed (2009) where tannin content of Mucuna seeds and Culantro plant increased after irradiation but was opposite to the report of Abu-Tarboush, (1998) and Villavicencio, et al., (2000), where decrease in the tannin content of plant seeds and Brazilian seeds were observed after irradiation. The increase in tannin might be because the irradiation

Statistical analysis

Obtained results were expressed as mean. Analysis of variance (ANO-VA) and Duncan's multiple range test for mean separations were according to Statistical Analysis System proprietary software (SAS, 2002). Significance accepted at P < 0.05.

induced oxidative stress in the vegetable and de novo synthesis of tannins by increasing the activity of phenylalanine ammonia lyase (PAL) an enzyme for the biosynthesis of tannins (Mohammed 2009). Increase in saponin content as a result of irradiation has been observed by other researcher who worked on effect of irradiation on plants (Vardhan and Shukla 2017, Elhaak et al., 2018). The enzyme squalene synthase (SS) and squalene epoxidase (SE) are the enzymes that catalyses the initial steps in the pathway of various triterpenoids biosynthesis. SS catalysed the biosynthesis of squalene from farnesyl diphosphate and SE the synthesis of oxidosqualene from squalene (Vardhan and Shukla 2017). The increase in the saponin content could be due to the increase in the coding genes for SS and SE in response to irradiation and consequently enhanced saponin content (Vardhan and Shukla 2017). In storage the antinutrients phytate and tannin reduced significantly in both irradiated and non-irradiated samples. This result is in agreement with the report of Adetuyi et al., 2008a that the phytate and tannin content of okra reduced in storage. The reduction in phytate and tannin in storage could be attributed to the action of phytase and polyphenol oxidase enzymes respectively.

Antinutrients	Un-irradiated	Irradiated	Stored Un-irradiated	Stored irradiated
Phytate mg/g	18.12 a	9.02 b	6.59 c	7.30 c
Saponin g/100g	0.68 c	1.42 a	0.35 d	1.00 b
Tannin g/100g	1.44 c	1.52 b	1.55 b	1.69 a

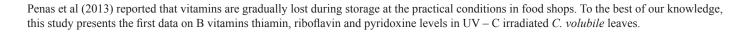
Table 1: Antinutrient content of UV-C irradiated and stored C volubile leaves

Values = mean triplicate readings.

Values with the same letter on the same row are not significantly ($P \le 0.05$) different.

Vitamin-B contents

The B vitamins Thiamine (B₁), Riboflavin (B₂) and Pyridoxine (B₆) content UV-C irradiation treated and stored C *volubile* leaves are presented in Table 2. Fresh C *volubile* leaves had significantly (p<0.05) higher vitamin B₆ (192.70 mg/100 g) than vitamin B₂ (72.16 mg/100 g) and vitamin B₁ (48.82 mg/100 g). UV-C irradiation of the leaves resulted in slight reduction in vitamin B₆ (1.3%), while vitamins B₁ and B₂ reduced by approximately 6%. Previous researchers reported similar reduction in B vitamins of two varieties of Brazilian beans after irradiation (Villavicencio, et al., 2000). The losses in B vitamins as result of irradiation are minimal in comparison to other processes of conservation of foods and the studies relating to the effects of radiation on the B vitamin content of foods are still inconclusive (Harder, *et al.*, 2016, Lima *et al.*, 2018). Storage of the leaves (irradiated and un-irradiated) resulted in significant reduction in vitamins B₁ and B₂, but pyridoxine (B₆) increased. Un-irradiated sample content increased from 192.70 mg/100 g to 237.88 mg/100 g while the irradiated sample increased from 190.22 mg/100g to 249.22 mg/100g.



Vitamins	Un-irradiated	Irradiated	Stored Un-irradiated	Stored irradiated
Thiamine (Vit. B_1)	48.82 a	45.69 b	41.33 d	43.15 c
Riboflavin (Vit. B_2)	72.16 a	68.12 b	67.33 b	66.42 c
Pyridoxine (Vit. B_6)	192.70 c	190.22 d	237.88 b	249.22 a

Table 2: Vitamin B content of UV-C irradiated and stored C. volubile leaves in mg/g

Values = *mean triplicate readings*.

Values with the same letter on the same row are not significantly (P < 0.05) different.

Mineral content

The mineral contents of irradiated and un-irradiated *C. volubile* leaves are presented in Table 3. Potassium (K), magnesium (Mg), sodium (Na) and calcium (Ca) were the most abundant elements in irradiated and un-irradiated *C. volubile* leaves. There was no significant change in the observed mineral content of the vegetable after UV-C irradiation with the exception of Mg which increased significantly from 8.11mg/g to 9.85 mg/g and K which decreased significantly from 16.23 mg/g to 13.97 mg/g. There are no comprehensive studies on the mineral content changes as a result of UV-C irradiation, but Olaiya *et al.*, (2016) reported that there were no significantly while the content of other observed minerals decreased significantly. The reason for the increase in P and Ca remains unclear. However, the decrease in other minerals in storage could be due to the physiological and metabolic activities going on within the cell of the vegetable (Adetuyi *et al.*, 2008b)

Minerals	Un-irradiated	Irradiated	Stored Un-irradiated	Stored Irradiated
Р	0.79 d	0.98 c	1.28 b	1.53 a
Zn	0.11 a	0.12 a	0.06 b	0.07 b
Fe	0.20 a	0.20 a	0.18 a	0.19 a
Mn	0.30 a	0.32 a	0.28 a	0.30 a
Κ	16.23 a	13.97 b	11.01 d	12.43 c
Na	1.83 b	2.07 a	1.69 c	1.89 b
Ca	1.59 d	1.78 b	1.66 c	2.11 a
mg	8.11 b	9.85 a	3.95 d	5.48 c

Table 3: Mineral content of UV-C irradiated and stored C. volubile leaves in mg/g

Values = *mean triplicate readings*.

Values with the same letter on the same row are not significantly (P < 0.05) different.

Mineral ratio

The absorption of minerals in the body is determined by the interactions of minerals present in the mineral source (Soetan *et al.*, 2010), therefore the mineral ratios reveals more than the mineral composition of the foods. When Ca : P ratio in food is high, Ca absorption in the intestine is favored for bones formation, contrary to that foods that are high in protein and P may cause Ca loss in urine (Adeyeye *et al.*, 2012, Adeoti *et al.*, 2013). The Ca : P ratio of samples *C. volubile* leaves in all observed conditions were greater than 1 (Table 4). The Ca : P ratio of the studied

vegetable *C. volubile* could promote Ca absorption with the purpose of the formation of bones and teeth since foods with Ca : P ratio greater than 1 are good source of Ca and with less than 0.5 are considered poor (Alinnor and Oze, 2011). The Na : K ratio of all the samples were less than 1. This is considered good for hypertensive patients since a good food should have Na : K ratio of less than one (Jacob et al., 2015). Vegetables with this kind of ratio when consumed will caused a reduction in the blood pressure of hypertensive patients because low Na and high

K in food intake, will help to reduce high blood pressure in hypertensive patients (Perez and Chang, 2014). When Ca : Mg ratio is high, Mg absorption efficiency reduces, movement of Mg into bone reduced and the activity of demineralizing parathyroid hormone increases (Mai et al., 2003). The Ca : Mg ratio reported in this study was less than 2. Mai et al., (2003) reported the Ca : Mg ratio of 2 as the recommended ratio for the balance absorption of Mg/Ca. The Ca : Mg ratio in this study will enhance increase in Mg absorption, increase in movement of Mg into bones and reduction in the activity of demineralizing parathyroid hormone since the Ca : Mg ratio was less than 2. The Ca : K ratio reported in this study was less than 4. A good source of Ca should have Ca : K ratio of 4:1 (Watts 2010). The calcium/potassium ratio is indicative of thyroid function. High Calcium over Potassium (>10) indicates a decreased thyroid effect at the cellular level. A low Ca/K ratio (<3) indicates an excess thyroid effect at the cellular level. The Ca : K ratio is considered the thyroid ratio since Ca and K are important in regulating the thyroid activity (Olagbemide et al., 2016). Zn and Fe

are the most abundant trace minerals in human body, they are assessed together, have the same dietary sources, their absorption is affected by the same factors and their deficiency occurs simultaneously (Lim et al., 2013). The Fe : Zn ratio of irradiated and un-irradiated vegetable were less than 2 but the Fe : Zn ratio of the stored samples were greater than 2. From the result of the irradiated and un-irradiated samples, the Zn absorption will be impaired but in the stored sample the Zn absorption will not be impaired because Fe will not impair the absorption of Zn when Fe : Zn is greater than 2. According to Pérès et al. (2001) iron did not impair zinc absorption at Fe: Zn ratio of 2:1. Milliequivalent ratios K : (Ca + Mg) of the stored irradiated and un-irradiated samples were higher than 2.2 but the UV-C irradiated sample has a value of 1.96 which was less than 2.2. This shows that the irradiated vegetable will discourage hypomagnesaemia in man (Adeyeye et al., 2012) since the consumption of the irradiated vegetable this will ensure the availability of more Mg in the body.

Mineral ratio	Un-irradiated	Irradiated	Stored Un-irradiated	Stored irradiated
Ca:P	2.01 a	1.82 b	1.30 c	1.38 c
Na:K	0.11 b	0.15 a	0.15 a	0.15 a
Ca:Mg	0.20 b	0.18 b	0.42 a	0.39 a
Ca:K	0.10 c	0.13 c	0.15 b	0.17 a
Fe:Zn	1.82 c	1.67 c	3.00 a	2.71 b
$[K:(Ca + Mg)]^X$	2.75 b	1.96 c	3.42 a	2.83 b

^x - miliequivalent

Values = mean triplicate readings.

Values with the same letter on the same row are not significantly (P < 0.05) different.

Molar ratio

The molar ratios of *C. volubile* irradiated and un-irradiated leaves are shown in Table 5. The phytate : Fe ratio ranged from 3.30 for stored un-irradiated vegetable to 7.00 un-irradiated vegetable. Iron absorption will not be inhibited by phytate when phytate : Fe ratios are less than 1.0 (Hurrell *et al.*, 2003). The values of phytate : Fe ratios in this study were all greater than 1, indicating poor Fe availability for absorption in the irradiated, un-irradiated and stored vegetables. The phytate : Zn molar ratio of the irradiated, un-irradiated and stored vegetable were less than 15 with the irradiated vegetable having the least value of 7.0. The phytate content in the irradiated, un-irradiated and stored vegetable were will not prevent Zn availability for absorption because the phytate : Zn molar ratios were below the critical value of 15 (Ferguson *et al.*, 1988). The Ca : phytate molar ratio in this study ranged from 1.39 un-irradiated vegetable to 4.82 stored irradiated vegetable, these values were less

than 6.0 indicating incomplete phytate precipitation. The determinant in the solubility of phytate and bound Zn is the dietary calcium (Ca), phytate precipitation is incomplete when dietary Ca : phytate molar ratios are less than 6.0. (Adetuyi *et al.*, 2011). There is a better index for predicting Zn availability for absorption, this is the calculated [Ca] [Phytate] / [Zn] molar ratio because of the interaction of Ca to phytate (Adetuyi *et al.*, 2011). There will be Ca interference with Zn availability for absorption when [Ca][Phy] / [Zn] molar ratio is greater than 0.5 mol/kg (Davies and Warrington, 1986). The [Ca][Phytate] / [Zn] molar ratio for the irradiated, un-irradiated and stored vegetable ranged from 0.0003 mol/Kg irradiated vegetable to 0.0006 mol/kg stored irradiated vegetable, these values were below 0.5 mol/kg, indicating that dietary Zn are available for absorption in the vegetable.

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Molar ratio	Un-irradiated	Irradiated	Stored Un-irradiated	Stored irradiated
Phy : Fe	7.00 a	3.50 b	3.30 c	3.67 b
Phy : Zn	14.00 a	7.00 c	10.00 b	11.00 b
Ca : Phy	1.39 d	3.21 c	4.20 b	4.82 a
[Ca] [Phy]/ [Zn] ^X	0.0005 b	0.0003 c	0.0004 c	0.0006 a

Table 5: Molar ratio of UV-C irradiated and stored C. volubile leaves

X - mol/Kg

Values = *mean triplicate readings*.

Values with the same letter on the same row are not significantly (P < 0.05) *different Phy - phytate.*

Conclusions

The results of this study, showed clearly that UV-C irradiation reduced significantly the content of phytates, but increased the content of saponin and tannin which possessed antinutritional and antioxidant activity in *C. volubile* leaves. The result also showed that UV-C irradiation caused reduction in the B vitamins, increase in the minerals P, Zn, Mn, Na, Ca and Mg but decrease in K and has no effect on the Fe content of *C. volubile* leaves. The mineral ratios and the milliequivalent ratios K: (Ca + Mg) as affected by UV-C irradiation were within the required standard for their availability for absorption. The [Ca][Phytate]/[Zn] molar ratio for the UV-C irradiated, un-irradiated and stored *C. volubile* vegetable were below 0.5 mol/kg. This therefore suggest that irradiating food just like other food processing methods will depend on what the food has to contribute to the total diet since UV-C irradiation caused reduction in the observed B vitamins but increase in some observed minerals.

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