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EFFECT OF STORAGE AT ROOM TEMPERATURE ON ANTINUTRIENT AND MINERAL PROFILE OF *Bacopa floribunda* LEAVES

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Abstract

In Nigeria, the local market women designed their own local way of preserving/storing their unsold vegetables for the second day sales usually by putting their vegetables in plastic basin, sprinkled with water, placed inside polypropylene bag and leaving the vegetables outside during the night. This study aimed at investigating the effect of market storage methods in Nigeria on the antinutrient and mineral profile of *Bacopa floribunda* leaves. The leaves were stored for 48 hours. The phytate contents reduced significantly in storage from 8.24 to 4.01 mg/g. There was no significant ($P < 0.05$) change in the saponin content of freshly harvested and stored *B. floribunda*. The tannin content 0.54% in freshly harvested *B. floribunda* reduced but not significant ($P < 0.05$) at the end of 48 hrs storage to 0.45%. Mg increased, Ca decreased but Zn, Fe and Mn content of *B. floribunda* has no significant change in storage. The mineral ratios Ca : P and Na : K of *B. floribunda* were within the required standard for healthy living.

Keyword: Phytate, Saponin, Tannin, Mineral ratio, Mineral content

Introduction

Bacopa floribunda belongs to the family Scrophuliaceae. *Bacopa floribunda* is an important herb in Ikale/Ilaje communities of Ondo State; it is used as decoction given to children and adults for memory retention and enhancement. *B. floribunda* leaf is being used in folklore for the management of cognitive dysfunction. Ethnobotanical survey of plants locally used for memory enhancement among the Yoruba people of South-western Nigeria indicated that *Bacopa floribunda* (Ewe Oniyemuye) appeared to be the most important (Olatunji *et al.,* 2017). Several line of study has revealed that medicinal plants confer holistic management to many human ailments including Alzheimer's disease (AD). They act as excellent source of antioxidants, anti-inflammatory and inhibitor of enzymes linked to AD such as cholinesterase and β-secretase inhibitors (Olatunji *et al.,* 2017). So many traditional medicine practitioners have claimed to have treated some of the neurodegenerative diseases like 'iyerira', 'aagana', 'olodeori' with some herbs especially when discovered at the early stages. The plants mentioned include *J. curcas* L., *Talinum triangulare* (Jacq.) Willd., *A. adianthifolia* (Schumach.) and *B. floribunda.* Many of these plants are obtained from the forest, while some of them have been

domesticated (Sonibare and Ayoola, 2015).

The most prominent and frequently mentioned species among the herbs that possessed potential anticholinesterase and neuroprotective actions is *B. floribunda* (Sonibare and Ayoola, 2015). Different plant parts are used, but predominantly the leaves and that the herbs are prepared in combination with other herbs. It would be efficacious when *B. floribunda,* is mixed with *S. mombin* and *D. horizontalis*. The administration of these leaves varies the dried leaves of *B. floribunda, S. mombin* and *D. horizontalis* can be boiled with water and made into a decoction or grinded together, mixed with pap and taken regularly. The leaves of *B. floribunda*, fruit of *A. melegueta* and the leaves of *Abrus precatorius* L. were sun dried and ground together or burnt together and taken with pap regularly. The dried leaves of *B. floribunda* could be ground or ground together with *S. mombin* and taken with pap or mixed with honey (Sonibare and Ayoola, 2015). In Nigeria herbs are consumed without regulation, these herbs contained other components different from the active component for the ailment; these other components when consumed without regulation could be injurious to the body example are antinutrients. The mineral profile of the herbs needs to be looked into because if it is not in the proper ratio, it could have adverse effect on the body. This means the dietary implication of consuming this herb cannot be over looked. In Nigeria, the local market women designed their own local way of preserving/storing their unsold vegetables for the second day sales usually by putting their vegetables in plastic basin, sprinkled with water, placed inside polypropylene bag and leaving the vegetables outside during the night (Adetuyi and Ogundahunsi 2010). This study is carefully, designed to evaluate the effect of local market storage method on the antinutrient and mineral profile of *Bacopa floribunda* leaves.

Methods

Sample collection and Storage

Fresh leaves of *Bacopa floribunda* were harvested from water side area of river Oluwa in Okitipupa, Ondo State, Nigeria, identified and authenticated at the herbarium of Biological Sciences Department, OAUSTECH. Leaves were sorted and rinsed to remove dirt. The leaves were subjected to Nigeria local market condition and stored with the market storage method for 48 hours. 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, it was removed from the polypropylene bag the following day and the process repeated the second day (Adetuyi and Ogundahunsi 2010).

Sample preparation.

The edible portion of the samples (50 g) were separated, washed, drained completely, chopped, air dried and analysed for antinutrients (Phytate, Saponin and Tannin), minerals and mineral availability. All analysis was carried out in triplicate.

Phytate determination

The Vaintraub and Lapteva (1988) method was adopted for phytate content determination. The sample (0.5 g) was taken, 10 mL of 0.2 N HCl added and allowed to stand for 1 hour at room temperature, it was centrifuged at 3000 × g for 30 mins. The clear supernatant (3 mL) and Wade reagent (2mL) were added together, homogenized and centrifuged at 3000 × g for 10 min. Absorbance measured at 500 nm with a UV spectrophotometer (JENWAY 6305, Barloworld Scientific Ltd., Dunmow, Essex, UK). Phytate was calculated using phytic acid as standard.

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 $MgCO_3$ 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% $FeCl_3$ 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 (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^{0}C using water-bath. The total polyphenols (as tannin) was determined using Folin ciocalteau reagent and 2.5 mL Na_2CO_3 solution. Absorbance measured at 725 nm. Total polyphenols (as tannin) calculated using the standard curve.

Minerals determination

The official method of AOAC (2005) was used for the mineral 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 Corp., USA.). The Na and K contents were determined by Flame photometry with NaCl and KCl as standards, Vanado-molybdate method for P.

Mineral and Molar ratio of antinutrients to minerals determination

The mineral ratio of Ca:P, Na:K, Ca:Mg, Ca:K, Fe:Zn, the milliequivalent ratio of [K:(Ca + Mg)] of the samples, the molar ratios of the phytate to Ca, Zn and Fe and Ca : Phy and [Ca] [phy]/ [Zn] molar ratios were calculated as described in Adetuyi *et al.,* (2019) [Phytate = 660, Fe = 56, Zn = 65.40, Ca = 40].

Statistical analysis

The result was expressed as mean of triplicate determination. Analysis of variance (ANOVA) was performed using Statistical Analysis System proprietary software (SAS version 8.3, SAS Institute Inc., Cary, NC, USA). Duncan's multiple range tests was used for mean separation ($P < 0.05$).

Result

Antinutrient content

The antinutrient content of freshly harvested and stored *B. floribunda* is presented in Table 1. It showed that the phytate content of *B. floribunda* reduced significantly ($P < 0.05$) in storage from 8.24mg/g (freshly harvested) to 4.01mg/g (48 hrs storage). There was no significant ($P < 0.05$) change in the saponin content of freshly harvested and stored *B. floribunda*. The tannin content 0.54% in freshly harvested *B. floribunda* reduced but not significant ($P < 0.05$) at the end of 48 hrs storage to 0.45%.

Mineral content

These minerals Ca, Mg and K were the most abundant in *B. floribunda*, there was no significant ($P < 0.05$) reduction in Zn, Fe and Mn in storage. The mineral Ca decreased while Mg increased significantly ($P < 0.05$) in storage (Table 2).

The mineral and molar ratio of minerals

Table 3 and 4 showed the mineral and molar ratios of minerals. It revealed that Ca : P, Ca : Mg and Ca : K mineral ratios reduced significantly ($P < 0.05$) in storage while there was no significant ($P < 0.05$) reduction in Na : K mineral ratio. The milliequivalent ratio of [K : (Ca + Mg)] increased significantly ($P < 0.05$) in storage. The molar ratios of Phy : Fe, Phy : Zn, Ca : Phy and [Ca][Phy]/[Zn] of *B. floribunda* reduced significantly ($P < 0.05$) in storage.

Discussion

The storage temperature and relative humidity play a very important role in reducing the degenerative processes occurring in vegetables during the postharvest stages (Kader, 2001). Phytate is also known as myoinositol 1,2,3,4,5,6-hexakis dihydrogen phosphate and is the main storage form of phosphorus in plant. It has antinutritive activities by blocking the absorption of minerals like Fe, Zn, and Ca (Gupta *et al.,* 2013). In this study, the phytate content of *B. floribunda* reduced in storage (Table 1) and this could be attributed to the action of the enzyme phytase cleaving and freeing the bound phosphorus from the phytate molecule, liberating Ca^{2+} and Mg^{2+} at the same time (Adetuyi and Ogundahunsi, 2010). Some of the most important bioactive constituents of medicinal plants are saponins. it has a characteristic of causing hemolysis of red blood cells (RBC's), form persistent froth if shaken with water, among its biological role and medicinal properties are anti-inflammatory, antibacterial, antifungal, antiviral, insecticidal, anticancer cytotoxic and molluscicidal actions, also it exhibit cholesterol-lowering action in animals and human (El Aziz *et al.,* 2019). This study revealed that there was no significant change in saponin contents of freshly harvested and stored *B. floribunda*. MinhThu and Le Son (2019) reported that the concentration of saponin slightly reduced in week 6 of storage and steadily declined in week 11, but still maintained almost same value after 16 weeks of storage. Tannin acts as antimutagenic, anticancer and antioxidant, it helps to diminish serum cholesterol and triglycerides, also suppress lipogenesis by insulin (Das *et al.,* 2020). The tannin content in freshly harvested *B. floribunda* reduced in storage but not significant as shown in this study. The tannin content of sorghum flour stored at 4° and 25° C showed no significant differences during storage. But at 40° C resulted in lower levels during storage. This reduction observed during storage may be due to interactions of tannins with proteins and carbohydrates, which decrease tannin extractability (Barros *et al.,* 2012).

Minerals have very important roles in the body ranging from developing strong bones to nerve impulses transmission and for healthy and lengthy life. Minerals are also essential in standard heartbeat regulation (Gharibzahedi and Jafari, 2017). It was observed in this work (Table 2) that Zn, Fe and Mn have no significant change in storage. This was similar to the result obtained by Zhang *et al.,* (2016) that there were no remarkable changes in mineral values of walnut male inflorescence, a traditional vegetable in China during the whole storage. The decreased in Ca and P of *B. floribunda* in storage could be due to the physiological and metabolic activities within the cell of the stored vegetable (Adetuyi *et al.,* 2019).

The availability of minerals in the body for absorption is dependent on the mineral to mineral interactions in the mineral source (Soetan *et al.,* 2010) therefore it is imperative to examine the mineral ratios which gives more information than the mineral composition of the foods (Adetuyi *et al.,* 2019). The Ca : P ratio of freshly harvested and stored *B. floribunda* were greater than 1 (Table 3). The Ca : P ratio in this study whether stored or freshly harvested could promote Ca absorption with the aim of bones and teeth formation because foods with Ca : P ratio greater than 1 are good source of Ca (Alinnor and Oze, 2011). The Na : K ratio (Table 3) were less than 1. This will cause a reduction in the blood pressure of hypertensive patients when consumed since low Na and high K in food intake of hypertensive patients caused reduction in blood

pressure (Perez and Chang, 2014). When Ca : Mg ratio is higher than 2, there is reduction in the efficiency of Mg absorption, decrease in Mg movement into bones and increase demineralizing parathyroid hormone activity (Mai *et al.,* 2003). The Ca : Mg ratio in the stored *B. floribunda* (Table 3) will enhance increase in Mg absorption and reduction in demineralizing parathyroid hormones activity because they have Ca : Mg ratio value of less than 2. The Ca : K ratio is the thyroid ratio because Ca and K are very important in regulating the thyroid activity (Olagbemide *et al.,* 2016). The Ca : K ratio reported for freshly harvested and stored *B. floribunda* in this work was less than 4 (Table 3). Food with Ca : K ratio of 4:1 is considered a good source of Calcium (Watts 2010).

The Fe : Zn ratio in this study were less than 2 (Table 3), from this result the Zn absorption will be impaired by Fe, implying that more Fe will be absorbed in preference to Zn because Fe will not impair Zn absorption when Fe : Zn is greater than 2 (Pérès *et al.*, 2001). This study reveals that Milliequivalent ratios K : (Ca + Mg) of the samples were lower than 2.2 (Table 3) which shows that the consumption of *B. floribunda* whether freshly harvested or stored will ensure the availability of Mg in the body and discourage hypomagnesaemia in man (Adeyeye *et al.,* 2012).

Phytate always inhibit the availability and absorption of Fe but only when phytate : Fe ratios are more than 1.0 (Hurrell *et al.,* 2003). The phytate: Fe molar ratio were less than 1.0 (Table 4) indicating good Fe availability for absorption when *B. floribunda* is consumed. The phytate: Zn molar ratio reported in this work were less than 15. This is an indication that the phytate content in *B. floribunda* will not prevent Zn availability for absorption because the phytate : Zn molar ratios were below 15 the critical value (Ferguson *et al.,* 1988). The Ca : phytate molar ratio in this study were more than 6.0 (Table 4) indicating that phytate was completely precipitated. Dietary calcium determines the solubility of phytate and bound Zn, when dietary Ca : phytate molar ratios are less than 6.0., phytate precipitation becomes incomplete (Adetuyi *et al.,* 2011). The [Ca][Phytate] / [Zn] molar ratio for freshly harvested and stored *B. floribunda* were less than 0.5 mol/kg, indicating that calcium will not interfere with dietary zinc availability for absorption. When [Ca][Phy] / [Zn] molar ratio is greater than the critical value (0.5 mol/kg) calcium will interact with dietary zinc and prevent zinc availability for absorption (Akindahunsi and Oboh 1999).

Table 1: Antinutrient content of stored *B. floribunda* leaves

Antinutrients	Freshly harvested	24 hrs storage	48 hrs storage
Phytate (mg/g)	8.29 a	5.30 b	4.01 c
Saponin (%)	8.35 a	7.94 a	8.40 a
Tannin (%)	0.54 b	0.52 a	0.45 b

Values = mean triplicate readings. Values with the same letter on the same row are not significantly ($P < 0.05$) different.

Table 2: Mineral content of stored *B. floribunda* leaves in mg/g

Mineral	Freshly harvested	24 hrs storage	48 hrs storage
P	0.4 a	0.2 b	0.2 b
Zn	2.9 a	2.5 a	2.6 a
Fe	0.9 a	1.1 a	0.7 a
Mn	0.2 a	0.2 a	0.2 a
K	7.7 b	9.2 a	6.7 b
Na	0.8 a	0.6 c	0.9 a
Ca	14.8 a	6.8 b	6.0 c
mg	7.1 c	7.8 b	9.0 a

Values = mean triplicate readings. Values with the same letter on the same row are not significantly ($P < 0.05$) different.

Table 3: Mineral ratio in stored *B. floribunda* leaves

Mineral ratio	Freshly harvested	24 hrs storage	48 hrs storage
Ca:P	37.0 a	34.0 b	30.0 c
Ca:K	1.92 a	0.74 c	0.90 b
Na:K	0.10 a	0.07 a	0.13 a
Ca:Mg	2.08 a	0.87 b	0.67 c
Fe:Zn	0.31 b	0.50 a	0.27 b
$[K:(Ca + Mg)]^X$	0.79 c	1.56 a	1.15 b

Values = mean triplicate readings. Values with the same letter on the same row are not significantly ($P < 0.05$) different.
^{X} - miliequivalent

Table 4: Molar ratio of stored *B. floribunda* leaves

Molar ratio	Freshly harvested	24 hrs storage	48 hrs storage
Phy : Fe	0.81 a	0.40 b	0.46 b
Phy : Zn	0.30 a	0.24 b	0.15 c
Ca : Phy	28.46 a	21.25 c	25.00 b
$[Ca]\ [Phy]/\ [Zn]^X$	0.109 a	0.04 b	0.023 c

Values = mean triplicate readings. Values with the same letter on the same row are not significantly ($P < 0.05$) different.
Phy - phytate, ^{X} – mol/Kg

Conclusions

In summary, this study demonstrates that phytate content of *B. floribunda* reduced significantly in storage but no significant change in the saponin content of freshly harvested and stored *B. floribunda*. The tannin content of *B. floribunda* reduced in storage but not significant. The mineral ratios Ca: P and Na: K were within the required standard for healthy living. Storage of *B. floribunda* discouraged demineralizing parathyroid hormones activity because they have Ca: Mg ratio value of less than 2 but encouraged the absorption of Fe because the Fe: Zn ratio were less than 2 and the phytate: Fe molar ratio were less than 1.0. The leaves of *B. floribunda* whether freshly harvested or stored will discourage hypomagnesaemia when consumed because the Milliequivalent ratios K: (Ca + Mg) of the samples were lower than 2.2. The [Ca][Phytate] / [Zn] molar ratio for freshly harvested and stored *B. floribunda* showed that calcium will not interfere with

dietary zinc availability for absorption.

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