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Effect of Soaking Time and Volume of Water on the Ascorbic Acid Content of Three Nigerian Green Leafy Vegetables

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Abstract
Freshly harvested leaves (*Heinsia crinata*, *Talinum triangulare*, and *Venonia amygdalina*) were subjected to different pre-processing treatments; slicing, slicing with salt and squeeze-washing; a portion of whole leaves was used as control. Each sample was soaked twice; in 1 litre and 2 litres of distilled water for 480 min. The 2, 6-dichlorophenol-indophenol Titrimetric method was used to determine the ascorbic acid (AA) content of the fresh leaves. During soaking, the trend of loss was monitored for each treatment every 120 min. The AA content of fresh *H. crinata* was 73.42 mg/100 g of which 49.7–71.9% was lost; fresh *T. triangulare* had 337.30 mg/100 g of which 54.9–93.2% was lost and fresh *V. amydalina* had 121.00 mg/100 g of which 49.9–89.1% was lost during soaking. The pre-processing treatments increased the degree of AA loss in the three leafy vegetables and the higher the soaking time, the higher the AA loss.
Keywords: Ascorbic acid, soaking, *Heinsia crinata, Talinum triangulare, Vernonia amygdalina*

1. Introduction

Ascorbic acid (AA) also known as vitamin C is an antioxidant which is readily soluble in water and it's widely distributed in many fruits and vegetables (Muller, 1988; Babalola *et al*., 2010). Vitamins are normally higher in the leafy portion of plants because this is the site of photosynthesis (Ihekoronye and Ngoddy, 1985). Hence, green leafy vegetables are good sources of vitamin C.

Vitamin C being water soluble is of particular interest to food processors from the standpoint of its retention in the final product as a result of pre-processing treatments (washing, cutting, slicing, squeezing, soaking, blanching), thermal exposure (drying, moist-and dry-heating, cooking), exposure to light or some metals such as copper or iron (Solanke and Awonorin, 2002). The route and rate of oxidation of AA is also influenced by several factors including pH, trace metals, enzymes, presence of oxygen as well as time and temperature (Babatola and Adewoyin, 2011).

The reported losses of vitamin C during blanching or cooking are enormous and have varied between 60 and 90% in some cooked vegetables when processed at 100^{0}C for 15 minutes (Solanke and Awonorin, 2002). Similar studies based on the effect of blanching and drying conditions on sensory quality, chlorophyll and AA retention of green leafy vegetables have been reported (Ariahu and Egwujeh, 2009). Babalola *et al*. (2010) also reported the effect of blanching, boiling, sun-drying and squeeze-washing on AA content of seven Nigerian leafy vegetables.

Though it is generally known that AA is soluble in water, but the degree of leaching with respect to time and even the volume of water used has hardly been formerly documented. This work an investigation of the effect of soaking time, volume of water used and some pre-processing treatment (slicing, squeeze-washing, soaking with brine solution) on AA contents of three Nigerian green leafy vegetables at ambient temperature.

2. Materials and methods

2.1 Material collection and treatments

All chemicals and reagents used were of analytical grade. Trichloroacetic acid (TCA) crystal was obtained from Loba Chemie India, L-Ascorbic acid and 2, 6-Dichlorophenol-indophenol (DCPIP) were obtained from Kem Light Laboratories PVT Ltd. India.

Between 800–1000 g each of freshly harvested *Heinsia crinata* (bush apple leaf), *Talinum triangulare* (water leaf) and *Vernonia amygdalina* (bitter leaf) were purchased from farmers and/or major holders in Mile 3 market, Diobu Area Port-Harcourt Nigeria. Each sample was sorted, cleaned and divided into 8 portions of 100 g per treatment as follow;

i. Whole leaf 1 (WHL1): A portion of untreated leaves soaked in 1 litre distilled water
ii. Whole leaf 2 (WHL2): A portion of untreated leaves soaked in 2 litres distilled water
iii. Sliced leaf 1 (SLL1): A portion of sliced leaves soaked in 1 litre distilled water
iv. Sliced leaf 2 (SLL2): A portion of sliced leaves soaked in 2 litres distilled water
v. Squeeze washed leaf 1 (SQW1): A portion squeeze-washed leaves in 1 litre distilled water

vi. Squeeze washed leaf 2 (SQW2): A portion squeeze-washed leaves in 2 litres distilled water
vii. Sliced and salted leaf 1 (SSL1): A portion of sliced leaves soaked in 2% salt solution (1 litre)
viii. Sliced and salted leaf 2 (SSL2): A portion of sliced leaves soaked in 2% salt solution (2 litres)

The set up were kept on the laboratory bench under ambient conditions for 8 hr while the ascorbic acid content was determined every 2 hr.

2.2 Determination of percentage moisture and dry matter contents

The moisture content (MC) of each sample was determined before soaking by drying known mass of each vegetable (5 g) in hot air oven preset at 105^0C until a constant weight was obtained (AOAC, 2000). Percentage dry matter (DM) was determined from the MC of the leafy vegetables by using the difference method (Solanke and Awonorin 2002);

% DM = 100 - % MC

2.3 Determination of ascorbic acid content (mg/100 g DM)

The 2, 6-dichlorophenol-indophenol titration method described by Ndawula *et al.* (2004) was adopted for the determination of ascorbic acid content. This method was slightly modified and used as follow; 2 g of sample was macerated in a mortar containing 10 ml of 5% TCA (extraction solution) and the content transferred into 100 ml volumetric flask. More extraction solution was added up to the mark. The content being mixed thoroughly, filtered immediately and aliquots (10 ml) of extract were titrated against standardized 2, 6-dichlorophenol-indophenol solution. An equivalent amount of the extraction solution was titrated against standard 2, 6-dichlorophenol-indophenol solution as blank.

2.4 Determination of percentage loss in ascorbic acid content (mg/100 g DM)

Percentage loss in ascorbic acid (AA) content (mg/100 g DM) was calculated for all the treatments by estimating the loss in the ascorbic acid content after 480 min soaking time using the relation below;

$$\text{Percentage (\%) loss in AA content (mg/100 g DM)} = \frac{\text{Initial} - \text{Final concentration}}{\text{Initial concentration}} \times 100$$

Where: Initial concentration = ascorbic acid concentration of raw/fresh sample
Final concentration = ascorbic acid concentration after soaking (480 min).

2.5 Statistical analysis

Data were analyzed by one-way analysis of variance using SPSS software package version 20.0 (IBM SPSS Statistics, IBM Corporation 2011). The difference between mean values was determined by New Duncan's Multiple Range Tests (Duncan, 1955). Significance was accepted at 5 % probability level.

3. Results and discussion

3.1 Moisture and dry matter contents of three Nigerian green leafy vegetables

The result of moisture and dry matter contents of freshly harvested three Nigerian green leafy vegetables is presented in Table 1. Moisture contents of the leafy vegetables examined ranged from 64.47–94.11% and dry matter contents was between 5.89–35.53%. The moisture content of *Talinum triangulare* was significantly ($p<0.05$) higher than other green leafy vegetables. Similarly, the dry matter content of *Heinsia crinata* was significantly ($p<0.05$) higher than the other vegetables. These results; high moisture content of *T. triangulare* and high dry matter content of *H. crinata* could be attributed to their texture.

Table 1: Moisture and dry matter contents of freshly harvested three Nigerian green leafy vegetables

Vegetable name		Moisture content (%)	Dry matter content (%)
Biological name	Common name		
Heinsia crinata	Bush apple leaf	$64.47^c \pm 3.96$	$35.53^a \pm 3.96$
Talinum triangulare	Water leaf	$94.11^a \pm 0.13$	$5.89^c \pm 0.13$
Vernonia amygdlina	Bitter leaf	$84.65^b \pm 0.69$	$15.35^b \pm 0.69$

Results show mean ± SD of triplicate determinations. Means with different superscripts along the same column differ significantly ($p<0.05$).

3.2 Ascorbic acid contents (mg/100 g DM) of three Nigerian green leafy vegetables

Ascorbic acid (AA) content (mg/100 g DM) of freshly harvested *H. crinata* and at different soaking time was presented in figure 1. The AA content of fresh *H. crinata* leaf before soaking was 73.42 mg/100 g DM. The result showed that after 480 min of soaking, there were significant ($p<0.05$) loss in the AA content of all the *H. crinata* samples. Increase in the volume of soaking water from one to two litres caused significant ($p<0.05$) loss and/or gain in the AA contents (mg/100 g DM) only in the sliced *H. crinata* samples (salted and unsalted) after

480 min soaking time.

The AA content (mg/100 g DM) of freshly harvested *T. triangulare* and at different soaking time is as presented in figure 2. The AA content of the fresh *T. triangulare* was 337.30 mg/100 g DM. Soaking for 120 min caused a significantly increase ($p<0.05$) in the AA content of WHL1 and WHL2 to 542.49 and 546.20 mg/100 g DM respectively. At the soaking time of 240 min, the AA content of WHL2 was 543.48 mg/100 g DM, which was significantly ($p<0.05$) higher than that of fresh sample. Further increase in soaking time to 480 min caused significant ($p<0.05$) loss in the AA contents of all the *T. triangulare* leaf samples. Change in the volume of soaking water from one to two litres did not affect the AA content of *T. triangulare* significantly ($p>0.05$) within 480 min.

The AA content of freshly harvested *V. amygdalina* was 121.00 mg/100 g DM (figure 3). Significant increase ($p<0.05$) was observed in the AA content of WHL1 (133.74 mg/100 g DM) at 120 min soaking time. Prolonged soaking to as high as 480 min caused significant ($p<0.05$) loss in the AA content of *V. amygdalina* leaf samples. Increase in the volume of soaking water from one to two litres caused a significant difference ($p<0.05$) between the AA contents of WHL1 and WHL2 while other treatments did not showed any significant ($p>0.05$) difference.

The AA contents of freshly harvested three Nigerian vegetables obtained in the present study are well compared with available reports. Ejoh *et al*. (2005) reported the AA content of fresh *V. amygdalina* as 166.5 mg/100 g; but a lower value was reported by Babalola *et al*. (2010). According to them, the AA contents of fresh *T. triangulare* and *V. amygdalina* were 9.30 and 42.40 mg/100 g respectively. However, the AA contents (mg/100 g) of all the freshly harvested three Nigerian green leafy vegetables examined under this present study were higher than the US daily reference intake of vitamin C; 75 and 90 mg/day for women and men respectively as reported by Szeto *et al*. (2002).

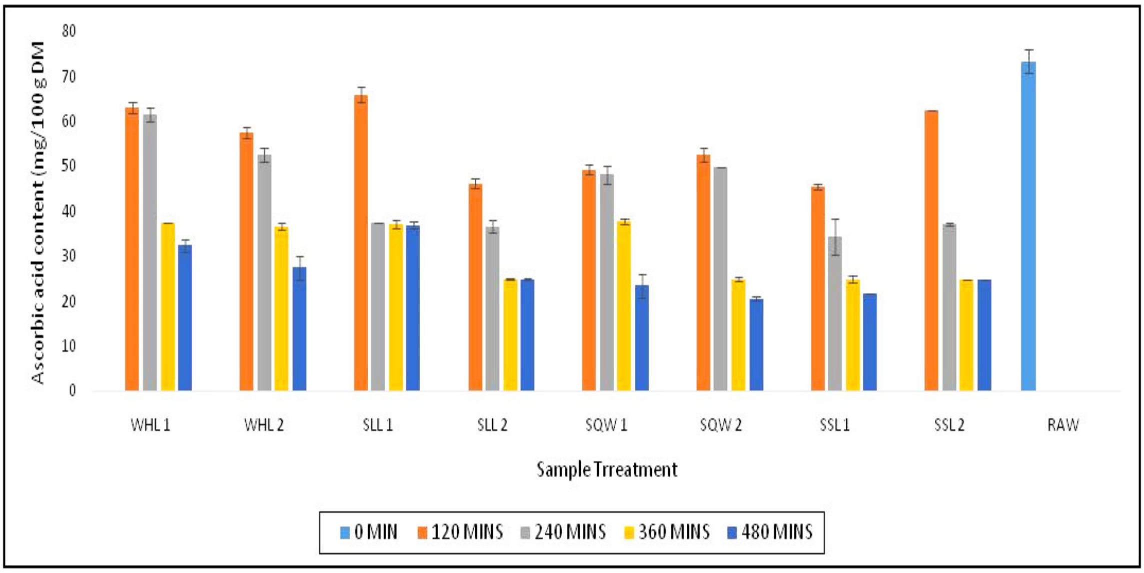

Figure 1: Ascorbic acid content (mg/100 g DM) of freshly harvested *Heinsia crinata* and at different soaking time in two separate volumes of water. Each bar represents mean of three separate readings. Error bars represent the standard deviations of the readings.

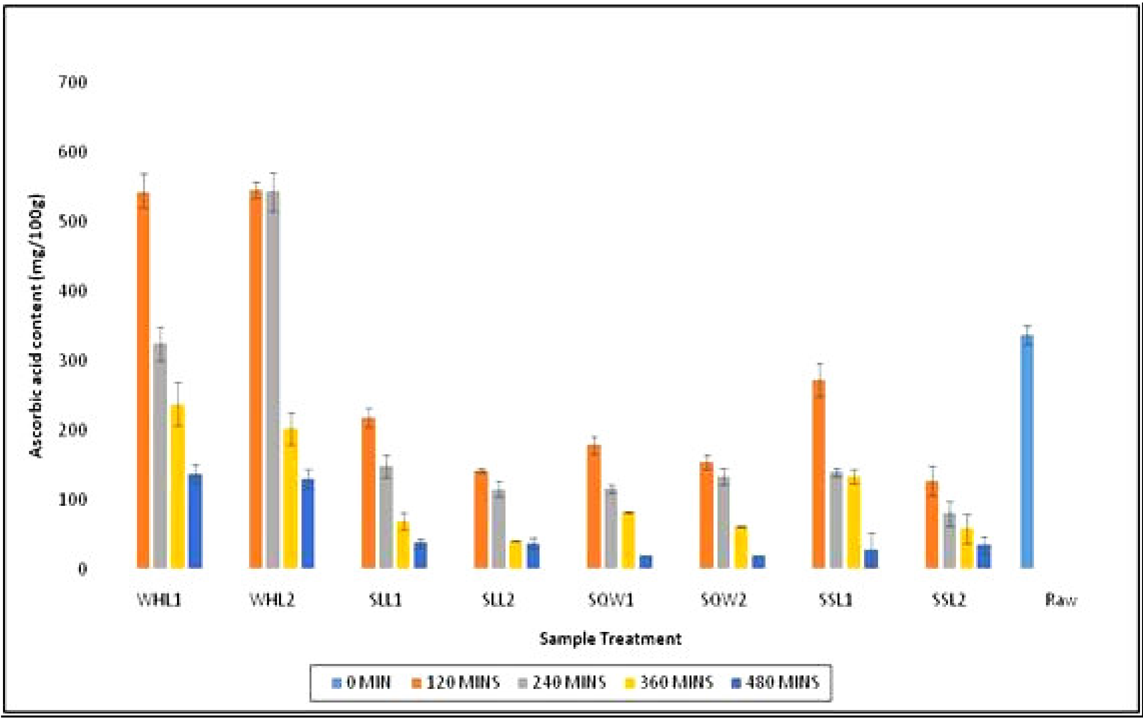

Figure 2: Ascorbic acid content (mg/100 g DM) of freshly harvested *Talinum triangulare* and at different soaking time in two separate volumes of water. Each bar represents mean of three separate readings. Error bars represent the standard deviations of the readings.

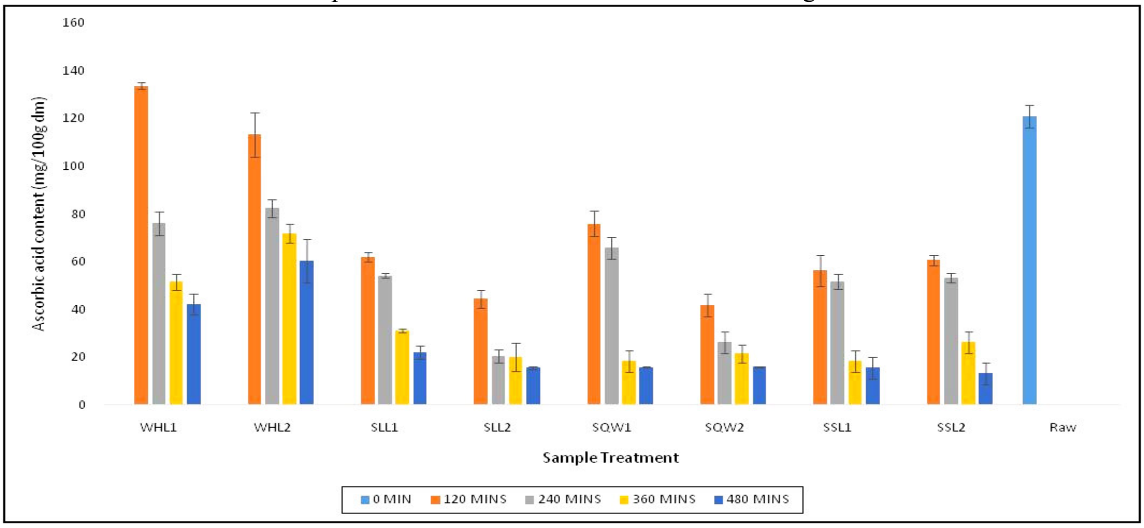

Figure 3: Ascorbic acid content (mg/100 g DM) of freshly harvested *Vernonia amygdlina* and at different soaking time in two separate volumes of water. Each bar represents mean of three separate readings. Error bars represent the standard deviations of the readings.

3.3 Percentage loss in ascorbic acid contents

The percentage loss in AA contents (mg/100 g DM) of *H. crinata*, *T. triangulare* and *V. amygdalina* within 480 min soaking time is shown in figure 4. *H. crinata* lost from 49.7–71.9% of the original AA content. Effect of pretreatment showed that squeeze-washing caused the greatest loss in AA of *H. crinata*. Also, *T. triangulare* lost 54.9–93.2% of its original AA during the 480 min of soaking. Squeeze-washing also caused the highest degree of loss in AA of *T. triangulare* within the experimental period (480 min). For *V. amygdalina*, the lost was 49.9–89.1% of its initial AA content within the 480 min soaking time. Slicing and soaking in salt solution caused the highest degree of AA loss of *V. amygdalina* leaf samples. Generally, the degree of loss in AA was high in *T. triangulare* than the other two samples and it was least in *H. crinata*.

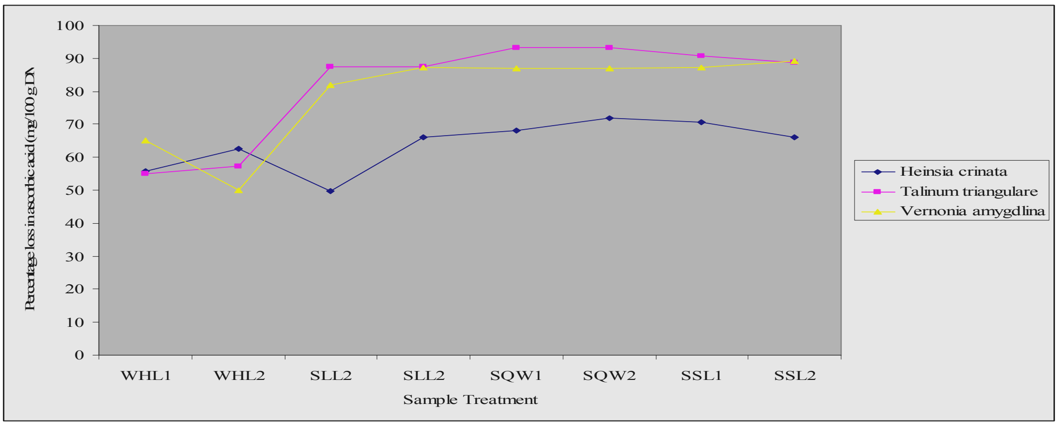

Figure 4: Percentage loss in ascorbic acid content (mg/100 g DM) of *Heinsia crinata, Talinum triangulare and Venonia amygladina* after 480 min soaking time in distilled water.

The losses of AA observed due to pre-processing and soaking were very high especially when the samples were sliced, squeeze-washed and soaked with or without salt. However, this result agrees with earlier report on AA loss of similar and/or other green leafy vegetables. For instance, reported losses of AA during blanching or cooking are as high as 60 to 90% in some leafy vegetables (Solanke and Awonorin, 2002). Ejoh *et al*. (2005) observed high losses (as much as 77%) when *V. amygdalina* species were squeeze-washed, squeeze-washed and boiled and squeeze-washed with natron. Also, according to Babalola *et al*. (2010), squeeze-washing reduced the AA content of *Telfaria occidentalis* leaf from 62.50 to 6.47 mg/100 g (89.65%) while squeeze-washing followed by boiling of *V. amygdalina* reduced the AA content from 42.40 to 2.18 mg/100 g (94.90%).

4. Conclusion

Freshly harvested green leafy vegetables are rich in ascorbic acid. However, the vitamin is lost due to soaking and pre-processing methods. Slicing, squeeze-washing and brine solution caused more losses in ascorbic acid than when the green leafy vegetables were soaked as whole leaves. Percentage loss in the ascorbic acid content of green leafy vegetables is directly proportional to the soaking time while increase in volume of soaking water had no clear cut correlation. Principally, soaking is done to remove field soil and other extraneous materials. It is therefore advisable to soak green leafy vegetables as whole leaves for a minimized period of time. To get the best out of these green leafy vegetables, it is advisable to take them when they are raw or minimally processed for short period of time.

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