Chlorophyll, ascorbic acid and total phenolic contents of sweet potato leaves affected by minimum postharvest handling treatment

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Abstract. There is a wide variety of native vegetables utilized by rural people in developing countries. However, information about their chemical content primarily that have functional properties and their degradation during handling is scarce and not well quantified. One example of those native leafy dark-green vegetables is sweet potato tips. Sweet potato leaves or tips are biomass waste after the sweet potato tubers are harvested. In addition, this leaves, as other leafy vegetables, are very susceptible to degradation leading to deterioration. Therefore, the objective of this research was to investigate the effect of minimum handling on some qualities of sweet potato tips. The three factors experiment was arranged in a completely randomized design with 4 replications. The first factor was dipping solution: Water or solution containing sucrose and citric acid. The second was solution changing or no changing. The third factor was basal stem cutting or no cutting. All the leaves were hold for 5 days at 30 ± 5 °C, and then analyzed for their chlorophyll, ascorbic acids, and phenolic contents. The results showed holding solution of 1.5% sucrose containing 300 ppm citric acid helped to reduced chlorophyll degradation of sweet potato tips. However, this treatment induced a higher degradation of ascorbic acid. Daily changing pulsing and daily basal stem cutting did not affect chlorophyll, but it helped to maintain ascorbic acid and induced higher phenolic content of the tips.

1. Introduction
Leafy dark-green vegetables have long been regarded as rich source of Vitamin A and C, fibre, iron as well as other minerals, protein and calories. Sweet potato leaves, usually regarded as biomass waste, are commonly consumed by rural poor people in some part of Indonesia, Malaysia, The Philippines, and other part of the world. In addition to its nutritive value, sweet potato leaves have been reported to content an appreciable amount of bioactive compounds such as polyphenol and flavonoids. Phenolic and other flavonoids are intensely studied for their physiological health benefits as functional foods. As other green leafy vegetables, sweet potato leaves are highly perishable food products. Water loss and postharvest decay account for most of their losses. Various studies reported these losses have been estimated to be more than 40% in the tropics and subtropics. In many tropical countries, postharvest waste among vegetables may range from 20 to 78% when transport is delayed. On daily bases losses range from 3 to 11%. These losses are due to ripening, decay, mechanical injury, weight loss,
trimming, culls, sprouting and browning which take place during harvesting, packing, transporting, grading, storage and retailing [6].

All vegetables continue to lose water through evaporation or transpiration after they are harvested. Water loss leads to shrivelling and wilting and turns the vegetables tough or mushy and, eventually inedible [7]. Therefore maintenance of an appropriate amount of water in the marketable commodity is vital to protect its quality. In addition, weight loss in fresh fruits and vegetables could cause a direct quantitative monetary loss, a quantitative loss in appearance of the product, its stability and also a nutritional loss involving flavor and vitamins.

The practice of dipping the stem cut flowers in preservative solutions for 12 to 24 hr to keep the quality and prolong life is known as pulsing, which has been practiced for years by the flower industry. In pulsing or holding, the most important ingredients in chemical solution are water and metabolic sugar such as sucrose. In addition to sugar, acidic solutions move more readily through the stem of cut flower than alkaline or neutral solutions. Therefore, an organic acid is often included in preservative formulation to reduce the pH to 3 or 3.5. Citric acid is most widely used at concentration of 50 to 800 ppm. The additional benefit of using citric acid is its capability to improve water balance between the stems and the solution and reduce stem plugging [8]. The aims of this study were to evaluate the effect of pulsing solution and basal stem cutting as minimal postharvest treatments on some qualities of sweet potato leaf tips. The qualities analyzed were chlorophyll, vitamin C (ascorbic acid), and total phenolic contents.

2. Materials and Methods
Local varieties of purple sweet potato tips for this study were obtained from sweet potato grown at South Lampung. Sweet potato leaf tips used in this study had the desirable attribute for human consumption. They had small stems and petioles they were glabrous, tender and showed some purple color. The 15 cm long tips were harvested 12 weeks after transplanting.

Fifteen sweet potato tip stems were dipped in 500 ml containers filled with water (control) or a solution containing 1.5% sucrose and 300 ppm citric acid. The solution of sucrose and citric acid was changed every 24 hr. In the control, the holding solution and water were not changed during the experiment. The basal stem cutting treatment consisted of cutting with a sharp knife 0.5 cm from the base of the stem every 24 hr. In the control, no cutting was performed.

The tips were held for 5 days at 30 ± 3°C. At the end of 5 days, the tips were removed from the holding media, steam blanched for 3 min, and cooled at room temperature. They were then cut into small pieces, put into plastic bags, and frozen at -15°C until analysis was performed. Initial samples were steam blanched and frozen immediately after harvest. The samples were evaluated for their chlorophyll, vitamin C (ascorbic acid), and total phenolic contents.

2.1 Chlorophyll
The samples (25 gram of frozen tips) were homogenized in 50 ml of deionized water. A 3 g subsample was mixed with 12 ml of 80% acetone (v/v) and centrifuged for 10 minutes at 3,500 rpm. The supernatant was recovered, adjusted with acetone to 15 ml, and the chlorophyll absorption was read at 645 nm and 663 nm. The following formula described by Arnon [9] was used to calculate total chlorophyll content:
Total chlorophyll (mg/l) = 20.2 A + 8.02 B
where : A = Absorbance at 645 nm
B = Absorbance at 663 nm

2.2 Vitamin C (Ascorbic acid)
Ascorbic acid was evaluated by the procedure of AOAC\(^{[10]}\) slightly modified as follows: the samples (12.5 g of frozen tips) were homogenized in 50 ml of 1% oxalic acid and filtered through Whatman number 1 filter paper. Then 1 part of filtrate was diluted with 4 parts of 1% oxalic acid. Determination of ascorbic acid was done by reading the absorbance of a 2-ml diluted sample reacted with 2 ml dichlorophenol indophenol dye at 520 nm. The ascorbic acid content of the sample was obtained by reading a standard curve developed with levels of pure ascorbic acid ranging from 0 to 16 µ per ml.

2.3 Total phenolics
Samples of 5 g frozen tips were homogenized in 25 ml of 80% methanol (v/v) and filtered through Whatman number 1 filter paper. One ml of extracted tissue was added to one ml of 0.25 N Folin-Ciocalteau solution and mixed well. After 3 min one ml of 1N sodium bicarbonate was added. Ten min later, it was adjusted to 10 ml with deionized water and allowed to stand for 2 hr. The absorbance of total phenolics was read at 725 nm. The phenolic content of the sample was obtained by using a standard curve developed with levels of chlorogenic acid ranging from 10 to 100 micrograms per ml. This method was described by Swain and Hillis\(^{[11]}\).

The data were analyzed as a factorial 2 x 2 x 2 (2 holding solutions, 2 changes, 2 cutting treatments) with 4 replications. Four replications of initial samples were steam blanched and frozen immediately after harvest in order to know the levels of chlorophyll and ascorbic acid and total phenolic content at the initiation of the experiment.

3. Results and Discussion
Holding solution treatment had significant effect on chlorophyll, ascorbic acid, and phenolic contents of sweet potato. The holding solution of sucrose and citric acid retained higher levels of chlorophyll, but reduced the ascorbic acid and increased phenolic contents as compared to the water holding control (Table 1). The ability of sucrose holding solution in delaying senescence of cut flower as reported by Halevy and Mayak\(^{[12]}\) and Acock and Nichols\(^{[13]}\), and the benefit of adding citric acid to lower the pH in holding solutions as discussed by Durkin\(^{[8]}\) and Reid\(^{[14]}\), showed the same effects on delaying chlorophyll degradation of sweet potato tips. The lower content of ascorbic acid was probably caused by the presence of sucrose in the holding solution. Greasy\(^{[15]}\) reported that sucrose can partially replace light and carbon dioxide as a promoter of phenolic synthesis. These phenolics will be oxidized to quinone, and since quinone is a powerful oxidizing agent, it may have oxidized some of ascorbic acid to dehydroascorbic acid which was not measured with the ascorbic acid assay used in this study.

Holding solution changes did not show significant effect on chlorophyll and ascorbic acid (Table 1). However, there was an interactive effect on ascorbic acid between holding solution change and type of holding solution. Daily change of water maintained high ascorbic acid content of the tips, while the opposite was observed in the sucrose and citric acid solution (Figure 1). These results were probably due to oxidation of ascorbic acid as a result of phenolic synthesis in the presence of sucrose\(^{[16]}\). Basal stem cutting had a significant effect only on ascorbic acid where the rate of degradation appeared to be slower as compared to the uncut control (Table 1). The effect of daily basal stem cutting in maintaining high levels of ascorbic acid was probably affected by a better water uptake due to the elimination of basal stem plugging. The possibility of stem plugging was suggested by the presence of
wilted leaves in the control no basal stem cutting. Ezell and Wilcox’s \cite{17} findings, indicating that wilting induces the loss of ascorbic acid, also support this possibility.

A significant second order interaction on ascorbic acid content of sweet potato tips also indicated that the water holding solution with or without daily change had the best effect on maintaining high levels of ascorbic acid in the basal stem cut tips. It was also observed that the development of roots around the basal stem was faster when the tips were held in water (1.5 cm) in comparison to that held in a solution of 1.5% sucrose and 300 ppm citric acid (0.5 cm).

Pulsing solutions, pulsing change, and basal stem cutting had a significant effect on the total phenolic content. Solution containing sucrose and citric acid increased higher levels of phenolic as compare to those pulse in water. This indicates that phenolic synthesis was favoured by pulsing, especially when sucrose and citric acid solution was used. Polyphenols are known as important antioxidants, therefore phenolic contained in sweet potato leaves could be expected as a source of bioactive compound serves as antioxidant, and consequently should be promoted as functional leafy vegetables in an attempt to promote health status, especially in developing countries.

Table 1. Effect of holding solutions, daily holding solution change and basal stem cutting on chlorophyll, vitamin C, and phenolic contents of frozen blanched-sweet potato tips\textsuperscript{1,2}.

| Treatments                  | Total chlorophyll mg/100g | Ascorbic acid Mg/100 g | Total phenolic µg/g |
|-----------------------------|---------------------------|------------------------|---------------------|
| A. Pulsing Solution         |                           |                        |                     |
| Water                       | 6.0\textsuperscript{b3}   | 20.4\textsuperscript{a} | 0.77\textsuperscript{a} |
| 1.5% sucrose + 300 ppm citric acid | 11.0\textsuperscript{a} | 17.1\textsuperscript{b} | 0.88\textsuperscript{a} |
| B. Pulsing solution change  |                           |                        |                     |
| Daily change                | 8.5\textsuperscript{a}    | 19.2\textsuperscript{a} | 0.88\textsuperscript{a} |
| No change                   | 8.6\textsuperscript{a}    | 18.3\textsuperscript{a} | 0.71\textsuperscript{a} |
| C. Basal stem cutting       |                           |                        |                     |
| Daily cut                   | 8.7\textsuperscript{a}    | 19.9\textsuperscript{a} | 0.88\textsuperscript{a} |
| uncut                       | 8.4\textsuperscript{a}    | 17.5\textsuperscript{b} | 0.72\textsuperscript{b} |
| Interactions                |                           |                        |                     |
| AxB                         | NS\textsuperscript{4}     | *                      | NS |
| AxC                         | NS                        | NS                     | NS |
| BxC                         | NS                        | NS                     | NS |
| AxBxC                       | NS                        | *                      | NS |

\textsuperscript{1}Initial level of chlorophyll, ascorbic acid, and phenolics at harvest time were12.0 mg/100 g, 24.8 mg/100 g, and 0.7 mg/ g frozen blanched-samples

\textsuperscript{2}Tips were held at 30±5°C for 5 days.

\textsuperscript{3}Mean separation within a column for each main effect by LSD 5% level.

\textsuperscript{4}NS, *are non significant or significant at 5% level.

Polyphenols are known as important antioxidants, therefore phenolic contained in sweet potato leaves could be expected as a source of bioactive compound serves as antioxidant, and consequently should be promoted as functional leafy vegetables in an attempt to promote health status, especially in developing countries, and further research on potential benefit of utilizing sweet potato leaf tips as vegetables is needed.
4. Conclusions
Holding solution of 1.5% sucrose and 300 ppm citric acid helped to reduce chlorophyll degradation and induced phenolic synthesis of sweet potato tips after 5 days of post-harvest holding at 30±3°C. However, this treatment induced a higher degree of ascorbic acid degradation. The opposite was observed in the water holding control. In general, minimum postharvest handling through using pulsing solution containing sucrose and citric acid, daily pulsing solution and daily basal stem cutting showed beneficial effects in maintaining ascorbic acid and inducing phenolic synthesis in sweet potato leaf tips.

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