is only a minimum value for the major fraction α-globulin and the value of ν_{Na^+} for the total protein would be much higher if one considers the amount of Na^+ bound to the 2S component also. However, at this point we have no definite information on the characterization of 2S component from sesame seed from the point of view of its hydrogen ion equilibria and amino acid composition. Nevertheless, the point is clear that this enormous amount of Na^+ bound to the total protein obviously need to shift the pH of minimum extractability to a more acidic region as the sodium chloride concentration increases. However, as the concentration of NaCl increases above 0.8 M the binding sites are all saturated and all the fractions are extracted. Hence, one does not see much change either in pH of minimum extractability or in the percent fraction of 2S and 11S components extracted above 0.8–1.0 M NaCl. Since initial studies from our laboratory have also indicated that the 2S component has much higher isoelectric pH (~6.0), it is conceivable that at low concentration of NaCl only the 2S component is extracted and the pH of minimum extractability is near pH 5.0. At higher NaCl concentration since α-globulin is preferentially extracted and has a lesser isoelectric pH (~4.5), it shifts the pH of minimum extractability toward acidic pH. It is shown by Nozaki et al. (1959) that the cations are bound at a carboxyl-rich locus, and since sesame total protein contains nearly 35% of acidic amino acids (Prakash and Nandi, 1978), it is conceivable that the value of ν_{Na^+} is large. These two effects in conjunction could be responsible for the drift in pH of minimum extractability as the NaCl concentration is increased during extraction of the protein.

The above result is only an indication of how sensitive some of these seed proteins are toward minor changes like ionic strength and effecting the macroscopic constants like "isoelectric pH" of the protein. However, other effects like conformational changes and association–dissociation reactions (Prakash and Nandi, 1977) might also be playing a dominant role in such interactions. These can only be investigated in pure systems and the results extrapolated to obtain meaningful interpretations.

Registry No. NaCl, 7647-14-5.

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Effect of Wilting on the Ascorbate Content of Selected Fresh Green Leafy Vegetables Consumed in Sri Lanka

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The loss of ascorbate in eight green leafy vegetables due to wilting, from harvest up to a period of 24 h at environmental temperatures (24.7–25.8 °C) and under refrigeration (4.4 °C), was studied. The ascorbate content in the fresh leaves ranged from 4 to 86 mg/100 g. The loss of ascorbate was appreciably reduced when the leafy vegetables were wilted under refrigerated conditions compared to that under environmental temperatures. The greatest loss in ascorbate content was observed in Gotukola (*Centella asiatica*) whereas Nivithi (*Basella alba*) showed the lowest loss during the 24-h wilting period. Wilting of these fresh leaves for 4, 8, 12, 16, 20, and 24 h lowered their ascorbate contents under environmental temperatures on the average by 30, 50, 58, 66, 72, and 80%, respectively. However, when refrigerated for the same periods, they lost 21, 41, 49, 55, 64, and 74% of ascorbate, respectively.

INTRODUCTION

Vitamin C (ascorbic acid) is required in the diet in greater amounts than all the other vitamins combined (FAO/WHO, 1974). However, it is notably labile and is more readily lost than most other food constituents.

Temperature and humidity are major factors in the shelf life of fresh vegetables (Ezell and Wilcox, 1959). Green leafy vegetables wilt rapidly in unfavorable environments due to their extensive and rather permeable surfaces. The amount of vitamin C available to the consumer depends on the environmental conditions the green leaves are subjected to, between harvest and the time of purchase. The effect of varying temperature and of slow, moderate and rapid wilting on the loss of vitamin C in vegetables have been reported by Ezell and Wilcox (1959). The loss of vitamin C in various vegetables on standing in different environmental temperatures, including refrigeration, has been reported in the literature (Cadwell and Gim Sai, 1973;

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Ryall and Lipton, 1972; Aberg, 1949; Scott and Kramer, 1949). The effect of home drying (solar drying) on the retention of vitamin C in horticultural products has also been studied (Addo and Adenike, 1981; Holmes et. al., 1979; Maeda and Salunkhe, 1981). However, the effect of wilting on the retention of ascorbic acid in tropical green leafy vegetables such as those consumed in Sri Lanka has not been reported yet.

In Sri Lanka, green leafy vegetables are grown in districts far removed from the populated urban centers. Even those grown relatively near are in the process of wilting by the time they appear in the market stalls and may lose much of their vitamin C, unless precautions are taken to preserve it. The objective of this study was to determine the effect of wilting under environmental (24.7–25.8 °C) as well as in refrigerated storage (4.4 °C) on the ascorbate contents of some of the common edible green leafy vegetables consumed in Sri Lanka. Being a developing third-world country, Sri Lanka lacks in modern packing and refrigerated transport facilities. Hence, local methods should be devised to minimize losses in vitamin C due to wilting during marketing. More knowledge on this aspect was a long-felt need, as it carries possible nutritional significance.

MATERIALS AND METHODS

Leafy Vegetables. The eight dark green leafy vegetables used in this study were Nivithi (*Basella alba*), Gotukola (*Centella asiatica*), Saladukola (*Lactuca sativa*), Thampala (*Amaranthus gangeticus*), Wattaka kola (*Cucurbita maxima*), Kankun (*Ipomea aquatica*), Mukunuwenna (*Alternanthera sessilis*), and Murunga kola (*Moringa oleifera*). They were obtained as fresh greens from the Agricultural Experimental Farm of the University of Peradeniya, Sri Lanka. The leaf samples were quickly brought to the laboratory and washed to remove sand and dirt, and their ascorbate contents were determined quickly on aliquots. The green leafy vegetables were tied into 300-g bundles with banana stem fibers to simulate their local marketing conditions. At 4-h intervals, 50-g portions were taken from each type of green leaves, divided into five equal lots, and used as samples for the determination of vitamin C.

Wilting of Leaves. The bundles of green leafy vegetables were allowed to wilt on wooden racks as done for selling in the local market stalls, under shade outside the laboratory. The maximum and minimum temperatures and the relative humidity were recorded throughout the 24-h wilting period. They were also placed in the refrigerator and allowed to wilt.

Determination of Vitamin C. Ten-gram leaves were ground with sand and 4% (w/v) metaphosphoric acid in a large pestle and mortar. The mascerate was kept covered by the acid to prevent its oxidation. This was extracted with 100 mL of an extracting solution containing 0.3% (w/v) trichloroacetic acid and 0.9 g of ethylenediaminetetraacetic acid to decolorize the solution. After this, the whole volume of extracted juice was measured, and the solids were removed by centrifugation and filtration. Aliquots of this filtrate were then analyzed colorimetrically with the 2,6-dichlorophenol–indophenol method (Freed, 1966).

RESULTS AND DISCUSSION

During the 24-h wilting period, the maximum temperature ranged from 24.7 to 25.8 °C, the minimum temperature ranged from 22.5 to 23.3 °C, and the relative humidity ranged from 75 to 83% (Table I).

The ascorbate contents of the eight fresh green leaves, (expressed as mg/100 g of fresh weight) ranged from 4.3 ± 2.7 mg in Wattaka kola (*C. maxima*) to 86.4 ± 2.1 mg in Nivithi (*B. alba*) (Table II). These values are slightly lower than those reported in Food Composition Tables (Medical Research Institute, Sri Lanka, 1979). This may be attributed to the variation in the maturity of the leafy vegetables and the different ascorbic acid assay methods employed. According to the FAO/WHO (1974) recommended dietary allowance for Southeast Asia, a moderately active adult should take in 30 mg of ascorbic acid to satisfy his daily metabolic needs. Of the eight green leafy vegetables studied, only Nivithi (*B. alba*) and Murunga kola (*M. oleifera*) are good sources of vitamin C. Consumption of about 35 g of these leaves could satisfy the recommended dietary intake (RDA) for vitamin C. In the case of Wattaka kola, one has to consume around 700 g of the leaves in order to satisfy the RDA for vitamin C. However, this quantity is too great to consume in 1 day. Further, for Sri Lankans, the per capita availability of all the vegetables is estimated around 114 g/day during the period 1969–1981 (Statistical Abstracts of Sri Lanka, 1982).

Table I. Temperature and Relative Humidity Recorded during 24-h Wilting of Green Leafy Vegetables

wilting time, h	max temp, °C	min temp, °C	rel humidity, %
0 (6.00 a.m.)	24.7	22.5	83
4 (10.00 a.m.)	25.8	22.5	75
8 (2.00 p.m.)	25.8	23.3	79
12 (6.00 p.m.)	25.5	23.3	83
16 (10.00 p.m.)	25.5	22.8	79
20 (2.00 a.m.)	25.0	22.8	83
24 (6.00 a.m.)	24.7	22.5	83

Table II. Ascorbate Contents of Fresh Green Leafy Vegetables

local name[a]	botanical name	ascorbate[b] content, mg/100 g of fresh wt
Nivithi	*Basella alba*	86.4 ± 2.1
Gotukola	*Centella asiatica*	7.8 ± 1.8
Saladu kola	*Lactuca sativa*	6.4 ± 1.7
Tampala	*Amaranthus gangeticus*	45.7 ± 2.4
Wattaka kola	*Cucurbita maxima*	4.3 ± 2.7
Kankun	*Ipomea aquatica*	14.3 ± 1.9
Mukunuwenna	*Alternanthera sessilis*	8.6 ± 1.7
Murunga kola	*Moringa oleifera*	82.9 ± 1.8

[a] Sinhala names. [b] Values were mean ± SEM; 10 determinations

The effect of wilting of the green leafy vegetables on the ascorbate loss is shown graphically in Figure 1. Wilting hastened the loss of ascorbic acid. Loss of vitamin C increased with wilting time up to 24 h after harvest. Most of these green leaves were observed to retain their lush appearance up to about 24 h after harvest, after which physical and visible wilting was observed. In the local market stalls, however, the traders sprinkle water or immerse the green leaves in water in an attempt to maintain their freshness.

Figure 1 shows that the loss of ascorbate by the green leaves continued as they were wilting, and the percentage loss per hour of wilting varied with different leaves. The rate of ascorbate loss was found to increase generally within 8 h after harvest in the case of Nivithi (*B. alba*) and Murunga kola (*M. oleifera*), after which the rate of loss decreased. However, in the case of the other leaves, the rate of loss of ascorbate increased throughout the 24-h wilting period. At the end of the 24-h wilting period, the greatest loss of vitamin C was observed with Gotukola (*C. asiatica*) (99%) and the lowest loss with Nivithi (*B. Alba*) (45%). These observations support the earlier findings by Ezell

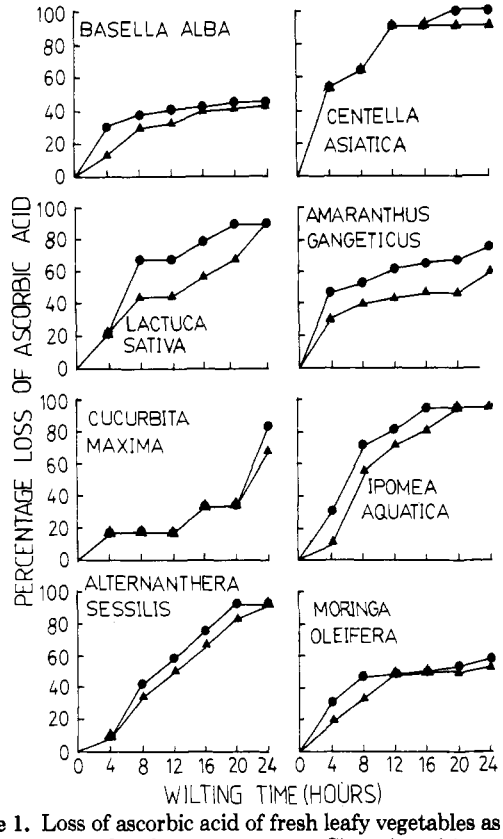

Figure 1. Loss of ascorbic acid of fresh leafy vegetables as related to wilting: ●, under shade (24.7–25.8 °C); ▲, in refrigerator (4.4 °C).

and Wilcox (1959) that wilting tends to increase the rate of loss of ascorbic acid in leafy vegetables. Of the 12 Nigerian leafy vegetables studied (Fafunso and Bassir, 1976), nine of them lost between 70 and 89% of their ascorbate content after 24-h wilting.

Figure 1 also shows that in the case of Wattaka kola (*C maxima*) and Thampala (*A. gangeticus*) at the end of 24-h wilting in the refrigerator reduced the loss of vitamin C by about 16 percentage points compared to the wilting at environmental temperature. However, in the case of the other six leafy vegetables, there was no appreciable conservation of vitamin C in refrigerated conditions compared to wilting under environmental temperatures at the end of the wilting period. Scott and Kramer (1949) reported that when asparagus spears were stored at 0 °C for a period of 1 week, the loss of ascorbic acid was only 50% compared to 90% loss when stored at 21 °C. The observations in the present study suggest that most of the green leafy vegetables studied were susceptible to chilling injury, thus reducing their ascorbate contents.

Table III shows the range of ascorbate loss by the fresh leaves when allowed to wilt for different periods under environmental and in refrigerated conditions. Within the first 8 h of wilting, between 8 and 55% loss of vitamin C occurred due to wilting under both environmental and refrigerated conditions. When the mean loss of ascorbate was considered, there was appreciable advantage in keeping the green leaves in the refrigerator compared to wilting under environmental conditions in conserving vitamin C.

Table III. Variations in the Percent Ascorbate Loss by Fresh Leafy Vegetables That Have Been Allowed To Wilt for Different Periods

wilting time, h	range of ascorbate loss, % under shade (24.7–25.8 °C)	in refrig (4.4 °C)
4	8–55 (30.33 ± 15.14)[a]	8–54 (21.49 ± 15.09)
8	17–70 (49.46 ± 17.64)	17–63 (40.95 ± 14.89)
12	17–91 (57.89 ± 23.09)	17–91 (49.34 ± 22.65)
16	33–95 (66.14 ± 22.52)	33–91 (54.76 ± 27.52)
20	33–99 (71.69 ± 25.71)	33–95 (63.85 ± 23.58)
24	45–99 (79.64 ± 19.14)	43–95 (73.82 ± 20.10)

[a] Values within parentheses indicate mean ± SD.

It was also observed that wilting of the fresh green leaves under environmental temperatures for 4, 8, 12, 16, 20, and 24 h reduced their ascorbate contents on the average by 30, 50, 58, 66, 72, and 80%, respectively. However, when they were wilted under refrigerated conditions for the same periods, they lost 21, 41, 49, 55, 64, and 74% vitamin C, respectively.

Registry No. Ascorbate, 50-81-7.

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