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Research article

Vitamin C content of commonly eaten green leafy vegetables in fresh and under different storage conditions

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Abstract: This study was an attempt to determine the consumption of popular green leafy vegetables (GLVs) available in Batticaloa district, Sri Lanka and to determine vitamin C content of fresh and stored (under room temperature 30±2ºC and at 4ºC for 4 days) GLVs. Vitamin C content was estimated in aqueous extracts using dichloroindophenol titrimetric method. Thirty one species of GLVs were consumed commonly by the subjects with an average consumption of 59%. Vitamin C content of fresh GLVs ranged from 5.25 mg/100 g for *Centella asiatica* to 433.13 mg/100 g wet weight for *Drgea volubilis. Drgea volubilis*, which had the highest amount of vitamin C, is consumed by 80% of the consumers followed by *Delonix elata*, which is consumed by 52% of the consumers interviewed. *Murraya koenigii*, which is used by almost all the consumers interviewed, is a poor source of vitamin C. Similarly *Centella asiatica*, which was claimed to be consumed by 90% of the consumers, too was a poor source of vitamin C. The decline in vitamin C content of all GLVs, ranging from 18% for *Aerva lanata* to 100% for *Moringa oleifera*, was higher and significant ($p = 0.000$ at 95% confident interval) when stored at room temperature for 4 days than stored at 4ºC (ranging from 2.5% for *Sauropus androgynus* to 70% for *Alternanthera sessilis*) except *Pisonia grandis*. Both *Drega volubilis* and *Delonix elata* showed 22.3 and 6.1% increase in vitamin C content respectively when stored at 4ºC.

Keywords: Vitamin C - Green leafy vegetables - *Drgea volubilis*.

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INTRODUCTION

Green leafy vegetables (GLVs) are rich sources of many nutrients and form a major category of vegetable group that are rich in health promoting phytochemicals. Their high antioxidant contents have attracted attention of several investigators (Boxin *et al*. 2002, Ismail *et al*. 2004, Gupta *et al*. 2005).

More than 90% of vitamin C in human diet is supplied by fruits and vegetables (Latif & El-Aal 2007). Vitamin C is required for the prevention of scurvy and has many biological activities in the human body (Antonelli 2002). Unfortunately, this vitamin has low thermo stability and high water solubility (Anna 2007). In view of this, refrigerated storage recommended for increased shelf life of fresh vegetables (Vina & Chaves 2006) would be helpful in retention of their vitamin C content. Latif & El-Aal (2007) reported that simple packaging of fresh green leafy vegetables in polythene was very effective in reducing weight and moisture losses during cold storage.

Inadequate number of studies, shortage of primary data and many information gaps exist regarding the consumption pattern of GLVs as well as the vitamin C content of the GLVs under the existing practice of storage. Therefore this study is an attempt to find the answers for the above concerns.

MATERIALS AND METHODS

Determination of percentage of consumption

Ten popular market places in Batticaloa district namely Arayampathy, Batticaloa, Chenkalady, Eravur, Kaluwanchikudy, Kallar, Kattankudy, Kiran, Oddamavadi and Valaichenai were selected for the study. During

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the preliminary study it was found that around 20,000 people regularly used these markets. Interviews were conducted for a period of 10 months and 5 consumers (subjects) were interviewed, on a random basis, during each visit to the selected market. A total of one thousand subjects were interviewed at the end of the survey (5 consumers × 2 times per month × 10 places × 10 months). Types of GLVs and percentage consumption of each GLV based on the interview was calculated

Preparation of GLVs, storage and packing

The GLVs collected from field were cleaned and shoots separated. The shoots were washed thoroughly under running tap-water for 5 minutes to remove soil particles and dirt. Then the washed shoots were placed in a plastic container with paper towel and allowed to drain for 5 minutes. The cleaned, washed, GLVs were left at room temperature to dry, and thereafter each GLV was wrapped with paper and then placed in polyethylene bags. They were stored both at room temperature (30± 2ºC) and 4ºC (refrigerator) for 4 days.

Estimation of vitamin C (Ascorbic acid)

All chemicals used for the study were of analytical grade and distilled water was used for the preparation of reagents. Samples of GLVs (10 g of each) were accurately weighed and ground using a mortar and pestle in 20 ml of metaphosphoric acid-acetic acid solution. The mixture was strained through a muslin cloth and the extract was made up to 100 ml with the metaphosphoric-acetic acid solution. The extracts were prepared using 3 different samples of each GLV and all analyses were carried out in triplicates.

Ascorbic acid content was estimated by the 2, 6-dichloroindophenol (DCP) titrimetic method (Nielsen 2010). Metaphosphoric acid-acetic acid solution (5 ml) was pipetted separately into three 50 ml Erlenmeyer flasks followed by 2 ml of the sample extract. The samples were titrated separately with the indophenol dye solution until a light rose pink colour persisted for 5 seconds. The 2, 6-dichlorophenolindophenol dye was standardized against standard ascorbic acid. The results were expressed as mg ascorbic acid/100 g wet weight (WW).

RESULTS AND DISCUSSION

Consumption of GLVs

Thirty one species of GLVs were consumed commonly as food by the subjects, which ranged from 15% for *Lactuca sativa* to 98% for *Murraya koenigii* and the average consumption was 59.35%. Among the GLVs, 16 species were consumed for more than 50 % and 15 species were consumed 50% or less by the subjects interviewed (Table 1). GLVs consumed more than 50% by the subjects were selected to determine the vitamin C content. The consumption of 16 selected GLVs ranged from 52 to 98% with an average of 79.1% (Table 1).

Table 1. Overall consumption of commonly consumed leafy vegetables for food purposes.

No	Name of GLVs	Vernacular names (T-Tamil, S- Sinhala, E-English)	Consumption (%)
1	*Aerva lanata**	Polpala (s) /Thaenkaipukerai (T) /Hongone	81
2	*Allmania nodiflora*	Kumatiya (S),Kumatti (T)	44
3	*Alternanthera sessilis**	Mukunuwenna/Ponnangani/Alligator weed	97
4	*Amaranthus caudatus**	Rana-Tampala/Kerai/Pendant amaranth	94
5	*Amaranthus spinosus*	Mudkerai(T), Spiny amaranth (E)	42
6	*Amaranthus viridis**	Kura/Kuppaikerai/Green amaranth	76
7	*Argyreia pomacea**	Manpanchan (T)	60
8	*Asteracantha longifolia*	Neer Mulli(T), Hydrophylla (E)	22
9	*Basella alba*	Nivithi kola (S),Pasali (T), Indian spinach (E)	60
10	*Borreria hispida*	Nathaisuri (T)	38
11	*Canthium parviflorum*	Kara (S),Kaarai (T), Wild jasmine (E)	28
12	*Cardiospermum halicacabum**	Penela-wel (S)/Mudakottan (T)/ Wintetcherry	82
13	*Centella asiatica**	Gotukola (S)/Vallarai (T), Indian penny ward (E)	88
14	*Cucurbita maxima*	Kumbala (S),Poosani (T), Pumpkin (E)	50
15	*Delonix elata**	Vatham-Nairaini (T),Yelow Gul-Mohur (E)	52
16	*Drega volubilis**	Anguna kola (S) Kurinja (T)/Sneez ward (E)	80

17	*Gymnema sylvestre*	Bin –nuga (S),Sirukurinja (T), Gymnema (E)	37
18	*Ipomoea aquatica*	Kangkong (S),Vallal (T), Water spinach (E)	49
19	*Lactuca sativa*	Salada (S), Salathu (T), Lettuse (E)	15
20	*Mollugo oppositifolia****	Hinipala (S)/Thirai (T)/Itch flower (E)	84
21	*Moringa oleifera****	Murunga (S)/Murungai (T)/ Drum stick (E)	95
22	*Murraya koenigii****	Karapincha (S)/Karuveppilai (T)/Curry leaf (E)	98
23	*Passiflora edulis*	Kodimathulai (T)	46
24	*Pisonia grandis****	Wathabanga (S) Ledchakaddai (T), Lettuse tree (E)	67
25	*Premna obtusifolia*	Sihin-Midi (S), Kananthi (T)	39
26	*Premna serratifolia*	Midi (S,) Earumai mullai (T)	24
27	*Premna latifolia*	Maha midi (S), Pasumullai (T)	40
28	*Rivex ornate*	Musutai (T), Midnapore creeper (E)	41
29	*Sauropus androgynus**	Japanbatukola (S)/ Thavasi murungai (T)	54
30	*Sesbania grandiflora****	Katuru murunga (S)/ Ahaththi (T)	76
31	*Solanam trilobatum****	Wel-tibbatu (S), Thuthuvilai(T), Heliotrope (E)	81
		Average	**59.35**

Note: Selected plant species used for determining vitamin C content.

Vitamin C (Ascorbic acid) content

The sixteen GLVs showed varying ascorbic acid content in fresh state and under two temperature storage conditions for 4 days (Table 2). The vitamin C content of fresh GLVs ranged from 5.25 mg/100 g for *Centella asiatica* to 416.2 mg/100 g for *Drega volubilis* on wet weight basis (W/W). Gupta & Prakash (2009) reported the ascorbic acid content of *C. asiatica* was 15.18 mg/100 g (W/W) using the same method as in the present study. However, the vitamin C content of *Centella asiatica* in the present study is much lower than that reported by Gupta & Prakash (2009).

Table 2. Vitamin C contents of fresh and stored (for 4 days) GLVs.

No	GLVs	Vitamin C content (mg/100g)		
		Fresh	**Room temp. 30±2 ºC**	**Refrigeration 4 ºC**
1	*Aerva lanata*	53.67 ± 5.10^{a}	43.75 ± 3.21^{c}	49.29 ± 4.50^{b}
2	*Alternanthera sessilis*	36.17 ± 3.42^{a}	6.71 ± 1.91^{c}	10.79 ± 1.58^{b}
3	*Amaranthus caudatus*	51.63 ± 4.15^{a}	5.83 ± 2.55^{c}	28.88 ± 5.41^{b}
4	*Amaranthus viridis*	35.58 ± 4.37^{a}	6.42 ± 2.32^{c}	24.50 ± 4.73^{b}
5	*Argyreia pomacea*	7 ± 1.86^{a}	0.29 ± 0.88^{c}	3.21 ± 1.16^{c}
6	*Cardiospermum helicacabum*	85.17 ± 11.07^{a}	27.71 ± 3.96^{c}	44.63 ± 5.08^{c}
7	*Centella asiatica*	5.25 ± 1.86^{a}	2.63 ± 1.31^{b}	$4.38\pm2.27^{a,b}$
8	*Delonix elata*	183.17 ± 13.43^{a}	77 ± 13.32^{b}	194.83 ± 16.54^{a}
9	*Drega volubilis*	416.2 ± 38.6^{b}	371 ± 29.14^{c}	505.46 ± 7.08^{a}
10	*Mollugo oppositifolia*	39.38 ± 10.08^{a}	7.29 ± 2.19^{c}	27.13 ± 10.66^{b}
11	*Moringa oleifera*	135.33 ± 5.10^{a}	0.00^{c}	123.08 ± 4.03^{b}
12	*Murraya koenigii*	22.75 ± 5.57^{a}	16.04 ± 5.16^{b}	23.92 ± 6.49^{a}
13	*Pisonia grandis*	17.50 ± 3.71^{a}	11.08 ± 2.55^{b}	12.54 ± 3.15^{b}
14	*Sauropus androgynus*	66.79 ± 6.58^{a}	47.71 ± 5.79^{b}	65.04 ± 5.53^{a}
15	*Sesbania grandiflora*	134.75 ± 14.07^{a}	42.88 ± 7.54^{c}	83.13 ± 9.64^{b}
16	*Solanam trilobatum*	10.5 ± 2.94^{a}	3.5 ± 2.27^{b}	6.42 ± 2.32^{b}

Note: Values are means of 3 replicates ± Standard deviation; Statistically significance at 5% level (Superscripts of letters a, b, c denotes statistically significant, superscripts of same letter denotes statistically not significant).

The vitamin C content of 14 common vegetables in Nigeria estimated by the Nielsen (2010) procedure was found to range from 400 to 692 mg/100 g dry weight and *Amaranthus caudatus* was reported to contain 400 mg/100 g on dry weight (Akindahunsi & Salawu 2005). *Amaranthus caudatus* was the only GLV investigated by Akindahunsi & Salawu (2005) and in the present study and a comparison could not be made because of the different procedures used. Oboh (2005) reported the vitamin C content of *Amaranthus cruentus* and *Solanum*

macrocarpon estimated by the Nielsen (2010) method as 70.0±0.5 mg/100 g and 43.57±0.6 mg/100 g respectively. The *Amaranthus* varieties used in the current study had lower vitamin C content than reported by Oboh (2005) and the difference could be due to different varieties studied. Similarly the vitamin C content of *Solanam trilobatum* used in the present study too was lower than the levels reported by Oboh (2005) for *Solanum macrocarpon.*

In general, ascorbic acid content of GLVs declined during 4 days of storage and the differences observed in vitamin C content among selected GLVs in fresh and under two storage conditions are significant ($p < 0.05$) (Table 2). *Drega volubilis* showed the highest content of ascorbic acid (416.2±38.6 mg/100 g, when fresh) in all three conditions followed by *Delonix elata*, *Sesbania grandiflora*, *Moringa oleifera* and *Sauropus androgynus*. Three species showed lower ascorbic acid content in all three conditions, such as *Argyreia pomacea*, *Centella asiatica* and *Solanam trilobatum* (Table 2). Vitamin C content of fresh GLVs such as *Centella asiatica*, *Delonix elata*, *Murraya koenigii* and *Sauropus androgynus* showed no notable changes when stored in a refrigerator (Table 2).

The decline in vitamin C content in all the GLVs, ranging from 18% for *Aerva lanata* to 100% for *Moringa oleifera*, was higher and significant when stored at room temperature for 4 days than when stored at 4ºC (ranging from 2.5% for *Sauropus androgynus* to 70% for *Alternanthera sessilis*) except for *Pisonia grandis* (Table 3). Both *Drega volubilis* and *Delonix elata* showed 22.3 and 6.1% increase in vitamin C content respectively when stored at 4ºC (Table 3).

Table 3: Loss of vitamin C of GLVs stored for 4 days at different temperatures.

No	GLVs	Loss during storage (%)	
		Room temp. 30±2 ^{0}C	Refrigeration 4 ^{0}C
1	*Aerva lanata*	18^a	8.04^b
2	*Alternanthera sessilis*	81.6^a	70.06^b
3	*Amaranthus caudatus*	88.93^a	44.4^b
4	*Amaranthus viridis*	82.3^a	31.51^b
5	*Argyreia pomacea*	96.30^a	53.70^b
6	*Cardiospermum helicacabum*	67.41^a	47.43^b
7	*Centella asiatica*	50.00^a	14.81^b
8	*Delonix elata*	58.23^a	-6.31^b
9	*Drega volubilis*	10.75^a	-22.29^b
10	*Mollugo oppositifolia*	81.48^a	32.93^b
11	*Moringa oleifera*	100.00^a	9.03^b
12	*Murraya koenigii*	30.13^a	-4.88^b
13	*Pisonia grandis*	36.27^a	28.27^a
14	*Sauropus androgynus*	37.69^a	2.47^b
15	*Sesbania grandiflora*	68.37^a	38.31^b
16	*Solanam trilobatum*	67.96^a	38.52^b

Note: Values are means of 3 replicates ± Standard deviation; Statistically significance at 5% level (Superscripts of letters a, b, c denotes statistically significant, superscripts of same letter denotes statistically not significant).

The vitamin C content of *Drega volubilis* increased significantly by 22.3% when stored in a refrigerator for 4 days compared to the fresh state (Tables 2 & 3). This should be further investigate any biochemical support to this increasing. Interestingly, Latif & El-Aal (2007) also found that the vitamin C content of fresh GLVs *Raphanus sativus* and *Anthum graveolans* increased during first 4 days of storage at 4ºC. The same observation was made by Latif & El-Aal (2007) for *Allium kurrat* stored at 4ºC for 4 days. *M. oleifera* showed complete loss of vitamin C at room temperature when stored for 4 days (Table 2 and 3). *Moringa oleifera* turned yellow and some decayed when stored under room temperature for 4 days. *Pisonia grandis* showed no notable changes in vitamin C when stored under room temperature and in a refrigerator.

More than 50% of vitamin C was lost in 10 species when stored at room temperature (30±2ºC) such as *Alternanthera sessilis*, *Amaranthus caudatus*, *Amarantus viridis*, *Argyeria pomacea*, *Cardiospermum halicacabum*, *Delonix elata*, *Mollugo oppositifolia*, *Moringa oleifera*, *Sesbania grandiflora* and *Solanam trilobatum.* Green leafy vegetables such as *Alternanthera sessilis*, *Cardiospermum halicacabum*, *Delonix elata*,

Moringa oleifera and *Sesbania grandiflora* turned yellow when stored at room temperature. *Alternanthera sessilis* and *Argyeria pomacea* lost more than 50% of vitamin C when stored at 4ºC (Table 3).

The highest percentage vitamin C loss when stored at room temperature was observed for *Moringa oleifera* (100%) whereas highest percentage of vitamin C retained when stored at 4ºC was 98% for *Sauropus androgynus*. Seven GLVs namely *Aerva lanata, Centella asiatica, Delonix elata, Drega volubilis, Moringa oleifera, Murraya koenigii* and *Sauropus androgynus* showed more than 80% of vitamin C retention (Table 3). Latif & El-Aal (2007) also reported that 80% of total vitamin C is retained when stored for 8 days, at 4ºC±1ºC packed in polyethylene bags. The rate of loss of vitamin C is low at cold temperature in the current study which is in agreement with the observation of Latif & El-Aal (2007) and Favell (1998) under similar packing conditions using polyethylene bags. The variation in decline in vitamin C content in GLVs appears to be due to species difference, pre and post-harvest conditions, initial vitamin C concentration, auto-oxidation, storage and enzymatic degradation (Latif & El-Aal 2007, Rivera *et al*. 2006, Howard 1999). Vitamin C loss continues during post-harvest handling, processing, cooking and storage (Moreira *et al*. 2006).

Drega volubilis, which had the highest amount of vitamin C is consumed by 80% of the subjects interviewed (Table 1). *Murraya Koenigii*, which is used by almost all the subjects interviewed, is a poor source of vitamin C. Similarly *Centella asiatica*, which was claimed to be consumed by 90% of the subjects, too was a poor source of vitamin C. Furthermore, packaging and storing the vegetables at 4ºC, which prevents wilting and retain the freshness of GLVs, preserves the vitamin C content compared to packaging and storing at room temperature. These observations are valuable in carrying out nutrition awareness among the public. *Delonix elata*, which was next to *Drega volubilis* in ascorbic acid content, was stated to be consumed by 52% of the subjects.

CONCLUSION

Thirty one species of GLVs were commonly consumed by the subjects. The average consumption was about 59% and ranged from 28 to 98%. GLVs consumed for medicinal purpose, ranged from 1.7% for *Amaranthus caudatus* to 48.9% for *Cardiospermum halicacabum* and the average consumption was 20.11%. GLVs consumed more than 50% by the subjects interviewed, were selected to determine the vitamin C content and such GLVs also showed a high consumption for their medicinal properties.

Vitamin C content of fresh GLVs ranged from 5.25 mg/100 g wet weight for *Centella asiatica* to 416.2 mg/100 g wet weight for *Drega volubilis*. Decline in vitamin C content was observed in GLVs stored at room temperature. GLVs wrapped with paper and then in polyethylene bags and stored at cool temperature retained more than 80% of vitamin C. Vitamin C content of *Drega volubilis* increased by 22% when stored in a refrigerator for 4 days. This needs further investigation.

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