Research Article

Received: 23 December 2008 Revised: 1 April 2009 Accepted: 20 April 2009 Published online in Wiley Interscience: 5 June 2009

(www.interscience.wiley.com) DOI 10.1002/jsfa.3644

Effects of storage condition and domestic cooking on the quality and nutrient content of African leafy vegetables (*Cassia tora* and *Corchorus tridens*)

Snehal Prabhu and Diane M Barrett*

Abstract

BACKGROUND: The main objective of this research was to determine changes in nutrient content of two African leafy vegetables, *Cassia tora* and *Corchorus tridens*, on cooking and storage under different temperature conditions, i.e. room storage (20 °C), refrigerated storage (4 °C) and frozen storage (−18 °C).

RESULTS: The leafy vegetables were analysed for moisture, colour (Hunter *L*, *a*, *b*), texture, total chlorophyll, ascorbic acid, dehydroascorbic acid and total phenolics. Results indicated that the degradation of ascorbic acid was highest as a result of frozen storage, followed by room temperature storage. The dehydroascorbic acid content was correspondingly high in frozen stored leafy vegetables, whereas it was undetectable in the room temperature and refrigerated stored materials. The total phenolic content of the leaves increased with storage time while the total chlorophyll content decreased under all storage conditions. Domestic cooking resulted in significant additional losses of ascorbic acid following storage under all temperature conditions, with only 1–10% retention in the leaves and 50–60% retention in the cooking water. The green colour of the leafy vegetables was retained best under refrigerated and frozen storage, while the peak force and toughness of the leaves increased upon storage under all conditions. The moisture content of the leafy vegetables did not show any significant difference on storage.

CONCLUSION: This study is one of few to report nutrient content changes on the same raw material stored under various temperature conditions and cooked domestically. Refrigerated storage resulted in the highest retention of ascorbic acid and green colour in the leafy vegetables.

Keywords: leafy vegetables; storage; refrigerated; frozen; cooking

INTRODUCTION

Sub-Saharan Africa grows an enormous variety of leafy vegetables. In many parts of Africa, leafy vegetables are used as a side dish to accompany a thick starchy gruel that is the primary carbohydrate source.[1,2] Enhanced consumption of vegetables has been known to alleviate micronutrient malnutrition that is a cause of chronic diseases. Vegetables are one of the most cost-effective and sustainable solutions to micronutrient deficiencies, which affect far more people than hunger alone, and this is crucial in most of sub-Saharan Africa.[3]

Development of appropriate postharvest handling techniques for many of these vegetable species is a critical component in promoting utilisation and commercialisation of these crops.[4] Postharvest losses account for as much as 50% of the total crop loss throughout the developing world owing to inadequate infrastructure and an oversupply of fresh produce that can go unused when market access is limited.[4] Understanding traditional postharvest practices, researching and adapting appropriate postharvest technologies and compiling basic postharvest physiological data will help to ensure successful promotion and commercialisation of these crops.

Owing to substantial loss of unused fresh produce, use of postharvest preservation methods such as refrigeration and freezing might extend their shelf life but may also affect the nutritional content of these crops. A literature review by Rickman *et al.*[5] also concluded that very few studies have monitored changes in nutritional parameters in the same commodity from harvest through storage and domestic cooking.

This research focused on determining the effects of postharvest preservation methods such as refrigeration and freezing on the nutritional quality of leafy vegetables. The study was carried out in collaboration with the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), and seeds of two cultivars of traditional leafy vegetables grown in Africa were provided by the research institute. The initial raw material for all studies

* *Correspondence to: Diane M Barrett, Department of Food Science and Technology, University of California, Davis, 1 Shields Avenue, Davis, CA 95616, USA. E-mail: dmbarrett@ucdavis.edu*

Department of Food Science and Technology, University of California, Davis, 1 Shields Avenue, Davis, CA 95616, USA

was grown under controlled conditions. After harvest, vegetables were stored at room temperature (20 °C) for 6 days, refrigerated temperature (4 °C) for 14 days and frozen temperature (−18 °C) for 90 days. The length of storage under different temperature conditions was based on preliminary shelf life study results in which it was determined that the leaves were not suitable for human consumption owing to microbial spoilage at the end of these periods. Leafy vegetables are typically cooked prior to consumption in Africa, so the other aspect of this study was to evaluate nutrient retention after cooking. Ascorbic acid, one of the most sensitive nutrients found in leafy vegetables, was evaluated as an indicator or nutrient loss. Ascorbic acid was the only nutrient evaluated following cooking.

EXPERIMENTAL

Leafy vegetable growth and harvesting

Plants of *Cassia tora* (family Caesalpinaceae), commonly called sicklepod, and *Corchorus tridens* (family Tiliaceae), commonly called Jew's mallow, were grown locally in the College of Agriculture and Environmental Sciences greenhouse (latitude 38° 32′ N, longitude 121° 46′ W) at the University of California, Davis (UC Davis).

Leaf colour was monitored throughout the growing period, and a five-point colour scale was developed using digital photographs of typical maturity stages. Harvesting of one specific colour/maturity stage was carried out during the pre-flowering stage. Leaves were harvested from 15 plants and placed randomly in 26.8 cm × 27.3 cm Ziploc bags (S.C. Johnson & Son, Inc., Racine, WI, USA). One leaf weighed approximately 1 g, and approximately 50 g of leaves were placed in each bag. Fifty bags of leaves were used for each storage study, and the entire study, from growing plants to harvesting and storage, was repeated twice. On the day of analysis, three bags were chosen randomly and leaves were randomly sampled from all three bags for each measurement.

Cassia tora growth and harvest

In study 1, seeds were sown in January 2007 and the leaves were harvested in July 2007 (growth period 9 months). In study 2, seeds were sown in April 2007 and the leaves were harvested in October 2007 (growth period 6 months).

Corchorus tridens growth and harvest

In studies 1 and 2, seeds were sown in January 2007 and the leaves were harvested in August and September 2007 respectively.

Average temperature and solar radiation recorded during the growth and harvest periods are shown in Fig. 1. A preliminary shelf life study was carried out to establish the duration of room temperature and refrigerated storage and to establish the experimental design (Table 1). All analyses were done in triplicate.

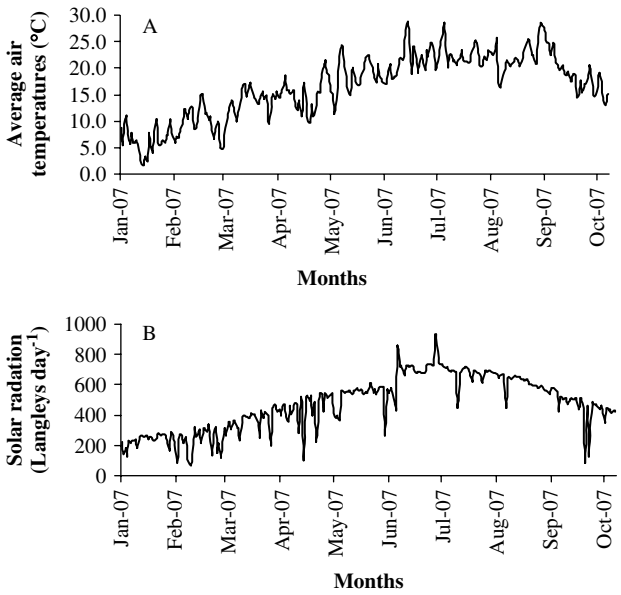

Figure 1. (A) Average air temperature and (B) solar radiation recorded during growth and harvest period of *Cassia tora* and *Corchorus tridens* (adapted from http://wwwcimis.water.ca.gov/cimis/frontDailyReport.do).

Storage conditions

The leafy vegetables were stored in plastic bags as described above and then placed in cardboard boxes in the dark under room temperature (20 °C), refrigerated (4 °C) and frozen (−18 °C) storage conditions. The room temperature storage took place in the laboratory, while the refrigerated and frozen storage took place in walk-in temperature-controlled rooms in the Department of Food Science and Technology, UC Davis. These rooms were continuously monitored by time–temperature control charts, and temperatures during the storage period fluctuated by approximately ±1.5 °C.

Sample preparation

For all analyses except texture, colour and cooking treatments, in which whole leaves were used, the fresh leafy vegetables were finely chopped using a cutting board and a knife immediately prior to analysis. For cooking evaluation, approximately 2 g of leaves were added to a beaker containing 50 mL of distilled water. The beaker was covered with aluminium foil to minimise vapour loss. The leaves were cooked by placing the beaker on a stirred heating plate (Model 51 450 Series, Cole & Parmer, Vernon Hills, IL, USA). It took approximately 10 min for the water to boil, and the leaves were boiled vigorously for 10 min, giving a total cooking time of 20 min. The cooked leaves were allowed to drain on a sieve at room temperature for 5 min following cooking. The drained water was collected in a beaker and also analysed. This cooking method was selected because it is typical of domestic cooking practices used in Africa.

Table 1. Experimental design for *Cassia tora* and *Corchorus tridens* storage studies

Room temperature storage			Refrigerated temperature storage			Frozen temperature storage		
Temp. (°C)	No. of days	Sampling days	Temp. (°C)	No. of days	Sampling days	Temp. (°C)	No. of days	Sampling days
20	6	0, 2, 4, 6	4	14	0, 2, 4, 6, 8, 10, 12, 14	−18	90	0, 30, 60, 90

Leafy vegetable analysis

Moisture

Moisture content was analysed using a Mettler Toledo HR 83 Halogen moisture analyser (Metler Toledo, Columbus, OH, USA). A clean aluminium dish was placed in the moisture analyser and tared. Approximately 2 g of finely chopped leaves were weighed into the aluminium dish and evenly distributed and the moisture content was determined by heating at 140 °C for 7–8 min. The analysis was carried out in triplicate.

Colour measurement

A Minolta CR-200 chromameter (Minolta Co., Ramsey, NJ, USA) was used to measure *L*, *a* and *b* values of the leaves. In the CIE colour system, positive *a* values describe the intensity of red colour, positive *b* values describe the intensity of yellow colour and the *L* value describes lightness (black = 0, white = 100). The chromameter was calibrated using an appropriate standard tile that most resembled the sample colour. The light projection aperture was positioned directly on top of the leaves, which were placed on a flat surface. One measurement was taken per leaf, to the right of the main vein. Samples were analysed in triplicate and 15 readings were taken for each replicate.

Total chlorophyll

This method was based on the work of Arnon.[6] The concentration of total chlorophyll was determined by measuring the absorption of 800 mL L^{-1} acetone chlorophyll extracts with a Shimadzu UV-2101PC spectrophotometer (Shimadzu, Columbia, MD, USA) at 652 nm in a 10 mm cuvette.

Approximately 2 g of finely chopped leaves were homogenised (Brinkmann Polytron PT 3000, Kinematica AG, Bohemia, NY, USA) in 20 mL of distilled water for 1 min at 28 000 rpm. Then 1–2 g of the homogenate was weighed into a 50 mL conical flask covered with Parafilm 'M' laboratory film (Pechiney Plastic Packaging, Chicago, IL, USA) and extracted with 20 mL of 800 mL L^{-1} acetone for 4–5 h in the dark at 4 °C with intermittent stirring every hour.

The extracted samples were centrifuged at 16.1 × *g* for 5 min in an IEC Centra CL 2 centrifuge (International Equipment Company, Waltham, MA, USA) and the absorbance of the clear supernatant was read at 652 nm using the Shimadzu UV-2101PC spectrophotometer. The following formula was used for the calculation of total chlorophyll based on the study by Arnon:[6]

$$\text{total chlorophyll (mg L}^{-1}) = D_{652} \times 1000/34.5$$

where D_{652} is the absorbance at 652 nm and 34.5 is the value of the specific absorption coefficient at 652 nm.

Texture

Texture measurements were carried out using a puncture test based on the method of Read and Sanson.[7] Frozen leaves were not analysed for texture owing to their fragile nature following freezing. Texture was measured with a TA-XT2 texture analyser (Version 2.64, Stable Microsystems, Scarsdale, NY, USA). The test was performed to 90% deformation of the original thickness (L_o) and the test speed was set to 1 mm s^{-1}. The maximum force, peak area and gradient were measured for each leaf. Strength is defined as the maximum force to fracture per unit area over which the force is applied. Toughness is defined as the work to fracture, measured as the area under the force–displacement curve. Stiffness is related to the initial slope of the curve (gradient), or stress per unit strain.[7] One measurement was taken per leaf, to the right of the main vein. Samples were analysed in triplicate and 15 readings were taken for each replicate.

Total phenolics

Total phenolic concentration was measured using the Folin–Ciocalteu assay.[8] Approximately 2 g of weighed sample was combined with 20 mL of distilled water and homogenised using the Brinkmann Polytron PT 3000 homogeniser (Kinematica AG) for approximately 1 min. Polyphenols were extracted from the homogenised sample by adding 2 parts acetone/water (1 : 1 v/v) to 1 part sample. The sample mixture was then vortexed for 1 min and allowed to stand for 10 min. After extraction the sample was centrifuged at 16.1 × *g* for 5 min using the IEC Centra CL 2 centrifuge.

A 100 µL aliquot of extract was added to 1.2 mL of 0.1 mol L^{-1} Folin–Ciocalteu reagent and the mixture was vortexed and allowed to stand for 2 min. A 2 mL aliquot of 75 g L^{-1} sodium carbonate was then added and the mixture was vortexed and incubated in a dry bath incubator (Fisher Scientific, Pittsburgh, PA, USA) for 5 min at 50 °C for colour development. After 5 min the mixture was allowed to cool to room temperature. The absorbance was read at 760 nm using the Shimadzu UV-2101PC spectrophotometer.

Ascorbic acid

Ascorbic acid and dehydroascorbic acid were determined based on the work of Zapata and Dufour.[9] Approximately 3 g of weighed sample, either fresh or cooked, was homogenised in 20 mL of 20 g L^{-1} oxalic acid for 1 min using the Brinkmann Polytron PT 3000 homogeniser (Kinematica AG). The homogenate was centrifuged at 16.1 × *g* for 10 min using an Eppendorf Model 5415D centrifuge (Eppendorf, Westbury, NY, USA). Supernatants were diluted with 20 g L^{-1} oxalic acid depending on their ascorbic acid content in the following ratios: for fresh leafy vegetables, 200 µL of supernatant was added to 400 µL of 20 g L^{-1} oxalic acid; for drained cooking water, 300 µL of supernatant was added to 300 µL of 20 g L^{-1} oxalic acid; for cooked leafy vegetables, 600 µL of supernatant was used without dilution.

All extracts were derivatised in order to determine their dehydroascorbic acid content, by adding 200 µL of 1,2-phenylenediamine (OPDA) to the 600 µL samples for 30 min in the dark before they were loaded onto a high-performance liquid chromatography (HPLC) system. Analysis was performed using a Shimadzu LC-10AD HPLC pump with 1300 psi back pressure. Reverse phase separation was attained using a Phenomenex Luna C18 (2) 100A HPLC column (150 mm × 4.6 mm, 5 µm) with a Phenomenex Luna C18 guard column (4 mm × 3 mm i.d.) (Phenomenex, Torrance, CA, USA). The mobile phase was 50 mL L^{-1} methanol with 5 mmol L^{-1} hexadecyltrimethylammonium bromide and 20 mmol L^{-1} potassium dihydrogen phosphate and the pH was adjusted to 4.59 using an Accumet Basic pH meter (Model AB 15, Fisher Scientific). The flow rate was maintained at 1.2 mL min^{-1} and the injection volume was 5 µL. A Shimadzu SPD-M10AVP diode array detector was used to detect ascorbic acid at 261 nm and dehydroascorbic acid at 348 nm.

Minerals

The leafy vegetable samples were randomly selected on the day of harvest and dried in a Fisher Senior Isotemp Oven (Fisher Scientific) at 65 °C for 3 days. The dry samples were ground into a fine powder with a mortar and pestle, and 100–125 mg of the ground sample

was weighed into a crucible and subjected to ashing in a muffle furnace (Model 126, Fisher Scientific) at 500 °C for 4 h. After ashing, the residue was dissolved in 2 mL of 0.5 mol L^{-1} HNO_3 and this solution was heated at 80 °C for 10 min. After being cooled to room temperature, the solution was filtered through Whatman No. 1 filter paper (Whatman, Piscataway, NJ, USA), and hot water (100 °C) was used for washing the filter paper to make up the volume of the filtrate to 10 mL. This solution was evaluated by the Division of Agricultural and Natural Resources (DANR) analytical laboratory at UC Davis for its content of calcium, zinc and iron. Inductively coupled plasma atomic emission spectroscopy (ICP-AES) was used for the detection of minerals.

Statistical analysis

All data points represent the mean ± standard error of three replicates. Analysis of variance (ANOVA) followed by Tukey tests with a significance level of $P < 0.05$ were performed on the data using SAS Version 6.21 software (SAS Institute, Inc., Cary, NC, USA).

RESULTS AND DISCUSSION

Quality and nutritional losses at 20 °C

Moisture content of the leafy vegetables stored at 20 °C did not change significantly (Table 2). Any changes in moisture may have been due to the biological variations in the leaves.

The *L* value increased significantly with storage time ($P < 0.05$), indicating a lightening in the colour of both leafy vegetable cultivars. The *b* value of *C. tora* leaves showed significant increases over storage time in study 1, indicating an increase in yellow colour, but *b* values did not change significantly in study 2. There was no significant change in the green or yellow colour of room temperature stored *C. tridens* leaves; however, at the end of storage, visual observation of the leaves found yellow to brown patches on the surface.

Initial chlorophyll contents of *C. tora* leaves at harvest in the two studies were 3.7 and 2.5 mg g^{-1} wet weight (WW) respectively, while those of *C. tridens* leaves were 2.9 and 2.8 mg g^{-1} WW respectively. The difference in initial chlorophyll content between the two studies of *C. tora* leaves may have been due to the different seasons in which the plants were grown and harvested. The higher chlorophyll content in study 1 can be related to the higher temperatures and solar radiation recorded during the growth and harvest period (Fig. 1). Earlier studies reported higher accumulation of chlorophyll in spinach grown at higher temperatures than in spinach grown under cooler conditions.[10]

Chlorophyll concentrations were significantly higher at harvest than after storage at 20 °C for 4 or 6 days (Fig. 2A). The retention of chlorophyll in leaves of both cultivars stored at 20 °C was approximately 92–97%. Declines in chlorophyll content have

Table 2. Quality parameters measured in replicate studies of (A) *Cassia tora* and (B) *Corchorus tridens* stored at room temperature (20 °C)

A

	Study 1 storage time (days)			Study 2 storage time (days)			
Quality parameter	0	2	4	0	2	4	6
Moisture (%)	75.2 ± 0.1a	76.3 ± 0.4a	77.2 ± 0.4a	80.2 ± 0.4a	80.8 ± 0.5a	81.3 ± 0.05a	81.0 ± 0.1a
Colour							
L	34.0 ± 0.1a	36.2 ± 0.3b	40.2 ± 1.1b	43.9 ± 0.3a	44.1 ± 0.1a	46.7 ± 0.5b	45.8 ± 0.8b
a	−10.2 ± 0.0a	−10.2 ± 0.3a	−10.3 ± 0.4a	−10.6 ± 0.0a	−10.3 ± 0.1a	−10.9 ± 0.7a	−10.0 ± 0.4a
b	7.4 ± 0.1a	8.1 ± 0.2b	10.9 ± 1.5b	15.4 ± 1.0a	13.0 ± 0.3a	14.8 ± 1.9a	15.2 ± 0.7a
Texture							
Force (N)	1.6 ± 0.0a	1.9 ± 0.0b	1.9 ± 0.1b	2.1 ± 0.0a	2.9 ± 0.1b	2.9 ± 0.1b	2.8 ± 0.1b
Area (N mm)	1.2 ± 0.0a	1.4 ± 0.1b	1.4 ± 0.1b	2.2 ± 0.1a	3.3 ± 0.3b	3.0 ± 0.1b	2.8 ± 0.2b
Gradient (N mm^{-1})	1.0 ± 0.0a	1.0 ± 0.1b	1.0 ± 0.1b	0.8 ± 0.0a	1.0 ± 0.1b	1.0 ± 0.0b	1.0 ± 0.0b

B

	Study 1 storage time (days)				Study 2 storage time (days)			
Quality parameter	0	2	4	6	0	2	4	6
Moisture (%)	75.6 ± 0.2a	79.0 ± 0.7a	78.7 ± 1.0b	77.8 ± 0.3b	78.4 ± 0.2a	78.7 ± 0.7a	79.3 ± 1.2a	78.9 ± 0.6a
Colour								
L	40.6 ± 0.7a	41.8 ± 0.1a	43.7 ± 1.0b	45.0 ± 0.2b	45.2 ± 0.6a	45.2 ± 0.3a	48.4 ± 0.6b	48.6 ± 0.5b
a	−14.6 ± 0.9a	−13.8 ± 0.1a	−14.0 ± 1.1a	−13.8 ± 0.3a	−14.3 ± 0.3a	−12.7 ± 0.6a	−14.1 ± 0.6a	−13.2 ± 0.7a
b	15.4 ± 0.9a	15.01 ± 0.2a	15.7 ± 1.8a	16.2 ± 0.6a	16.0 ± 1.0a	16.0 ± 0.3a	17.2 ± 0.9a	17.3 ± 1.3a
Texture								
Force (N)	2.1 ± 0.1a	2.5 ± 0.0a	2.3 ± 0.1a	2.3 ± 0.2a	1.8 ± 0.0a	2.3 ± 0.1a	1.8 ± 0.0a	2.1 ± 0.1a
Area (N mm)	2.3 ± 0.2a	3.0 ± 0.2b	2.9 ± 0.2b	2.8 ± 0.4b	2.0 ± 0.1a	2.9 ± 0.4b	1.8 ± 0.1a	2.3 ± 0.1a
Gradient (N mm^{-1})	0.9 ± 0.0a	0.9 ± 0.0a	0.8 ± 0.1a	0.8 ± 0.1a	0.6 ± 0.0a	0.7 ± 0.0a	0.7 ± 0.0a	0.7 ± 0.1a

Means followed by the same letter in each group are not significantly different ($P > 0.05$).

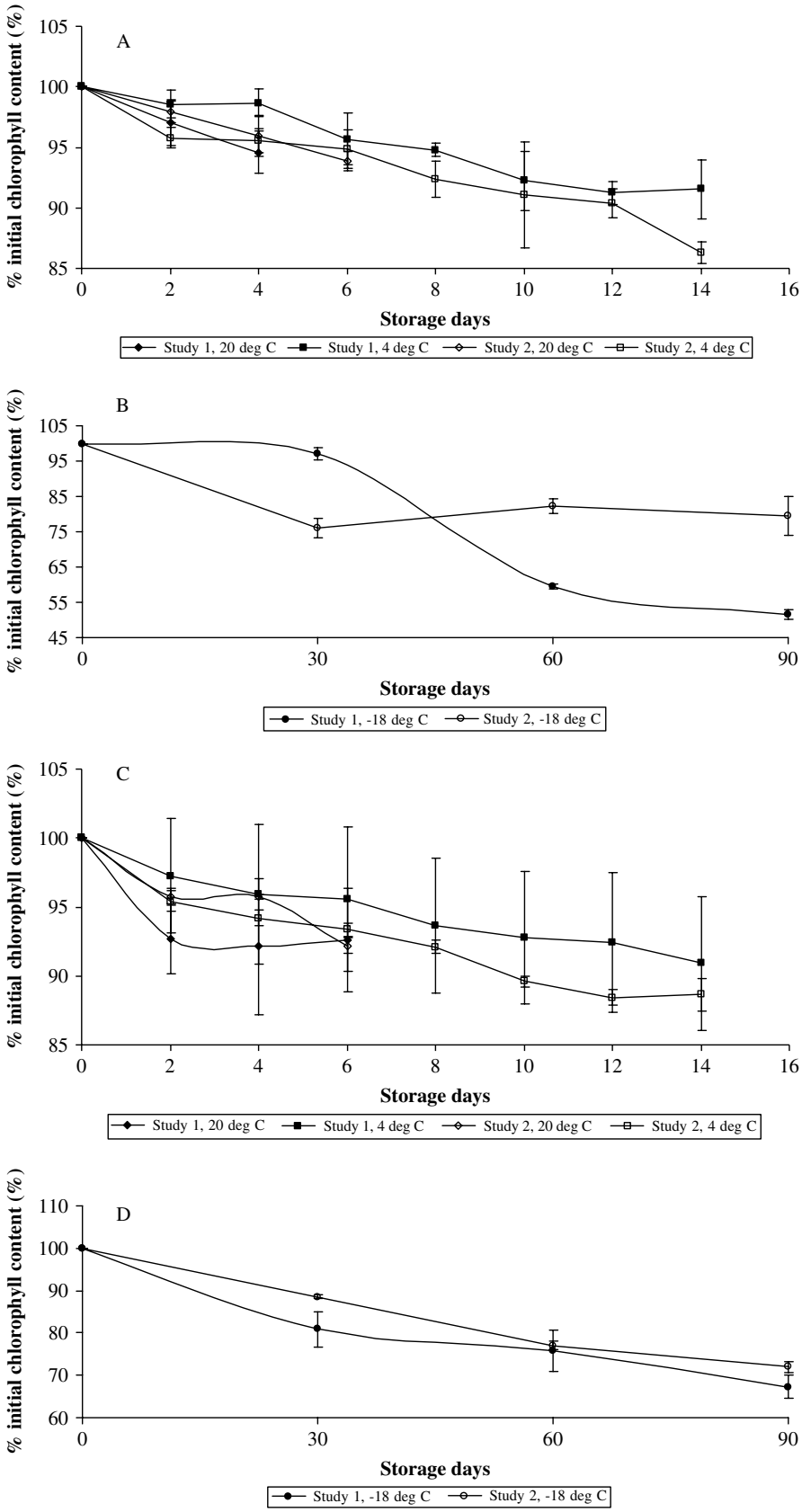

Figure 2. Percentage change in chlorophyll content (mg g^{-1} wet weight) of leaves in replicate studies of (A) *Cassia tora* stored at room temperature (20 °C) and refrigerated temperature (4 °C), (B) *Cassia tora* stored at frozen temperature (−18 °C), (C) *Corchorus tridens* stored at room temperature (20 °C) and refrigerated temperature (4 °C) and (D) *Corchorus tridens* stored at frozen temperature (−18 °C).

been observed in many vegetables on storage. Wang *et al*.[11] reported a similar value of 94.3% retention of chlorophyll content after storage of asparagus in air at room temperature for 11 days. Greater losses (as low as 35% retention) were recorded by Ferrante and Maggore[12] during storage of *Valeriana* leaves at 10 °C for 15 days. Vina and Chaves[13] observed only 50% retention of total chlorophyll in fresh-cut celery stored for 1 week at 10 °C, a temperature lower than that used in the present study. The lower retention of chlorophyll reported in those studies may be due to the lower initial chlorophyll contents of *Valeriana* leaves (1.75 μg g^{-1}) and fresh-cut celery (115 μg g^{-1}) in comparison with those of *C. tora* and *C. tridens*. The samples in the present study were stored for a shorter duration, i.e. 4–6 days, and the leaf surface area was lower than that of the other leafy vegetables studied. Another reason for the differences may be that the leafy vegetables utilised in the present study were grown in a university greenhouse on site and were analysed and stored immediately after harvest, which was not the case with the other published studies.

Texture profiles were analysed for peak force, area and gradient or slope (Table 2). In *C. tora* leaves, peak force increased significantly in both studies from freshly harvested leaves to those stored at 20 °C for 2 days ($P < 0.05$) but did not change further during storage. Area measurements, which correlate with toughness, and gradient measurements, which indicate tissue stiffness, showed exactly the same trends as peak force, changing significantly in the time period between harvest and 2 days of storage ($P < 0.05$). Interestingly, there were no significant changes in peak force or gradient values in *C. tridens* leaves, but there was a significant change in area measurements.

The increases in leaf firmness, toughness and stiffness that occurred within 2 days of harvest may be attributed to an increase in the degree of lignification of the tissue. Immature tissues with high respiratory rates often exhibit hardening and lignification during storage.[13] It has also been observed that lignin biosynthesis constitutes one of the defence mechanisms that may be activated in plants facing injury or spoilage.[14] The fact that *C. tridens* leaves did not change in textural attributes during storage indicates that these leaves may have lower rates of respiration or may for some other reason not undergo as much lignification.

Total phenolic contents of *C. tora* leaves in the two studies at harvest were 12.7 and 8.5 mg gallic acid equivalent (GAE) g^{-1} WW respectively, while those of *C. tridens* leaves were 8.2 and 11.2 mg GAE g^{-1} WW respectively. The concentration of total phenolics in both cultivars was significantly higher after storage in comparison with the initial content at harvest (Fig. 3). The increase in total phenolics after 4 or 6 days of storage at 20 °C was 24–25% in *C. tora* and 20% in both studies of *C. tridens*.

Research over the past few years has indicated that an increase in total phenolics in plants postharvest is catalysed by various abiotic and biotic stresses such as exposure to sunlight, insect and pathogen attack, nutrient deficiency and temperature variations during harvesting and storage.[15] Total phenolic accumulation also occurs as a result of postharvest senescence.[16] This may explain the increase in total phenolic content during storage in the present study.

Other researchers have also reported postharvest increases in total phenolics. A 12.2% increase in total phenolic content was recorded by Leja *et al*.[16] in broccoli stored at 20 °C for 3 days. Ferrante and Maggore[12] observed a 13% increase in total phenols in *Valeriana* leaves stored for 5 days at 10 °C. The slightly higher levels of total phenolics measured in the present storage study may be due to differences in the stresses to which each plant was exposed at harvest and during postharvest handling and storage.

Ascorbic acid contents of *C. tora* leaves at harvest were 2.6 and 2.8 mg g^{-1} WW in studies 1 and 2 respectively, while that of *C. tridens* leaves was 1.2 mg g^{-1} WW in both studies. The concentrations were significantly higher at harvest than after storage (Fig. 4). The retention of ascorbic acid in *C. tora* leaves was between 50 and 71%, while that in *C. tridens* leaves was between 62 and 66%.

It has been well documented that ascorbic acid levels frequently decline in harvested fruits and vegetables during storage.[17–20] Such losses can be due to a number of factors, such as oxidation, pH changes, relative humidity, ascorbic acid oxidase activity and temperature during storage.[21] A review of the literature illustrates that other investigators have found ascorbic acid to be both more and less stable in similar leafy vegetables. Favell[22] observed greater losses, e.g. only 10% retention in spinach within 3 days of storage at 20 °C, the same temperature as evaluated in this study. Yadav and Sehgal[23] determined that in spinach stored at 30 °C for 24 and 48 h there was 55.4 and 35.0% retention of ascorbic acid respectively. The same authors also observed 44.8 and 34.1% ascorbic acid retention in amaranth leaves stored at 30 °C for 24 and 48 h respectively. Watada *et al*.[24] observed 46% ascorbic acid retention in green beans stored at 20 °C for 6 days, 78% retention in spinach stored at 20 °C for 4 days and 75% retention in bell peppers stored at 20 °C for 4 days. The differences in the rate of loss of ascorbic acid between the present study and other studies may be due to differing vulnerabilities of the vegetables investigated, e.g. surface area and mechanical damage, differing ascorbate oxidase enzyme activities or, to a certain extent, differences in storage temperature. Conditions pre- and postharvest also affect the rate of loss as well as the initial ascorbic acid content, which was higher in the present study than in other studies. As stated earlier, another reason for the differences may be that the leafy vegetables utilised in the present study were grown in a university greenhouses on site and were analysed and stored immediately after harvest, which was not the case with the other published studies.

The contents of calcium, zinc and iron in *C. tora* leaves were 28.3 ± 19.4, 27.4 ± 1.2 and 99.0 ± 4.5 mg kg^{-1} respectively in study 1 and 38.8 ± 25.8, 16.9 ± 0.3 and 77.7 ± 4.7 mg kg^{-1} respectively in study 2, while those in *C. tridens* leaves were 21.2 ± 74.5, 27.3 ± 0.4 and 103.3 ± 6.0 mg kg^{-1} respectively in study 1 and 20.8 ± 119.7, 21.9 ± 0.6 and 86.4 ± 1.1 mg kg^{-1} respectively in study 2. Cooking resulted in 20–62.3% loss of calcium, 13–27% loss of zinc and 3–11% loss of iron, which may be attributed to leaching of these nutrients into the cooking water. In this study the cooking water was not analysed for minerals. However, previous studies on cooking of tropical root crops from the South Pacific[25] have reported that, if the recipe includes retention of the cooking water, as in preparing a soup or a stew, the loss of these minerals is minimised.

Quality and nutritional losses at 4 °C

Changes in quality and nutrient content were much less pronounced in the leafy vegetables stored at 4 °C than in those stored at 20 °C. Moisture content did not change significantly (Table 3) during storage at 4 °C in either study 1 or study 2. Similar results were obtained by Yoko *et al*.,[26] who studied three leafy vegetables, namely spinach, komatsuna and kaiware. When these leafy vegetables were stored at 4 °C for 14 days, there were no significant changes in their moisture content.

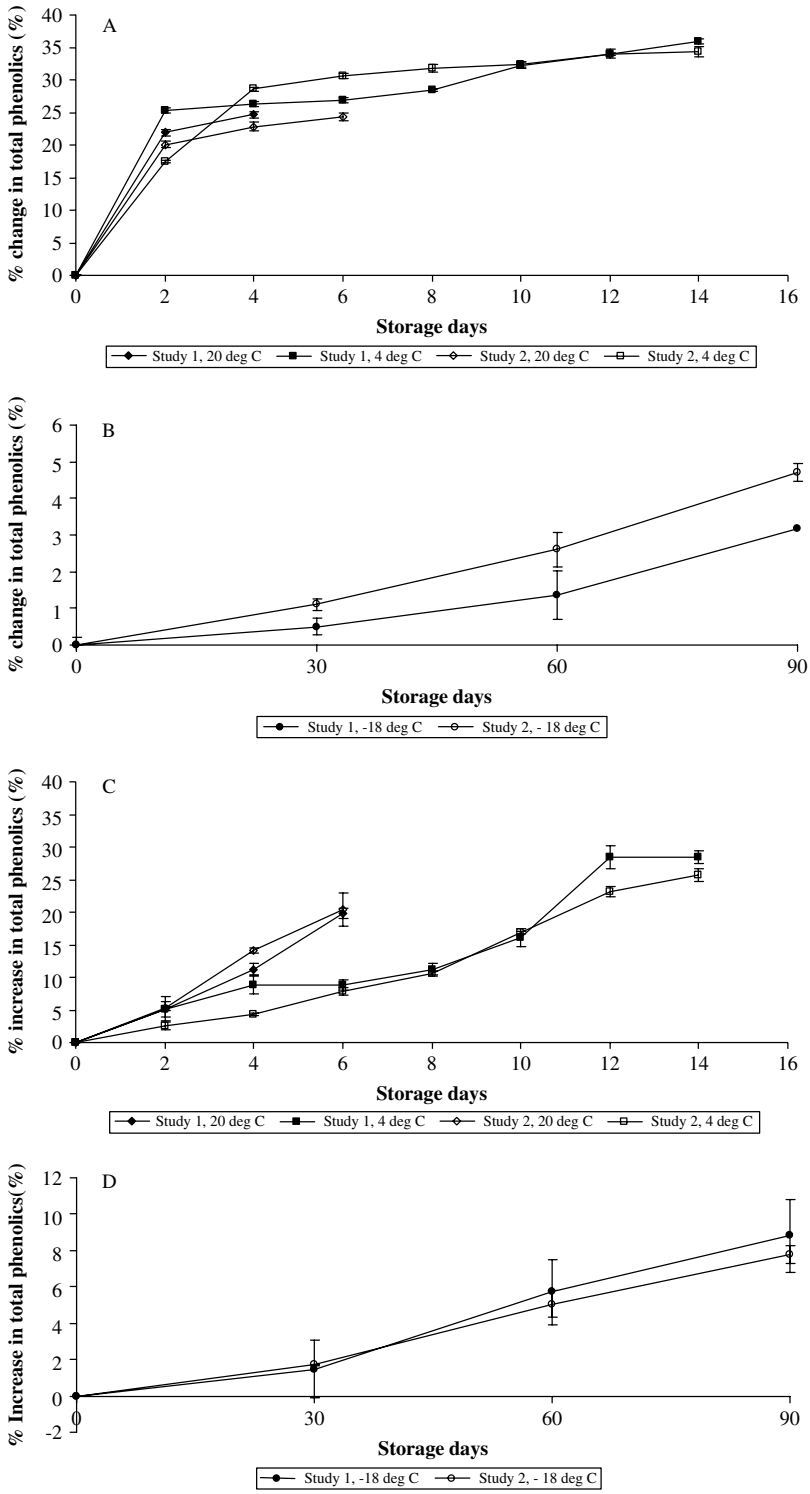

Figure 3. Percentage change in total phenolic content (mg gallic acid equivalent g^{-1} wet weight) of leaves in replicate studies of (A) *Cassia tora* stored at room temperature (20 °C) and refrigerated temperature (4 °C), (B) *Cassia tora* stored at frozen temperature (−18 °C), (C) *Corchorus tridens* stored at room temperature (20 °C) and refrigerated temperature (4 °C) and (D) *Corchorus tridens* stored at frozen temperature (−18 °C).

Colour parameters (*L*, *a* and *b*) did not change significantly during storage of either *C. tora* or *C. tridens* leaves. However, at the end of storage, visual inspection of the leaves showed a few yellow to brown patches on the surface.

Chlorophyll concentrations were significantly higher at harvest than after storage at 4 °C for 14 days (Fig. 2) in both studies. *Cassia tora* leaves retained between 86 and 91% and *C. tridens* leaves between 89 and 91% of their initial chlorophyll content.

Chlorophyll losses have been reported by Ferrante and Maggore,[12] who found 78% retention of chlorophyll after storage of *Valeriana* leaves at 4 °C for 15 days. Bergquist *et al.*[10] reported

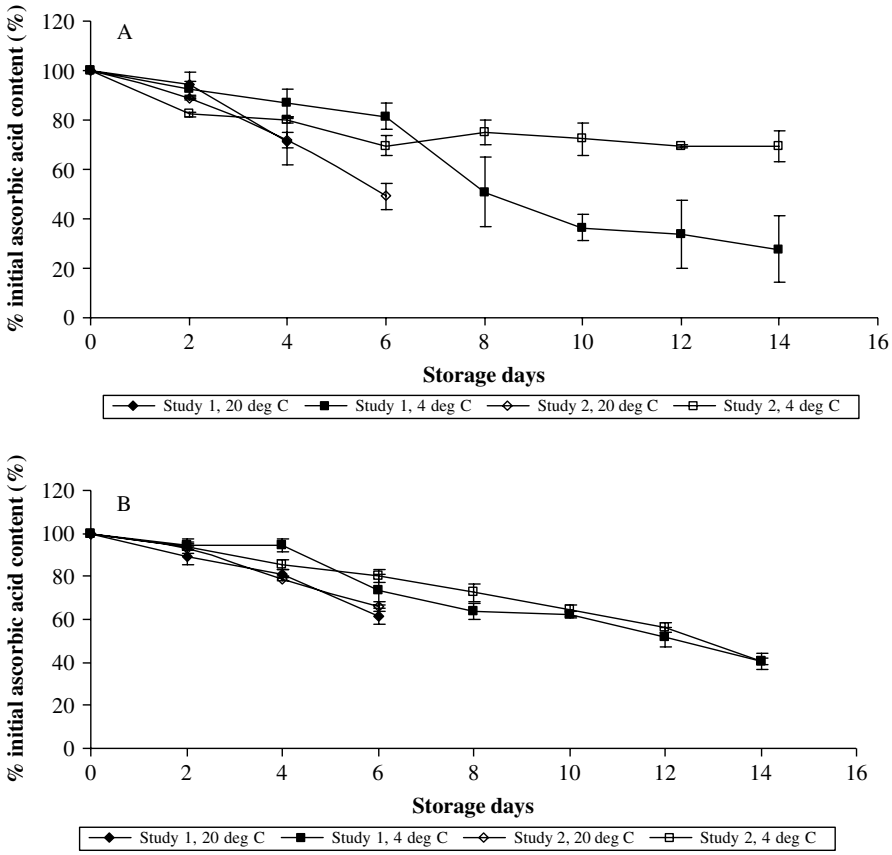

Figure 4. Percentage change in ascorbic acid content (mg g^{-1} wet weight) of leaves in replicate studies of (A) *Cassia tora* stored at room temperature (20 °C) and refrigerated temperature (4 °C) and (B) *Corchorus tridens* stored at room temperature (20 °C) and refrigerated temperature (4 °C).

81 and 89% retention, similar to the present study, in baby spinach harvested and stored at 2 °C for 9 days in June and July respectively. This was a lower storage temperature than that used in the present study, which should result in greater retention of chlorophyll. The slightly higher chlorophyll levels observed in the present study may result from greenhouse production on site and rapid analysis following harvest, which was not the case with the other studies cited.

Texture, i.e. peak force, area and gradient or slope, did not change significantly in either *C. tora* or *C. tridens* leaves during storage at 4 °C. This is in contrast to the changes observed in *C. tora* leaves stored at 20 °C, which toughened significantly. Total phenolic concentration increased significantly during storage at 4 °C (Fig. 3). The increase in total phenolics after 14 days was between 34 and 36% in *C. tora* leaves and between 26 and 28% in *C. tridens* leaves. Zhang and Hamauza[27] also reported a relatively large increase of 23.3–25.0% in total phenolics in iceberg lettuce stored at 4 °C for 14 days, which is similar to our results. A study by Ferrante and Maggore,[12] however, found only a 5.0% increase in total phenols in *Valeriana* leaves stored for 10 days at 4 °C. These relatively smaller increases in total phenolics compared with those determined in the present study may be due to differences in the stresses to which each plant was exposed at harvest or during postharvest handling.

Ascorbic acid concentrations were significantly higher at harvest than after storage at 4 °C for 14 days (Fig. 4). *Cassia tora* leaves retained between 28 and 69% and *C. tridens* leaves between 40 and 41% of their initial ascorbic acid content during storage. Favell[22] reported only 20% retention of ascorbic acid in spinach stored under refrigerated temperature (4 °C) for 7 days. Howard *et al.*[28] reported 87% retention of ascorbic acid in broccoli stored at 4 °C for 21 days. The differences in ascorbic acid retention observed between the present study and other sudies may be due to several factors, such as conditions under which the produce was harvested and analysed, exposure to heat and/or oxygen or perhaps higher or lower ascorbate oxidase activity in the vegetable. Although enzyme activity was not analysed in the present experiment, greater losses may be attributed in part to higher enzyme activity.

Quality and nutritional losses at −18 °C

Moisture content of the leafy vegetables stored at −18 °C for up to 90 days did not change significantly in either study 1 or study 2 (Table 4). Similar results were obtained by Labib *et al.*[29] during storage of Jew's mallow and spinach at −18 °C for 3 months. Colour parameters (*L*, *a* and *b* values) also did not change significantly during frozen storage of *C. tora* and *C. tridens* leaves.

Chlorophyll concentrations were significantly higher at harvest than after storage at −18 °C for 90 days (Fig. 2). *Cassia tora* leaves retained between 52 and 80% and *C. tridens* leaves between 67 and 72% of their initial chlorophyll content at various times during storage.

Lisiewska and Gebczynski[30] reported 78.7% retention of chlorophyll in kale and 78.5% in spinach stored at −20 °C for 4 months; these results are similar to those observed in study

2 of the present project. Martins and Silva[31] observed only 10% retention of chlorophyll content in green beans stored at −18 °C for 60 days. Lisiewska and Kmiecik[32] reported 80–85% chlorophyll retention in parsley stored at −18 °C for 9 months. Several authors have suggested that the efficient elimination of enzymes by blanching or cooking prior to freezing results in good retention of chlorophylls during frozen storage.[29,32,33] However, other researchers have found no effect of blanching on loss of chlorophyll.[30,32]

Although chlorophylls were degraded under both refrigerated and frozen storage, the colour of the leaves seemed to be preserved better than with room temperature storage. One possible explanation for this phenomenon might be the formation of metal (copper or zinc)–chlorophyll compounds, which retain a

Table 3. Quality parameters measured in replicate studies of (A) *Cassia tora* and (B) *Corchorus tridens* stored at refrigerated temperature (4 °C)

A

Quality parameter	Storage time (days) 0	2	4	6	8	10	12	14
Moisture (% w/w)								
Study 1	75.2 ± 0.1a	76.5 ± 0.5a	76.6 ± 0.2a	76.4 ± 0.2a	76.5 ± 0.2a	75.5 ± 0.3a	77.2 ± 0.3a	76.5 ± 0.3a
Study 2	80.2 ± 0.4a	78.3 ± 0.5a	79.4 ± 0.1a	80.3 ± 0.4a	80.4 ± 0.5a	79.3 ± 0.2a	81.0 ± 0.1a	78.0 ± 0.7a
Colour								
L								
Study 1	34.0 ± 0.1a	36.8 ± 0.8ab	38.8 ± 0.3b	39.7 ± 0.3b	40.3 ± 0.5b	38.9 ± 0.6b	39.9 ± 0.2b	39.7 ± 0.4b
Study 2	43.9 ± 0.3a	44.6 ± 0.6ab	44.2 ± 0.5ab	45.3 ± 0.3b	44.1 ± 0.1ab	45.9 ± 0.5b	44.3 ± 0.6ab	44.9 ± 0.9b
a								
Study 1	−10.2 ± 0.0ab	−9.4 ± 0.3ab	−8.5 ± 0.2ab	−8.5 ± 0.0ab	−8.9 ± 0.6ab	−10.5 ± 0.5a	−9.0 ± 0.5bc	−8.2 ± 0.6c
Study 2	−10.6 ± 0.0a	−12.8 ± 0.8a	−12.7 ± 0.4a	−12.1 ± 0.1a	−11.3 ± 0.5a	−11.4 ± 0.3a	−10.2 ± 1.0a	−8.2 ± 0.6a
b								
Study 1	7.4 ± 0.1a	8.3 ± 0.4bc	7.9 ± 0.2abc	7.6 ± 0.2abc	8.4 ± 0.6abc	8.3 ± 0.6abc	7.5 ± 0.4abc	6.7 ± 0.5c
Study 2	15.4 ± 0.9a	12.7 ± 1.4a	10.8 ± 2.3a	13.7 ± 0.6a	14.1 ± 0.6a	13.7 ± 0.3a	10.8 ± 2.0a	10.7 ± 0.6a
Texture								
Force (N)								
Study 1	1.6 ± 0.0a	1.7 ± 0.1a	2.1 ± 0.1b	1.9 ± 0.0b	1.9 ± 0.0b	2.2 ± 0.1b	1.6 ± 0.2a	1.6 ± 0.1a
Study 2	2.1 ± 0.0a	2.5 ± 0.3a	2.6 ± 0.2b	2.6 ± 0.2b	1.9 ± 0.0b	1.7 ± 0.1b	2.0 ± 0.1a	2.1 ± 0.1a
Area (N mm)								
Study 1	1.1 ± 0.0a	1.2 ± 0.2ab	0.8 ± 0.1ab	1.5 ± 0.0ab	1.5 ± 0.0ab	1.9 ± 0.1ab	1.3 ± 0.2ab	1.1 ± 0.1ab
Study 2	2.2 ± 0.1ab	3.4 ± 0.5a	2.6 ± 0.1a	2.5 ± 0.2a	1.7 ± 0.0ab	1.4 ± 0.1ab	1.2 ± 0.1ab	1.0 ± 0.1b
Gradient (N mm^{-1})								
Study 1	0.9 ± 0.0a	0.8 ± 0.1a	1.0 ± 0.1a	1.0 ± 0.0a	0.9 ± 0.0a	0.9 ± 0.0a	0.8 ± 0.1a	0.9 ± 0.1a
Study 2	0.8 ± 0.0abc	0.9 ± 0.1ab	0.9 ± 0.0a	0.9 ± 0.1a	0.8 ± 0.0ab	0.8 ± 0.0abc	0.6 ± 0.0bc	0.5 ± 0.0c

B

Quality parameter	Storage time (days) 0	2	4	6	8	10	12	14
Moisture (% w/w)								
Study 1	75.6 ± 0.2a	76.9 ± 0.1a	76.5 ± 0.4a	78.8 ± 0.7a	78.7 ± 0.8a	78.9 ± 0.9a	79.2 ± 0.9a	79.8 ± 0.3a
Study 2	78.4 ± 0.2a	81.6 ± 0.1a	79.4 ± 1.0a	78.5 ± 0.5a	79.0 ± 0.7a	79.0 ± 0.7a	78.9 ± 1.2a	78.8 ± 0.1a
Colour								
L								
Study 1	40.6 ± 0.7a	41.3 ± 0.1b	43.6 ± 0.3b	45.5 ± 0.6b	44.1 ± 0.7b	42.5 ± 0.9b	42.6 ± 1.0b	42.6 ± 0.4b
Study 2	45.2 ± 0.6a	46.3 ± 0.2b	46.5 ± 0.8b	46.1 ± 0.4b	46.2 ± 0.7b	47.0 ± 1.3b	47.2 ± 1.4b	47.0 ± 0.4b
a								
Study 1	−14.6 ± 0.9a	−12.8 ± 0.1ab	−12.7 ± 0.4ab	−12.1 ± 0.1ab	−11.3 ± 0.5b	−11.4 ± 0.3b	−10.1 ± 1.0b	−8.2 ± 0.6b
Study 2	−14.3 ± 0.4a	−13.7 ± 0.1ab	−12.4 ± 0.4ab	−12.2 ± 1.6ab	−12.9 ± 0.1b	−9.8 ± 0.8b	−8.9 ± 0.1b	−9.1 ± 0.0b
b								
Study 1	15.4 ± 0.9a	15.7 ± 1.4a	15.8 ± 2.3a	16.7 ± 0.6a	16.1 ± 0.6a	16.7 ± 0.3a	16.8 ± 2.0a	16.7 ± 0.5a
Study 2	16.0 ± 1.0a	16.0 ± 0.3a	16.5 ± 0.5a	16.9 ± 1.7a	17.2 ± 0.7a	17.1 ± 1.1a	17.7 ± 0.3a	17.7 ± 0.0a

(*continued overleaf*)

Table 3. (*Continued*)

B

Quality parameter	Storage time (days) 0	2	4	6	8	10	12	14
Texture								
Force (N)								
Study 1	2.1 ± 0.1ab	2.5 ± 0.2a	2.6 ± 0.2a	2.3 ± 0.1a	1.9 ± 0.1ac	1.8 ± 0.1bc	1.2 ± 0.1c	1.3 ± 0.1c
Study 2	1.8 ± 0.1ab	1.9 ± 0.1a	2.6 ± 0.2a	2.1 ± 0.2a	1.7 ± 0.1abc	1.5 ± 0.1bc	1.5 ± 0.1c	1.4 ± 0.0c
Area (N mm)								
Study 1	2.3 ± 0.2ab	2.8 ± 0.4a	0.9 ± 0.1a	2.7 ± 0.3a	2.0 ± 0.2abc	2.1 ± 0.1bc	1.3 ± 0.1c	1.4 ± 0.1c
Study 2	2.0 ± 0.1ab	2.3 ± 0.1a	0.6 ± 0.0a	2.5 ± 0.3a	1.9 ± 0.1abc	1.6 ± 0.1bc	1.6 ± 0.1c	1.3 ± 0.0c
Gradient (N mm^{-1})								
Study 1	0.8 ± 0.0a	0.9 ± 0.1a	0.9 ± 0.0a	0.9 ± 0.0a	0.8 ± 0.0a	0.7 ± 0.0ab	0.5 ± 0.0b	0.5 ± 0.0b
Study 2	0.6 ± 0.0a	0.6 ± 0.0a	0.9 ± 0.0a	0.7 ± 0.1a	0.6 ± 0.0a	0.6 ± 0.0a	0.6 ± 0.0a	0.6 ± 0.0a

Means followed by the same letter in each group are not significantly different ($P > 0.05$).

Table 4. Quality parameters measured in replicate studies of (A) *Cassia tora* and (B) *Corchorus tridens* stored at frozen temperature (−18 °C)

A

Quality parameter	Study 1 storage time (days) 0	30	60	90	Study 2 storage time (days) 0	30	60	90
Moisture (%)	75.2 ± 0.1a	75.2 ± 0.1a	75.8 ± 0.0a	76.3 ± 0.0a	80.2 ± 0.4a	80.9 ± 0.1a	80.6 ± 0.0a	80.5 ± 0.1a
Colour								
L	34.0 ± 0.0ab	36.0 ± 0.8ab	36.0 ± 0.1a	40.3 ± 0.3b	43.9 ± 0.3a	41.4 ± 1.0a	40.0 ± 0.8a	39.8 ± 0.5a
a	−10.2 ± 0.0a	−8.8 ± 0.4a	−8.3 ± 1.4a	−6.0 ± 0.4a	−10.6 ± 0.0a	−12.2 ± 0.9a	−11.1 ± 0.3a	−11.9 ± 0.6a
b	7.4 ± 0.1a	9.7 ± 1.2ab	10.8 ± 1.6b	6.8 ± 0.6ab	15.4 ± 0.9a	14.1 ± 0.7a	13.7 ± 0.1a	13.4 ± 0.9a
Ascorbic acid (mg g^{-1})	2.6 ± 0.1a	0.9 ± 0.0b	0.7 ± 0.0b	0.5 ± 0.0b	2.8 ± 0.0a	0.7 ± 0.1b	ND	ND
Dehydroascorbic acid (mg g^{-1} WW)	ND	0.1 ± 0.0a	0.1 ± 0.00a	1.6 ± 0.1b	ND	0.8 ± 0.0a	1.2 ± 0.2a	1.4 ± 0.2b

B

Quality parameter	Study 1 storage time (days) 0	30	60	90	Study 2 storage time (days) 0	30	60	90
Moisture (%)	75.6 ± 0.2a	75.8 ± 0.1a	79.3 ± 1.3a	79.3 ± 1.2a	78.4 ± 0.2a	79.5 ± 1.3a	80.9 ± 1.1a	80.9 ± 1.1a
Colour								
L	40.6 ± 0.7a	39.9 ± 1.1a	40.9 ± 1.0a	40.4 ± 2.3a	45.2 ± 0.6a	45.6 ± 0.6a	44.8 ± 0.6a	44.4 ± 1.2a
a	−14.6 ± 0.9a	−15.7 ± 0.8a	−15.0 ± 0.5a	−13.8 ± 0.9a	−14.3 ± 0.4a	−15.0 ± 0.2a	−15.5 ± 0.9a	−13.4 ± 0.7a
b	15.4 ± 0.9a	17.7 ± 0.9a	18.3 ± 0.5a	16.3 ± 1.6a	16.0 ± 1.0a	18.9 ± 0.8a	20.2 ± 1.5a	16.5 ± 1.9a
Ascorbic acid ($mg.g^{-1}$)	1.2 ± 0.0a	0.1 ± 0.0b	ND	ND	1.2 ± 0.0	ND	ND	ND
Dehydroascorbic acid (mg g^{-1} wet weight)	ND	1.9 ± 0.1a	1.4 ± 0.0a	1.4 ± 0.0a	ND	0.8 ± 0.0a	0.9 ± 0.0a	1.0 ± 0.0a

Means followed by the same letter in each group are not significantly different ($P > 0.05$). ND, not detected.

stable green colour at low temperatures.[31] Another explanation might relate to reduced activity of chlorophyllase at lower temperatures.

Total phenolic concentration increased significantly during 90 days of storage at −18 °C (Fig. 3). The increase in total phenolics after 90 days was between 3 and 5% in *C. tora* leaves and between 8 and 9% in *C. tridens* leaves.

Ascorbic acid concentrations were significantly higher at harvest than after storage at −18 °C (Table 4). For *C. tora* leaves the retention of ascorbic acid in study 1 was only about 18% after

90 days of storage and that in study 2 was about 28% after 30 days of storage. Only about 9% of the original ascorbic acid content of *C. tridens* leaves was retained in study 1 and the level was undetectable in study 2 after 30 days.

Ascorbic acid was not detectable in frozen stored *C. tora* on days 60 and 90 in study 2, nor was it detectable in frozen stored *C. tridens* on days 60 and 90 in study 1 or days 30, 60 and 90 in study 2. This result may be explained by the complete oxidation of ascorbic acid to 2,3-diketogulonic acid.[34]

Several previous studies[28,35–37] have considered the effects of frozen storage for 6–12 months on vegetables such as broccoli, carrots, green beans, green peas and spinach and reported between 37 and 80% retention of initial ascorbic acid. Favell[22] reported changes in ascorbic acid due to frozen storage of several vegetables for 12 months at −18 °C on a dry weight basis. He found negligible losses in carrots and 80 and 70% retention in broccoli and green peas respectively, possibly because the vegetables were blanched prior to freezing. Blanching vegetables before freezing purportedly results in greater ascorbic acid retention during frozen storage owing to the inactivation of ascorbate oxidase, which causes the oxidation of ascorbic acid to dehydroascorbic acid.[22,38]

Dehydroascorbic acid (Table 4) was undetectable under room temperature and refrigerated storage conditions, but it was detected in leafy vegetables stored under frozen conditions. The inability to detect dehydroascorbic acid in the leafy vegetables stored at 20 and 4 °C may be due to the fact that the plant tissues were still intact throughout storage. Yamaguchi *et al.*[38] reported previously that the enzyme ascorbate oxidase, which is responsible for the oxidation of ascorbic acid to dehydroascorbic acid, would most likely have been compartmentalised and therefore separated from its substrate under these warmer storage conditions. Freezing, however, causes cells to rupture, allowing the enzyme to come into contact with its substrate and oxidation to take place. Another reason for not detecting dehydroascorbic acid may be that it was completely oxidised to 2,3-diketogulonic acid during room temperature and refrigerated storage and this was not accounted for in the present study.

Table 5. Effect of cooking on wet weight ascorbic acid content of (A) *Cassia tora* and (B) *Corchorus tridens* stored at room temperature (20 °C), refrigerated temperature (4 °C) and frozen temperature (−18 °C)

A

		Ascorbic acid			
		Cooked leaves (μg g^{-1})		Cooking water (mg g^{-1})	
Storage temperature	Storage days	Study 1	Study 2	Study 1	Study 2
Room (20 °C)	0	26.0	28.2	1.3	1.4
	2	24.5	22.5	1.2	1.2
	4	18.6	20.2	0.9	1.0
	6		13.9	1.1	0.7
Refrigerated (4 °C)	2	21.4	26.1	1.0	1.3
	4	20.5	24.5	0.9	1.2
	6	18.1	23.0	0.9	1.1
	8	19.5	14.3	0.9	0.7
	10	19.0	10.3	0.9	0.5
	12	18.1	9.5	0.9	0.5
	14	18.0	7.8	0.9	0.4
Frozen (−18 °C)	30	8.0	7.7	0.4	0.1
	60	7.0		0.3	
	90	4.0		0.2	

B

		Ascorbic acid			
		Cooked leaves (μg g^{-1})		Cooking water (mg g^{-1})	
Storage temperature	Storage days	Study 1	Study 2	Study 1	Study 2
Room (20 °C)	0	47.0	49.7	0.6	0.6
	2	42.6	46.1	0.5	0.6
	4	38.6	39.2	0.5	0.5
	6	29.3	32.8	0.4	0.4
Refrigerated (4 °C)	2	45.1	46.7	0.6	0.6
	4	45.1	42.6	0.6	0.5
	6	35.1	36.9	0.4	0.5
	8	30.3	35.2	0.4	0.4
	10	29.6	27.4	0.4	0.3
	12	24.7	17.6	0.3	0.2
	14	19.3	14.8	0.2	0.2
Frozen (−18 °C)	30	4.3		0.1	
	60				
	90				

Effect of cooking on ascorbic acid content

Ascorbic acid is sensitive to heat and oxygen and is also soluble in water, therefore leaching into the cooking water may occur during processing, resulting in the potential for losses during either industrial processing or domestic cooking. In addition, leaves may absorb a large amount of cooking water and this can lead to dilution and therefore a reduced level of vitamins in the cooked product.

In this study the effect of cooking on the ascorbic acid content of African leafy vegetables was analysed. Cooking of both *C. tora* and *C. tridens* leaves resulted in a dramatic loss of ascorbic acid, with only 1–4% retention of the fresh content following cooking of leafy vegetables stored at room, refrigerated or frozen temperature (Table 5). The drained cooking water was also analysed for ascorbic acid content and it was observed that approximately 50–60% of the original ascorbic acid was leached into the cooking water of leafy vegetables stored at room, refrigerated or frozen temperature.

While the results presented in Table 5 are on a wet weight basis, moisture loss during storage and cooking makes it important to also evaluate these results on a dry weight basis. Initial dry weight concentrations of ascorbic acid in *C. tora* in studies 1 and 2 were 10.5 and 14.3 mg g^{-1} leaf respectively, while those in *C. tridens* were 4.9 and 5.8 mg g^{-1} leaf respectively. Losses of ascorbic acid as a result of both storage and cooking in both studies for these two leafy vegetables are reported in Table 6. It is evident from these data that the most significant storage loss in both leafy vegetables is higher following frozen storage, while concentrations in vegetables stored at room or refrigeration temperature declined more slowly. This may be due to loss of integrity in the plant cell as a result of the freezing process, as well as greater activity of ascorbic acid oxidase and exposure to oxygen. As illustrated in the wet weight results, cooking of either of the stored vegetables under the conditions used in this study resulted in almost complete loss of ascorbic acid.

Table 6. Effect of cooking on dry weight ascorbic acid content of (A) *Cassia tora* and (B) *Corchorus tridens* stored at room temperature (20 °C), refrigerated temperature (4 °C) and frozen temperature (−18 °C)

A

Storage temperature	Storage days	Ascorbic acid (mg g^{-1})			
		Uncooked leaves		Cooked leaves	
		Study 1	Study 2	Study 1	Study 2
Room (20 °C)	0	10.5	14.3	0.2	0.2
	2	9.2	13.1	0.1	0.5
	4	8.9	10.0	0.1	0.3
	6		8.6		0.2
Refrigerated (4 °C)	2	9.3	12.0	0.1	0.6
	4	8.6	11.7	0.1	0.5
	6	7.7	11.7	0.2	0.5
	8	8.5	7.3	0.1	0.3
	10	7.7	5.0	0.1	0.1
	12	7.9	5.0	0.1	0.2
	14	7.7	3.6	0.1	0.2
Frozen (−18 °C)	30	3.7	3.7	0.1	0.1
	60	3.0	3.0	ND	ND
	90	1.9	1.9	ND	ND

B

Storage temperature	Storage days	Ascorbic acid (mg g^{-1})			
		Uncooked leaves		Cooked leaves	
		Study 1	Study 2	Study 1	Study 2
Room (20 °C)	0	4.9	5.8	0.2	0.3
	2	5.1	5.2	0.2	0.3
	4	4.5	4.8	0.3	0.2
	6	3.3	3.5	0.1	0.2
Refrigerated (4 °C)	2	4.9	6.2	0.2	0.3
	4	4.8	5.2	0.1	0.2
	6	4.2	4.3	0.2	0.1
	8	3.5	4.4	0.2	0.2
	10	3.5	3.0	0.1	0.1
	12	3.0	2.1	0.2	0.1
	14	2.4	1.6	0.2	0.1
Frozen (−18 °C)	30	0.5	ND	0.0	ND
	60	ND	ND	ND	ND
	90	ND	ND	ND	ND

Comparable results were reported by Kala and Prakash[39] for spinach, where the retention of ascorbic acid following cooking for 12 min was approximately 5%. Yadav and Sehgal[23] found only 4–8% retention of ascorbic acid in spinach and amaranth leaves cooked for 30 min in an open pan with hydrogenated fat and spices. Klein *et al.*[34] reported about 50% ascorbic acid retention in spinach cooked for 7 min. This indicates that reduced cooking times may lead to a significantly greater retention of ascorbic acid. The range of reported retentions of ascorbic acid during cooking may be attributed to different amounts of initial ascorbic acid in leafy greens and also to the conditions used during storage and cooking. Shorter cooking times, less exposure of material to atmospheric oxygen, not chopping leaves prior to cooking, and lower water/vegetable ratios should all result in greater retention of the vitamin during cooking.

CONCLUSIONS

Storage of the leafy vegetables at room (20 °C), refrigerated (4 °C) and frozen (−18 °C) temperatures after harvest resulted in several changes in quality and nutritional parameters of *C. tora* and *C. tridens* leaves. There were significant changes in chlorophyll, total phenolics and minerals during storage and significant changes in ascorbic acid during both storage and cooking.

A uniform decrease in leaf chlorophyll content was observed under all storage temperatures. Room temperature and refrigerated stored leaves showed similar losses after 6 and 14 days respectively, but greater losses of chlorophyll were observed in frozen stored leaves. The colour of the leaves was better retained at lower temperatures (4 and −18 °C) than at 20 °C.

A significant increase in firmness and toughness of *C. tora* leaves was observed under room temperature storage conditions. The observed increase may have been due to lignification of the plant tissue.

Total phenolic content of the leaves showed a marked increase under all storage temperatures. This increase was approximately the same under room and refrigerated temperatures but was very low under frozen storage conditions.

Ascorbic acid content of the leafy vegetables decreased under all storage temperatures, with greater losses occurring under frozen and room temperature storage. Previous studies have reported that lowering the storage temperature results in greater retention of ascorbic acid, but oxidation continues even under frozen storage conditions owing to the activity of the enzyme ascorbate oxidase. Greater retention of ascorbic acid was observed during postharvest storage at 4 °C.

Cooking leafy vegetables also resulted in dramatic losses of ascorbic acid. The mineral content of the leafy vegetables, i.e. calcium, zinc and iron, showed a decrease on cooking, but this may have been due to leaching into the cooking water. The cooking water was also analysed for ascorbic acid in the present study and it was observed that most of the nutrients are leached into the cooking water. Blanching prior to frozen storage may reduce the activity of enzymes catalysing the continual loss of both ascorbic acid and chlorophyll during storage. Thus further research might address leaves stored blanched and unblanched under frozen temperatures.

To minimise qualitative and nutritive losses, consumers should store leafy green vegetables such as *C. tora* and *C. tridens* at 4 °C after harvest. In the present study the quality retention was superior at this storage temperature. Consumers may also consider using shorter cooking times, cooking vegetables in less water and consuming the water used for cooking as a part of the diet.

ACKNOWLEDGEMENTS

We would like to thank the International Crops Research Institute for Semi-Arid Tropical Crops (ICRISAT) for providing seeds for this project. We would also like to thank Drs Patrick Brown and Hening Hu for their valuable assistance with growing the vegetables in the UC Davis greenhouse. We would also like to thank and acknowledge MSc Eduardo Gutierrez for sharing his texture technique for leafy greens and assistance with other initial phases of experimental set-up.

REFERENCES

1 Maundu P, A publication about agricultural biodiversity – traditional African leafy vegetables: from despised to prized. [Online]. IPGRI (2006). Available: http://news.bioversityinternational.org.
2 Mwangi S and Kimathi M, African leafy vegetables evolve from underutilized species to commercial cash crops. *Research Workshop on Collective Action and Market Access for Smallholders*, Cali, pp. 1–19 (2006).
3 IPGRI, Neglected no longer: a fact sheet on neglected and underutilized species in Sub-Saharan Africa. [Online]. Available: http://news.bioversityinternational.org. [accessed:20 July 2008].
4 Brown P, Lumpkin T, Barber S, Hardie E, Kraft KH, Luedeling E, *et al*, Global horticulture assessment. *Scripta Hort* **3**:1–134 (2005).
5 Rickman JC, Barrett DM and Bruhn CM, Nutritional comparison of fresh, frozen and canned fruits and vegetables. Part 1. Vitamins C and B and phenolic compounds. *J Sci Food Agric* **87**:930–944 (2007).
6 Arnon DI, Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiol* **24**:1–15 (1949).
7 Read J and Sanson GD, Characterizing sclerophylly: the mechanical properties of a diverse range of leaf types. *New Phytol* **160**:81–99 (2003).
8 Lowry OH, Protein determination using the Folin–Ciocalteu reagent. *J Biol Chem* **193**:265–275 (1951).
9 Zapata S and Dufour JP, Ascorbic, dehydroascorbic and isoascorbic acid simultaneous determinations by reverse phase ion interaction HPLC. *J Food Sci* **57**:506–511 (1992).
10 Bergquist SAM, Gertsson UE and Olsson ME, Influence of growth stage and postharvest storage on ascorbic acid and carotenoid content and visual quality of baby spinach (*Spinacia oleracea* L.). *J Sci Food Agric* **87**:2170–2171 (2007).
11 Wang SS, Haard NF and DiMarco GR, Chlorophyll degradation during controlled-atmosphere storage of asparagus. *J Food Sci* **36**:657–661 (1971).
12 Ferrante A and Maggore T, Chlorophyll a fluorescence measurements to evaluate storage time and temperature of *Valeriana* leafy vegetables. *Postharv Biol Technol* **45**:73–80 (2007).
13 Vina SZ and Chaves AR, Texture changes in fresh cut celery during refrigerated storage. *J Sci Food Agric* **83**:1308–1314 (2003).
14 Vance CP, Kirk TK and Sherwood RT, Lignification as a mechanism of disease resistance. *Annu Rev Phytopathol* **18**:259–288 (1980).
15 Zhao X and Edward EC, Influences of organic fertilization, high tunnel environment, and postharvest storage on phenolic compounds in lettuce. *Hort Sci* **42**:71–76 (2007).
16 Leja M, Mareczek A, Starzynska A and Roz S, Antioxidant ability of broccoli flower buds during short-term storage. *Food Chem* **72**:219–222 (2001).
17 Hodges DM and Forney CF, The effects of ethylene, depressed oxygen and elevated carbon dioxide on antioxidant profiles of senescing spinach leaves. *J Exp Bot* **551**:645–655 (2000).
18 Hodges DM, Wismer WV and Forney CF, Antioxidant responses in harvested leaves of two cultivars of spinach differing in senescence rates. *J Am Soc Hort Sci* **126**:611–617 (2001).
19 Yamauchi N and Kusabe A, Involvement of ascorbate–glutathione cycle in senescence of stored broccoli (*Brassica oleracea* L.). *J Jpn Soc Hort Sci* **70**:704–708 (2001).
20 Zee JA, Carmichael L, Codere D, Poirier D and Fournier M, Effect of storage conditions on the stability of vitamin C in various fruits and vegetables produced and consumed in Quebec. *J Food Compos Anal* **4**:77–86 (1991).
21 Yahia EM, Contreras PM and Gonzalez AG, Ascorbic acid content in relation to ascorbic acid oxidase activity and polyamine content in tomato and bell pepper fruits during development, maturation and senescence. *Lebensm Wiss Technol* **34**:452–457 (2001).
22 Favell DJ, A comparison of the vitamin C content of fresh and frozen vegetables. *Food Chem* **62**:59–64 (1998).
23 Yadav SK and Sehgal S, Effect of domestic processing on ascorbic acid and β-carotene content of spinach (*Spinacia oleracea*) and amaranth (*Amaranthus tricolor*) leaves. *Plant Foods Hum Nutr* **47**:125–131 (1995).
24 Watada A, Kim S, Kim K and Harris T, Quality of green beans, bell peppers and spinach stored in polyethylene bags. *J Food Sci* **52**:1637–1641 (1987).
25 Bradbury JH, Bradshaw K, Jealous W, Holloway WD and Phimpisan T, Effect of cooking on nutrient content of tropical root crops from the South Pacific. *J Sci Food Agric* **43**:332–342 (1987).
26 Yoko H, Garucia P, Sonoko A, Keiko H and Atsuko S, Effect of storage attitude on the quality of leaf vegetables kept in a refrigerator. *J Cook Sci Jpn* **33**:66–71 (2000).
27 Zhang D and Hamauzu Y, Antioxidant activity and total phenolics in post-harvest iceberg lettuce (*Lactuca sativa*). *Acta Hort* **628**:687–691 (2003).
28 Howard LA, Wong AD, Perry AK and Klein BP, β-Carotene and ascorbic acid retention in fresh and processed vegetables. *J Food Sci* **64**:929–936 (1999).
29 Labib AAS, Abd El-Latife SA and Omran H, Quality indices of Jew's mallow and spinach during frozen storage. *Plant Foods Hum Nutr* **50**:333–347 (1997).
30 Lisiewska Z and Gebczynski P, Effects of the methods of pre-treatment before freezing on the retention of chlorophylls in frozen leaf vegetables prepared for consumption. *Eur Food Res Technol* **226**:25–31 (2007).
31 Martins RC and Silva CLM, Frozen green beans (*Phaseolus vulgaris*, L.) quality profile evaluation during home storage. *J Food Eng* **64**:481–488 (2004).
32 Lisiewska Z and Kmiecik W, Effect of freezing and storage on quality in Hamburg and leafy parsley factors. *Food Chem* **60**:633–637 (1997).
33 Dong H, Jiang Y, Wang Y, Liu R and Guan H, Effects of hot water immersion on storage quality of fresh broccoli heads. *Food Technol Biotechnol* **42**:135–139 (2004).
34 Klein BP, Kuo CHY and Boyd G, Folacin and ascorbic acid retention in fresh raw, microwave, and conventionally cooked spinach. *J Food Sci* **46**:640–641 (1981).
35 Murcia MA, López-Ayerra B, Martinez-Tomé M, Vera AM and García CF, Evolution of ascorbic acid and peroxidase during industrial processing of broccoli. *J Sci Food Agric* **80**:1882–1886 (2000).
36 Korus A, Lisiewska Z and Kmiecik W, Effect of freezing and canning on the content of selected vitamins and pigments in seeds of two grass pea (*Lathyrus sativus* L.) cultivars at the not fully mature stage. *Nahrung/Food* **46**:233–237 (2002).
37 Lisiewska Z, Korus A and Kmiecik W, Changes in the level of vitamin C, beta-carotene, thiamine, and riboflavin during preservation of immature grass pea (*Lathyrus sativus* L.) seeds. *Eur Food Res Technol* **215**:216–220 (2002).
38 Yamaguchi T, Mamiko K, Yuka O, Junji T, Kazuki K, Shunji O, *et al*, Influence of polyphenol and ascorbate oxidases during cooking process on the radical-scavenging activity of vegetables. *Food Sci Technol Res* **9**:79–83 (2003).
39 Kala A and Prakash J, Nutrient composition and sensory profile of differently cooked green leafy vegetables. *Int J Food Prop* **7**:659–669 (2004).

