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SHORT COMMUNICATION

L-ASCORBIC ACID LOSSES IN KENYAN VEGETABLES DURING COOKING AS DETERMINED BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT. The loss of L-ascorbic acid (L-AA) in 14 different cooked local vegetables found in Nairobi markets was determined by high performance liquid chromatography. The effect of quantity of water on the loss of L-AA during cooking was studied with cowpea leaves. It was found that more L-AA was lost when larger amount of water was used than when smaller amount was used. The effect of the sharpness of the knife on the loss of L-AA was studied with spinach. It was found that more loss of L-AA occurred when a blunt (edge thickness 0.08 cm) knife was used for cutting the vegetables than when a sharp knife (edge thickness 0.04 cm) was used during cooking. L-AA was also determined when vegetables were cooked in different size pieces (surface are >1 cm^2 and <1 cm^2) using cabbage. It was found that more losses occurred when cabbage was cooked in small pieces than when cooked in large pieces.

KEY WORDS: L-Ascorbic acid, Kenyan vegetables, High performance liquid chromatography, Loss of L-ascorbic acid during cooking

INTRODUCTION

L-Ascorbic acid (L-AA, vitamin C) widely distributed in plants and animals being synthesized both biologically and chemically from D-glucose. It is an essential nutrient for health maintenance. The main sources of L-AA in humans are fruits and vegetables. The exact content depends on species, variety and stage of development. It is mainly used in the prevention and treatment of scurvy. It is often added during the manufacture of juices or soft drinks to improve the nutritional value, for attracting consumers or to act as an antioxidant to prolong the shelf-life of products. However, its concentration in fruit juices requires careful regulation as too low a concentration will affect its antioxidant properties but too high concentration can cause certain disadvantages such as accelerated colour fading [1, 2].

Many analytical techniques have been used for the assay of L-AA. The two official methods: dye-titration method and microfluorimetric method, recommended by the Association of Official Analytical Chemists (AOAC) for L-AA analyses in foods, have been compared [3, 4]. The dye-titration method determines L-AA only and is hence considered unreliable due to a range of potentially interfering compounds. The other AOAC method, microfluorimetry is preferred from nutritional point of view as it determines the total vitamin C activity and is less susceptible to interference, but is a lengthy and elaborate analytical technique. Lately, polarographic methods [5-7] have been employed for the assay of L-AA, but are not suitable for routine analysis. Chromatographic methods [8, 9] are more commonly used because they are comparatively more sensitive and rapid.

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Vegetables being sold in the Nairobi markets come from different parts of the country. In the present study, loss of the L-AA content of 14 cooked different vegetables originating from different areas in the country being sold in Nairobi markets was studied in the year 1994. The effect of quantity of water, sharpness of the knife and the sizes of the pieces of vegetables used in the cooking were studied using cowpea leaves, spinach and cabbages, respectively.

EXPERIMENTAL

Apparatus. A distillation still (Fistreem Cyclon, Loughbororough, England), centrifuge (Gallen Kamp, England), flask shaker (Griffin S36-690, Great Britain), food blender (Waring, England) and pH meter (Jenway 3020, Great Britain) were used.

High performance liquid chromatography. Beckman Gradient liquid Chromatograph Model 332 equipped with Gow Mac recorder Model 70-700 was used. The column used was reversed phase Partisil 10 ODS-3 250 x 4.6 mm C_{18} (particle size 5 μm), with a Brownlee labs Newguard RP-18 15 x 3.2 mm pre-column (particle size 7 μm). Variable wavelength UV detector and a sample loop of 10 μL were used. The mobile phase delivery systems consisted of two Winchester bottles with a degasser attached to each of them to remove dissolved air.

Reagents. A 6% aqueous metaphosphoric acid was used as an extraction reagent. A 1 M phosphate buffer, made from potassium dihydrogen phosphate and disodium hydrogen phosphate, whose pH was adjusted to 6.0 by 6% metaphosphoric acid was used as a diluting agent for the extract. Aqueous tetrabutylammonium hydroxide (1%) in aqueous methanol (70%) was used as the mobile phase. All reagents were of analytical grade and were bought from Kobian Chemical Company, Nairobi.

Sampling method. Random sampling method [10] was used to collect the samples. Vegetables were bought from the local markets in or around Nairobi in bundles, for example, kale, cowpea leaves, pumpkin leaves, etc. (~ 250 g) or in heaps, for example, carrots, tomatoes, potatoes, etc. (~ 300 g). Five samples were prepared from each vegetable and five replicate determinations were performed on each sample and the mean L-AA content calculated. The mean contents of the samples were averaged to give the mean L-AA content of the particular type of vegetable.

Sample preparation. The preparation of the sample involved cutting the vegetable into pieces, weighing and crushing with the mortar and pestle (for large samples a food blender was used). This was then mixed with a measured volume of the extracting reagent in the ratio 1:5 (w/v). The resulting mixture was then put on the mechanical shaker for about 20 min to obtain a homogeneous mixture which was centrifuged for 20 min to obtain a clear solution. Traces of the suspension, if any, were finally removed by filtering through a Whatman filter to obtain a solution free of any suspensions to prevent blockage of the column. The solutions were then stored in the deep freezer until the time of the analysis.

Cooking. The vegetable in each sample lot (250 g) was cooked for 25 min and the amount of water used was 1000 mL unless otherwise indicated. For cooking of each vegetable, identical conditions, for example, container size, covering with a lid, etc. were maintained. The spinach was cut into pieces with knives of different sharpness. The blunt knife had an edge thickness of 0.08 cm while the sharp knife had edge thickness of 0.04 cm. The cabbage was cooked in two different sizes

large and small pieces (surface area greater than 1 cm^2 and less than 1 cm^2, respectively). The cooked vegetables were allowed to cool, mixed, blended and extracted as described below. The cowpeas leaves were sampled at various time intervals, blended and extracted. The mean content of L-AA of the particular vegetable was calculated as described latter.

Extraction and purification. The cooked vegetable in each sample lot was homogenized, and the homogenate pooled for analysis. 3-15 g samples were placed in about 50 mL (sample:extraction reagent, 1:5) and shaken mechanically for 20 min. The mixture was then centrifuged at 5000 rpm at room temperature (23 ^{0}C) for 20 min and any suspensions formed were filtered through Whatman No. 542 paper and then through a 0.22 µm Millipore filter before injection into the LC column for analysis.

Standard preparations. A stock solution of L-AA was prepared by dissolving 0.1 g of Analar L-AA in 100 mL of the 0.1 M phosphate buffer whose pH was adjusted to 6.0. Five working standards of 1-5 mg/100 mL were prepared by serial dilution of the stock solution.

Working conditions. The mobile phase was 70% methanol in distilled deionised water brought to pH 6.0 with tetrabutylammonium hydroxide (1%). The mobile phase was purged with nitrogen (99.9%) to remove dissolved oxygen. The flow rate was 2.0 mL/min and detection was at 273 nm. The mobile phase was allowed to flow through the column until a constant zero reading was recorded on the detector.

Analysis. Five replicate determinations were performed on each sample and the mean content determined. These were later averaged to give the mean content of the particular type of vegetable. Determinations were performed on cooked vegetables within one week from sampling so as to avoid the alteration of the L-AA by light, heat, humidity, etc. Samples were run with the standard working solutions intermittently.

Calculations. The concentration of L-AA in the unknown was calculated by determining the response factor of the working standards of known concentrations and determining the response factor of the unknown and getting the concentration from the calibration graph.

The concentrations and percentage recoveries were calculated from equations 1 and 2, respectively:

$$C = \frac{(A_2 C_s D_1 V)}{A_1 M} \qquad (1)$$

where C is the sample concentration, C_s is the standard concentration, A_1 is the standard area, A_2 is the sample area, D_1 is the dilution factor, V is the volume of the sample and M is the mass of the sample.

$$\% \text{ recovery} = \frac{(Z\text{-}X) \times 100}{A} \qquad (2)$$

where X is the concentration of the sample, Z is the concentration of the sample plus a known concentration of added L-AA and A is the concentration of the added L-AA.

RESULTS AND DISCUSSION

Ion-pair reversed phase HPLC with tetrabutylammonium hydroxide as the source of the counter ions was used for L-AA determination [11]. The retention time was 6.0 min and L-AA was detected at 273 nm because it showed maximum absorption at this wavelength. The chromatograms showed that there were no interferences at the chosen wavelength for monitoring the L-AA.

There was a linear dependence of the peak area of L-AA on the concentration in the range of 1-5 mg/100 mL. The reproducibility of the determination was satisfactory, each value of the peak area was determined as the mean of five measurements with a RSD of 10%. The quantification limit was found to be 0.4 mg/100 mL (the minimum amount of the sample that could be detected under the working instrumental conditions).

Table 1 lists L-AA levels determined in 14 different types of cooked vegetables originating from different localities. The highest loss was recorded in cowpea leaves (87.4%) while the least loss occurred in gynandra (29.2%) followed by English potatoes. Different vegetables had different losses because of their difference in their physiological make up.

Table 2 lists the losses of L-AA on boiling at various time intervals in cowpea leaves. It was observed that more losses occurred when boiling was in large amount of water than in small amount of water. This is due to the fact that large amount of water offers large surface area for the dissolution of the L-AA. The highest mean loss was 99.4% after 60 min in 1500 mL.

In Table 3, the effect of sharpness of the knife on the loss of L-AA in spinach was studied at various time intervals. It was observed that more losses occurred when a blunt knife was used in cutting than when a sharp knife was used. This is because a blunt knife destroys a lot of cells thus releasing more L-AA oxidase that oxidizes L-AA to dehydro L-AA and consequently more losses. The largest loss was 87.5% after 60 min.

Table 4 lists a comparison of L-AA contents of cabbages when cooked as small and large pieces. It was observed that more losses occurred when cabbage was cooked in small pieces. This is because small pieces increase the surface area for the dissolution of L-AA. The highest loss was 97.9% after 60 min.

Table 1. The % loss of L-AA after cooking for 25 min.

Vegetable	L-AA in raw sample (mg/100 g)	% loss after cooking
Kale	220.6±1.8	76.0
Cabbages	34.0±1.2	42.9
Spinach	39.6±1.0	49.5
Carrots	20.0±0.8	35.0
Cowpea leaves	333.0±2.7	87.4
Pumpkin leaves	135.8±2.0	66.9
Gynandra	226.0±1.4	29.2
Chorchoris	205.0±2.0	38.0
Amaranthus	380.0±2.3	72.4
Solanum	216.0±2.0	81.8
Crotalaria	215.0±2.8	38.6
Green peas	98.0±2.0	32.7
English potatoes	21.0±0.6	31.8
Potatoes	63.0±2.0	44.4

Table 2. The effect of quantity of water on the loss of L-AA on cooking of cowpea leaves at various time intervals.

Quantity of water (mL)	Cooking time (min)	% loss of L-AA
1500	0.0	0.0
1000	0.0	0.0
1500	10.0	84.4
1000	10.0	78.1
1500	20.0	88.0
1000	20.0	81.1
1500	30.0	97.6
1000	30.0	94.9
1500	40.0	97.9
1000	40.0	96.7
1500	50.0	98.5
1000	50.0	97.3
1500	60.0	99.4
1000	60.0	98.0

In Table 3, the effect of sharpness of the knife on the loss of L-AA in spinach was studied at various time intervals. It was observed that more losses occurred when a blunt knife was used in cutting than when a sharp knife was used. This is because a blunt knife destroys a lot of cells thus releasing more L-AA oxidase that oxidizes L-AA to dehydro L-AA and consequently more losses. The largest loss was 87.5% after 60 min.

Table 3. Effect of the sharpness of the knife on the loss of L-AA in spinach at various time intervals.

Type of knife	Cooking time (min)	% loss of L-AA
Sharp	0.0	0.0
Blunt	0.0	0.0
Sharp	10.0	56.3
Blunt	10.0	62.5
Sharp	20.0	64.1
Blunt	20.0	75.0
Sharp	30.0	73.4
Blunt	30.0	82.8
Sharp	40.0	78.1
Blunt	40.0	84.4
Sharp	50.0	79.7
Blunt	50.0	85.9
Sharp	60.0	84.4
Blunt	60.0	87.5

Table 4 lists a comparison of L-AA contents of cabbages when cooked as small and large pieces. It was observed that more losses occurred when cabbage was cooked in small pieces. This is because small pieces increase the surface area for the dissolution of L-AA. The highest loss was 97.9% after 60 min.

Table 4. Comparison of L-AA contents of cabbages when cooked as small and large pieces.

Cooking time (min)	Small pieces (% loss)	Large pieces (% loss)
0.0	0.0	0.0
10.0	70.6	55.5
20.0	85.3	73.5
30.0	88.2	85.3
40.0	94.1	91.1
50.0	97.1	94.1
60.0	97.9	95.0

CONCLUSION

A reversed phase ion pair HPLC method was devised for the study of L-AA loss during cooking. It was found that there was loss of L-AA during cooking either through dissolution or destruction by heat. The more time taken in cooking, the more L-AA was lost. More L-AA was lost when large amount of water was used (because of the increased surface area for dissolution) and when a blunt knife was used in cutting the vegetables this was because of many cells which are destroyed and hence release a lot of L-AA oxidase which oxidizes L-AA to dehydro L-AA. When vegetables are cooked as small pieces more L-AA is lost for the same reason. Thus to prevent the loss of L-AA during cooking, low temperature, small amount of water, sharp knife for cutting and large pieces of vegetables should be used.

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REFERENCES

1. Agarwal, V.K. *J. Assoc. Off. Anal. Chem.* **1988**, 71, 466.
2. Backheet, E.Y.; Emara, K.M.; Askal, H.F.; Saleh, G.A. *Analyst* **1991**, 116, 861.
3. Deutsch, M.J.; Weeks, C.E. *J. Assoc. Off. Anal. Chem.* **1965**, 48, 1248.
4. Tee, E.S.; Young, S.I.; Ho, S.K.; Mizura, S.S. *Pertanika* **1988**, 11, 39.
5. Fogg, A.G.; Summan, A.M. *Analyst* **1985**, 108, 691.
6. Lindquist, J.; Farroha, S.M. *Analyst* **1975**, 100, 377.
7. Wekesa, N.M.N.; Chhabra, S.C.; Thairu, H.M. *Bull. Chem. Soc. Ethiop.* **1996**, 10, 165.
8. Bushway, R.J.; King, J.M.; Perkings, B.; Krishna, M. *J. Liq. Chromatogr.* **1988**, 11, 3418.
9. Sood, S.P.; Sartoli, L.E.; Wittmar, D.P.; Haney, W.G. *Anal. Chem.* **1976**, 48, 796.
10. Miller, J.C.; Miller, J.N. *Statistics for Analytical Chemistry*, Ellis Horwood: London; **1988**; p 170.
11. Chhabra, S.C.; Wekesa, N.M.N.; Thairu, H.M.; Nyagah, G. *J. Agr. Sci. Tech.* **1999**, 2, 22.