Determination of L-Ascorbic Acid in Various Kenyan Fresh and Processed Fruits and Vegetables by High Performance Liquid Chromatography

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ABSTRACT

Liquid chromatography methodology for determination of L-ascorbic acid (L-AA) in fresh and processed fruits and vegetables has been developed. The method uses a C_{18} column and an RP-18 precolumn for the stationary phase. The high-performance-liquid-chromatographic solvent is 0.1M phosphate buffer (pH=6). The flow rate is 2.0 ml/min, and UV detection at 273 nm. Ten different varieties of fruits, 13 different varieties of vegetables, 13 different types of processed fruits and 4 different types of commercial soft drinks were analysed. Results obtained show that Ribena contains the highest (410.0 mg/100 ml) mean level of L-AA while Hey-ho has the lowest (3.8 mg/100 ml).

KEY WORDS

HPLC; vitamin C, L-Ascorbic acid, fruits, vegetables, commercial soft drinks.

1.0 INTRODUCTION

Dietary sources of vitamin C (L-ascorbic acid) include most fruits, vegetables and processed fruits. The exact content depends on climate, species, variety and the stage of development. Therefore, there is a need to know the levels of L-AA in various Kenyan fresh and processed fruits and vegetables as found in Kenyan markets, so that the information could be used by nutritionists and dieticians to educate the public about simple ways of treating and preventing scurvy. In the present study a liquid chromatography method was developed and used to determine L-AA content in both fresh fruits and vegetables as found in various Nairobi markets. The L-AA content was also determined in processed fruits and commercial soft drinks. The techniques that have found application in the determination of vitamin C in fruits, processed fruits and vegetables include fluorometric (Balla, 1979), colorimetric (Tokuichiro and Yoshihisa, 1987; White and Fitzgerald, 1972), volumetric (Baez et al. 1987; Bushway et al. 1988; Tee et al. 1988), enzymatic (Lee and Dawson, 1979;

spectrophotometric (Backheet et al. 1991; Mohammed et al. 1975), microfluorimetric (Frigola et al. 1988), polarographic (Imer et al. 1989; Sahbaz and Somer, 1992) and chromatographic methods (John et al. 1983; Romero et al. 1992; Sood et al. 1976; Vandemark and Schmidt, 1981). Chromatographic separation methods with sensitive and selective detection techniques are currently most widely used because they are accurate and less susceptible to interfering compounds from samples.

High performance liquid chromatographic (HPLC) methods using different parameters, for example, C_8 bonded phases (Vandemark and Schmidt, 1981) and C_{18} reversed phase with RP-18 precolumn (Romero et al. 1992), have been used for the separation of vitamins, for example, vitamin A, vitamin C, etc. in pharmaceutical preparations and organic acids, for example, malic and citric acids, in various fruits. Ion pair chromatography on a reversed phase column has also been used for determination of L-ascorbic acid (L-AA) in selected foods and multivitamin products. The most convenient counter ion used was tridecylammonium formate (Sood et al. 1976). The above techniques use UV detection method. However, other workers have used electrochemical detection after LC separation for L-AA quantification. Here detection is either amperometric using carbon paste electrode (Pachla and Kissinger, 1979) or direct current sampled polarography (Danuta et al. 1984).

In the present paper, L-AA has been determined accurately in various Kenyan fresh and processed fruits and vegetables by high performance liquid chromatography using a C_{18} column and an RP-18 precolumn as stationary phase.

The fruits studied were tangerine (*Citrus reticulata*), papaws (*Carica papaya*), pineapples (*Ananas comosus*), white and red passions (*Passiflora edulis*), white and red guavas (*Psidium guajava*), oranges (*Citrus sinensis*), mangoes (*Mangifera indica*) and lemons (*Citrus limon*) while processed fruits were Black currant, Treetop, Quencher whole orange, Hey-ho, Ribena, Lecol pure lemon, Mango rubicon, Realananas, Realorange, Rubicon exotic passion, Kengold, Zesta passion and Zesta mango. Commercial soft drinks studied were Sprite, Sonic fanta, Fanta and Coca-Cola. The fresh vegetables studied were spinach (*Beta vulgaris*), cabbage (*Brassica oleracea*), tomatoes (*Lycopersicon esculentum*), carrots (*Dancus carota*), kale (*Brassica oleracea*), pepper (*Capsicum annum*), gynandra (*Gynandropsis gynandra*), cowpea leaves (*Vigna unguiculata*), pumpkin leaves (*Cucurbita pepo*), onions (*Allium cepa*), English potatoes (*Solanum tuberosum*), solanum (*Solanum nigrum*) and dania (*Coriandrum sativum*).

The fresh fruits and vegetables were obtained from the main vegetable markets in and

around Nairobi (Kenya) while the processed fruits were obtained from main supermarkets in Nairobi. All processed fruits were locally manufactured except Lecol pure lemon, Mango rubicon and Rubicon exotic passion which were manufactured by Rubicon Product Ltd., a company based in United Kingdom.

2.0 MATERIALS AND METHODS

2.1 Apparatus and reagents

A Fistreem Cyclon (Loughborough, England) distillation still, centrifuge (Gallen Kamp, England), flask shaker (Griffin S36-690, Great Britain) and food blender (Waring, England) were used.

2.2 Liquid chromatograph

The method used is a modification of the one used by Romero et al. (1992) in the separation of vitamins in pharmaceutical preparation and organic acids. A gradient liquid chromatograph Bruker Model LC 23-3 (Bremen, German) equipped with a programmable pump and a Rheodyne injector fitted with 20 μl injection loop, a Bruker variable wavelength UV-VIS detector, A Bruker type LC 411 integrator connected, via Labnet to an AT-compatible personal computer (Epson PC Model Q801A) controlling a WINNER data acquisition system. The LC column used was a Partisil 10 ODS-2 250 X 4.6 mm C_{18} column with a Brownlee Labs Newguard RP-18 15 X 3.2 mm precolumn (particle size 7 μm). The flow rate was 2.0 ml/min and UV detection set at 273 nm. The mobile phase was 70% HPLC grade methanol (May and Baker) in water acidified to pH 6.0 with phosphoric acid.

L(+)-Ascorbic acid (vitamin C) (Merck Art. 127), HPLC grade water obtained by distillation and purified in a Millipore Milli-Ro/Milli-Q apparatus, metaphosphoric acid (Merck Art. 546), 100% glacial acetic acid, potassium dihydrogen phosphate, disodium hydrogen phosphate (May & Baker) and methanol HPLC grade (May & Baker) were used.

Stock standard solutions (1 mg ml^{-1}) of L-ascorbic acid are prepared as follows. In 100-ml volumetric flasks, 0.100 g of L-ascorbic acid was dissolved and made upto the mark with 0.1M phosphate buffer (pH=6.0). Working standard solutions in the range of 0.01-0.06 mg ml^{-1} were prepared by serial dilution of aliquots of the stock solution in 0.1M phosphate buffer.

2.3 Sampling method

Random sampling method (Miller and Miller, 1988) was used to collect the samples.

Fresh fruits and vegetables were bought from the local markets in or around Nairobi. Fifteen fruits of each type (for example, mangoes, oranges, etc.) were analyzed for vitamin C and the mean content determined. Five samples were prepared from each fruit extract 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 variety of fruit.

Vegetables were bought either in bundles, for example, kale, cowpea leaves, pumpkin leaves, etc. (approx. 250 g) or in heaps, for example, carrots, tomatoes, potatoes, etc. (approx. 300 g). Leaves from vegetables were plucked off, mixed, blended and extracted while other vegetables were just blended and extracted as described latter. The mean content of L-AA of the particular vegetable was calculated as described above.

For processed fruits, a minimum of five cans were taken in each case. Five samples were prepared from each batch and analyzed for L-AA. Five replicate determinations were performed on each sample and the mean L-AA content calculated as described above.

2.4 Extraction and purification

Determinations were performed on fresh fruits and vegetables within one week from sampling so as to avoid the alteration of L-AA acid by light, heat, humidity, etc. The determination of L-AA in processed fruits was done about 4 to 5 months before the expiry dates. The fruit or vegetable in each sample lot (500 g) was homogenized and the homogenate pooled for analysis. 3-15 g Samples were placed in about 50 ml (sample: extraction reagent = 1:5) of the solution of 6% metaphosphoric acid and shaken mechanically for 20 min. The mixture was then centrifuged at 5000 rpm at room temperature (23°C) for 20 min and any white 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. Five replicate analyses were performed on each sample.

2.5 Recoveries

The mean percentage recoveries in triplicate analysis of L-AA in fresh fruits were above 95.9% while for fresh vegetables were above 95.7%. This means that quantitation was possible with these high recoveries and the matrix did not have a significant effect. Spike levels were within the expected amounts (50-100 mg/100 g) and allowed to equilibrate for not less than 2 h for equilibration to occur.

2.6 Validation

The concentration range studied was between 0.187 and 3.750 mg g^{-1} for fresh fruits and 0.160 and 3.300 mg g^{-1} for fresh vegetables. For processed fruits, the concentration range was between 0.038 and 4.100 mg g^{-1} while for commercial soft drinks the range was between 0.04 and 0.20 mg ml^{-1}. Data validation was performed by confirming the response linearity in the concentration ranges coupled with a study of the reproducibility and repeatability analysis of the analytical data of homogenised samples. As the response of L-AA is known, the amounts in the homogenised samples were calculated by comparing the chromatographic peak areas with those of the standards. These tests were carried out by the same researcher using the same equipment on different days.

Statistical analysis of the areas measured was performed to verify the linearity and the sensitivity (AFNOR, 1985). Repeatability and reproducibility were tested according to the recommendations of the standard NX X06-04 (AFNOR, 1987).

2.7 Calculations

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

$$C=(A_2C_sD_1V)\div A_1M \quad \ldots\ldots 1$$

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

$$\% \text{ recovery} = (Z-X)\times 100\div A \quad \ldots\ldots 2$$

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

3.0 RESULTS AND DISCUSSION

Vitamin C content was determined in ten different varieties/species of fresh fruits (Figure 1), thirteen different types of processed fruits (Figure 2), four commercial soft drinks (Figure 3) and thirteen different types of vegetables (Figure 4) as obtained from markets in and around Nairobi (Kenya). L-AA standard gave maximum absorbance at 273 nm when run in the UV-VIS range between 200-500 nm.

The calibration graph was linear (r^2 = 0.99918) and can be described by the equation peak area = 0.400 (concentration of L-AA) + 0.0020607 in the concentration ranges between

0.01 and 0.06 mg ml^{-1}. Figure 5 shows a typical LC chromatogram for an orange sample run for L-AA. This shows that there were no interferences at the chosen wavelength for monitoring the L-AA. All matrices gave a similar chromatogram, since the colours are in the visible.

The specificity of the method for L-AA is shown by the absence of response to dehydroascorbic acid at working wavelength of 273 nm. The quantification limit was evaluated as the smallest amount of L-AA assayed during the validation and was 0.004 mg g^{-1}. The detection limit (at 3 times standard deviation of the blank) was evaluated as 0.018 mg ml^{-1}. The red guavas gave a threefold difference in L-AA content compared to the white guavas. This difference in varieties was however not as marked in white passions compared to red passions. Pineapples had the lowest amount of L-AA among the fruits tested and gave comparable amounts to tangerines. L-AA content of Ribena was the highest among the processed fruits and was almost eleven times than that of black currant. Therefore for prevention of cure of scurvy ribena should be recommended. In the present study more L-AA content than their respective unprocessed fruits because they contain additional L-AA as a preservative.

Lecol pure lemon and Realorange had comparable amounts of L-AA and were two to three times higher than the other processed fruits analysed in the present study. Among commercial soft drinks, Sonic Fanta had the highest content of L-AA while Coca-Cola and Fanta had the lowest contents and were comparable. Among the vegetables studied, cowpea leaves and dania contained comparable values of L-AA and were the highest.

Among the fresh fruits studied, all were found to be within the levels reported by other workers (Sonja et al. 1988; Herman, 1984) from other regions with the exception of red passions and red guavas whose levels were higher. Red guavas and passions were found to have higher mean levels than their white counterparts. This is because the mean levels of L-AA in fresh fruits depend on the variety (Sonja et al. 1988). In processed fruits the most widely used preservatives are sodium benzoate, sulphur dioxide, among others (Carnevale, 1980). These preservatives are used to deactivate L-ascorbic acid oxidase which is a copper containing enzyme that is responsible for the oxidation of L-AA. Among the vegetables, carrots had the lowest content of L-AA. However, for dania, solanum, pumpkin leaves, cowpea leaves and gynandra, there were no reported values in literature for comparison.

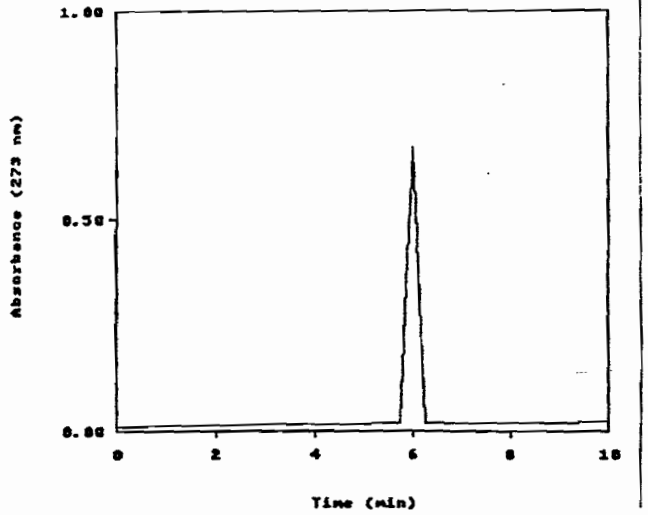

Fig. 1 Chromatograph showing L-AA in LC analysis of an orange juice sample

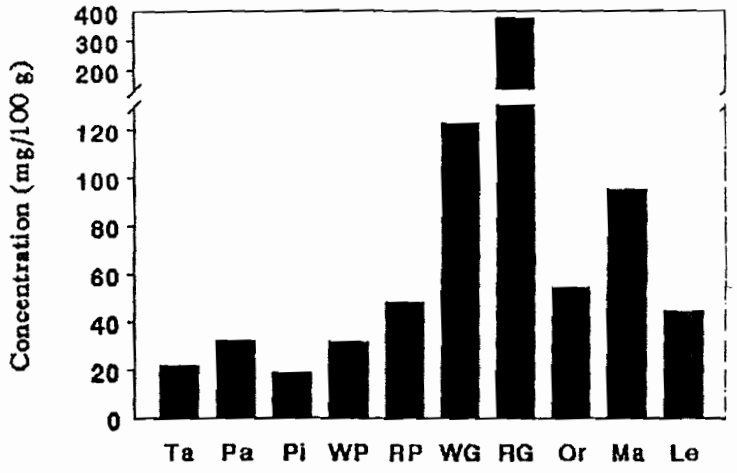

Fig. 2 Bar graph for the concentrations of fresh fruits

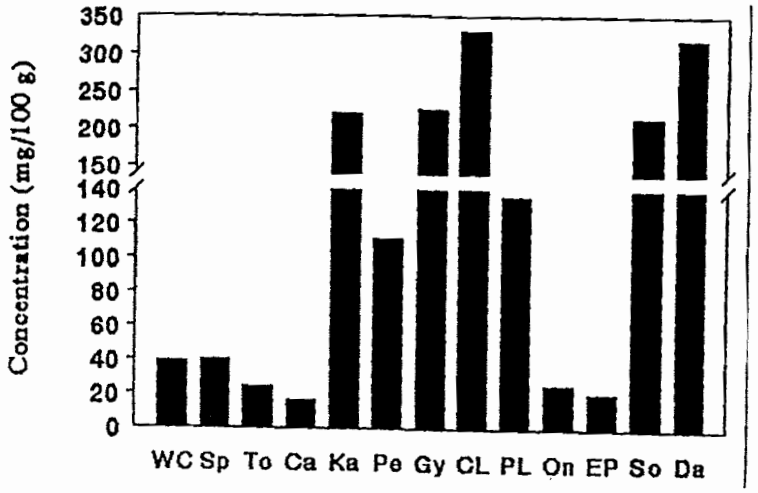

Fig. 3 Bar graph for the concentrations of processed fruits

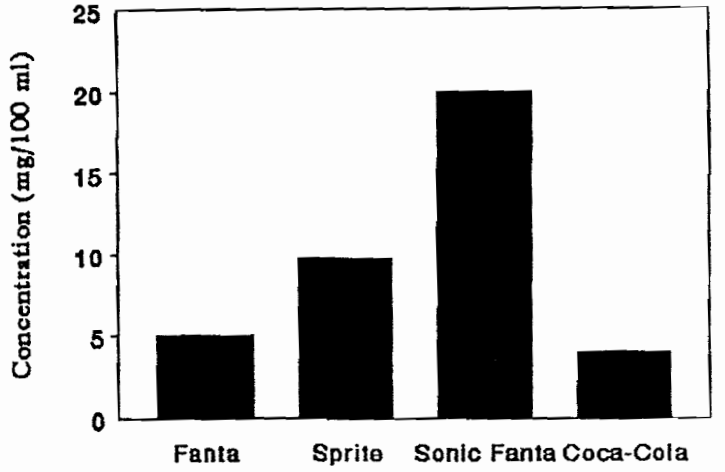

Fig. 4 Bar graph for the concentrations of commercial soft drinks

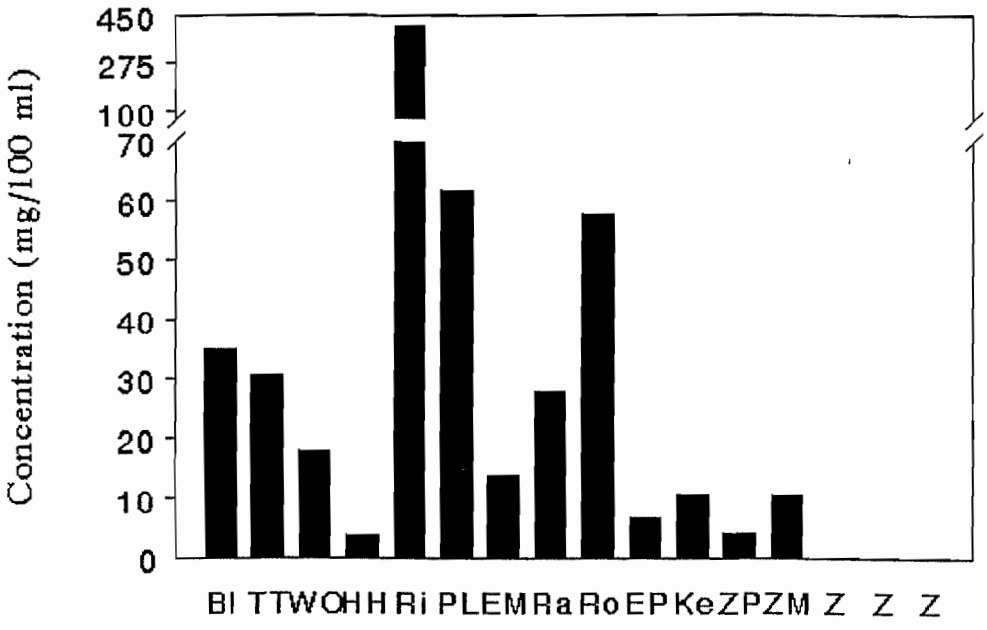

Fig. 5 Chromatograph showing L-AA in LC analysis of an orange juice sample

4.0 CONCLUSION

An RP-LC method with UV-VIS detection (λ=273 nm) for the determination of L-AA with 0.1M phosphate buffer and flow rate of 2 ml/min of the mobile phase has been developed and applied for the analysis of L-AA in fresh and processed fruits and vegetables.

Among the fruits studied, red guavas variety of Kenya had the highest mean content (375.0 mg/100 g) while pineapples had the lowest (18.7 mg/100 g). In vegetables cowpea leaves had the highest mean level (330.0 mg/100 g) while carrots had the lowest (16.0 mg/100 g). In processed fruits Ribena had the highest mean level (410.0 mg/100 g) while Hey-ho had the lowest (3.8 mg/100 ml). In commercial soft drinks Sonic fanta had the highest mean level (20.0 mg/100 ml) while Coca-Cola had the lowest (4.0 mg/100 ml).

ACKNOWLEDGEMENTS

Dr. L. Dossaji and Mr. D. Laur, National Museum of Kenya, Nairobi for assisting in the use of HPLC.

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