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Determination of Vitamin C in Fresh Fruits and Vegetables Using the Dye-titration and Microfluorometric Methods

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ABSTRAK

Kandungan vitamin C dalam 19 jenis buah-buahan segar dan 24 sayur-sayuran telah dikaji dengan kaedah-kaedah rasmi AOAC, iaitu titratan pewarna dan mikrofluorometrik. Seperti dijangkakan, hasil yang telah diperoleh daripada kaedah kedua, yang menentukan asid askorbik dan asid dehidroaskorbik (DHAA), adalah lebih tinggi daripada yang didapati daripada kaedah titratan, yang hanya menentukan asid askorbik. Terdapat perbezaan yang agak besar pada hasil-hasil yang diperoleh daripada kedua-dua kaedah, bergantung kepada kandungan DHAA dalam makanan. Perbezaan yang lebih besar telah didapati pada sayur-sayuran. Min hasil bilas daripada kaedah titratan adalah lebih tinggi daripada yang diberi oleh kaedah fluorometrik ($p < 0.01$). Kajian kebolehulangan menunjukkan bahawa perbezaan varians di antara kedua-dua kaedah tidak bererti ($p < 0.05$). Jika hanya kadar asid askorbik diperlukan, kaedah titratan dapat memberi hasil yang memuaskan, dan ia dapat dijalankan dengan menggunakan peralatan makmal yang mudah. Sebaliknya, jika alat fluorometer diperoleh, kadar jumlah vitamin C boleh ditentukan, dan ini adalah lebih berguna dari segi pemakanan.

ABSTRACT

The vitamin C content of 19 types of fresh fruits and 24 vegetables was determined by the official AOAC methods of dye-titration and microfluorometry. As expected, values obtained by the latter method, which estimated ascorbic acid plus dehydroascorbic acid (DHAA), were clearly higher than those given by the titration method, which determined only ascorbic acid. There were considerable differences in the values obtained by the two methods, depending on the concentration of DHAA in the foods. Larger differences were obtained for the vegetables. The mean recovery value obtained by the dye-titration method was significantly higher than that given by the fluorometric method ($p < 0.01$). Reproducibility studies showed that the two methods did not give significantly different variances ($p < 0.05$). If only ascorbic acid values were required, the titrimetric procedure would give good results, and it may be carried out rapidly using simple laboratory equipment. If a fluorometer was available, total vitamin C values, which would be more useful from the nutritional point of view, could be determined.

INTRODUCTION

Vitamin C or ascorbic acid is a water-soluble vitamin, well recognized as an anti-scorbutic food factor. Chemically, it is a six carbon sugar, with a diol grouping at carbons 2 and 3 which is readily oxidised to a diketo group to form dehydroas-

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corbic acid (DHAA) (Bender, 1982). Its ready oxidation to DHAA is the most prominent chemical property of the vitamin.

The two biologically active forms of vitamin C present naturally in foods are L-ascorbic acid and L-dehydroascorbic acid (DHAA). The form most often encountered is L-ascorbic acid, but small amounts of its primary oxidation product, DHAA, may also be present (Christie and Wiggins, 1978).

Procedures for the determination of vitamin C in foods have continued to draw the attention of analysts. Recent reviews in this subject include those of Christie and Wiggins (1978), Cooke and Moxon (1981), Sauberlich *et al.* (1982) and Pachla *et al.* (1985). The complex biological relationship between the compound(s) possessing vitamin C activity, as well as the chemical similarity of these compounds to others that are inactive, has made the existence of a single, simple, and specific method close to impossible (Sauberlich *et al.*, 1982). Thus, there has been a proliferation of methods developed, resulting in hundreds and possibly thousands of papers reported. However, they mostly describe minor variations on a fairly limited range of possible procedures, based essentially on the chemical reactions of ascorbic acid and DHAA (Cooke and Moxon, 1981).

The Association of Official Analytical Chemists (AOAC) has designated two official methods for the determination of vitamin C: the dye-titration method and the microfluorometric method (AOAC, 1984). The former method makes use of the reducing power of the vitamin, and employs 2, 6-dichlorophenolindophenol (DCIP) as the redox indicator for the determination of ascrobic acid. In the micro-fluorometric method, ascorbic acid is oxidised with active carbon to dehydroascorbic acid to form a fluorescent quinoxaline compound which is measured in a fluorometer.

This laboratory has been employing the dye-titration method using DCIP for the determination of ascorbic acid, values of which have been reported in the Food Composition Table published by the Division (Tee, 1985). Due to problems encountered with the method, mainly with foods which yielded coloured extracts (e.g. fruits and vegetables), an initial study was carried out on the microfluorometric method. The foods were also analysed simultaneously using the dye-titration procedure. This paper reports the results obtained from the study. It serves to demonstrate and quantitate the differences in vitamin C values obtained by the two methods for a variety of locally available foods. Since fruits and vegetables are the major sources of the vitamin in most diets, the study concentrated on the analysis of these foods.

MATERIALS AND METHODS

Samples of fresh fruits and vegetables were purchased from the local markets for analysis. Edible portions of each food sample were analysed by both methods, in duplicate, on the same day of purchase.

The dye-titration method used was essentially that of the AOAC procedure (AOAC, 1984). Metaphosphoric acid extracts of the foods were prepared and pH adjusted to about 1.2. The reducing capacity of the extracts was then measured by titrating with 2, 6-dichlorophenolindophenol (DCIP). In this oxidation-reduction reaction, ascorbic acid in the extract was oxidised to DHAA and the indophenol dye reduced to a colourless compound. End point of the titration was detected when excess of the unreduced dye gave a rose pink colour in acid solution. For samples which yielded highly intense coloured extracts, repeated ether extraction was carried out to facilitate detection of the end-point (details in Tee *et al.*, 1987).

In the microfluorometric method (also as given in AOAC, 1984), food samples were also first extracted into metaphosphoric acid solutions. Ascorbic acid in the extracts was oxidised to dehydroascorbic acid with Norit (active carbon). Aliquots were next reacted with σ-phenylenediamine to give a fluorescent quinoxaline derivative, which, on activation at 350 nm, fluoresces at 430 nm. For each food sample analysed, a specific sample blank was carried out by the addition of boric acid.

For each batch of analyses using either method, recovery studies were carried out by adding 10 mg of ascorbic acid to the food being analysed, and treating in the same manner as the food sample. Mean recovery values for the two methods were compared using t-test. Reproducibility studies were also studied using 4 types each of fruits and vegetables, and carrying out six analyses on

TABLE 1
Vitamin C content of fresh fruits (mg per 100 g edible portion)

	Dye-titration method (1)	Microfluorometric method (2)	Difference (2) – (1)
Pear, yellow *(Pyrus sinensis)*	1.6 ± 0.0 [a,b]	3.0 ± 0.1 [b]	1.4
Apple red *(Pyrus malus)*	2.2 ± 0.1 [a,b]	3.6 ± 0.4 [b]	1.4
Grapes, green *(Vitis vinifera)*	2.9 ± 0.0	1.2 ± 0.1	– 1.7
Coconut, young *(Cocos nucifera)*	5.0 ± 0.0	4.9 ± 0.1	– 0.1
Apple, green *(Pyrus malus)*	5.1 ± 0.2	2.4 ± 0.0	– 2.7
Watermelon, red *(Citrullus lanatus)*	5.2 ± 0.0	5.4 ± 0.3	0.2
Water apple *(Eugenia aquea)*	6.4 ± 0.0 [a]	6.7 ± 0.1	0.3
Banana *(Pisang nangka) (Musa paradisiaca)*	10.2 ± 0.4	28.0 ± 0.0	17.8
Orange, local *(Citrus suhuiensis)*	12.2 ± 0.2 [b]	17.9 ± 0.3 [b]	5.7
Pineapple *(Ananas comosus)*	14.3 ± 1.0 [b]	18.0 ± 1.1 [b]	3.7
Sapodilla *(Achras zapota)*	18.9 ± 1.1 [b]	26.6 ± 2.4 [b]	7.7
Starfruit *(Averrhoa carambola)*	25.4 ± 1.8 [b]	39.4 ± 2.5 [b]	14.0
Lime *(Limau nipis) (Citrus aurantifolia)*	41.7 ± 1.2	41.3 ± 0.1	– 0.5
Orange *(Citrus nobilis)*	42.7 ± 1.5	51.7 ± 2.2	9.0
Lemon *(Citrus limon)*	46.8 ± 0.0	60.2 ± 1.5	13.4
Mango *(Mangifera indica)*	56.5 ± 1.9	77.1 ± 0.5	20.6
Papaya *(Carica papaya)*	67.9 ± 0.6	64.3 ± 1.3	– 3.6
Rambutan *(Nephelium lappaceum)*	69.8 ± 5.2 [b]	86.7 ± 2.8 [b]	16.9
Guava, big *(Psidium guajava)*	98.4 ± 10.9 [b]	101.4 ± 8.4 [b]	3.0

[a] end point of titration difficult to determine due to coloured extracts

[b] mean of 6 determinations; all others, mean of duplicate analyses

each food sample. Analytical process standard deviations of the two methods were compared using the F-ratios method (Wernimont, 1985).

RESULTS AND DISCUSSION

A total of 43 types of fresh fruits and vegetables were studied. Vitamin C content of 19 types of fruits is tabulated in Table 1, whilst those for the 24 fresh vegetables are given in Table 2. Results in both tables are tabulated in ascending order of the vitamin content as obtained by the dye-titration method.

It is clear from results presented in the tables that there was a definite difference between vitamin C values obtained by the two procedures. With a few exceptions, values obtained by the fluorometric method were higher than those given by the dye-titration method. This is to be expected, since in the latter method, only reduced ascorbic acid was determined. In the fluorometric method, the total ascorbate estimated was derived from ascorbic acid as well as preformed DHAA present in the food. The differences in values obtained by the two methods (column 3 in both tables) may then be taken as the content of DHAA present in the fruits and vegetables.

In the case of the fruits analysed, there appeared to be no major difference in the vitamin C content determined by either method for 6 types of fruits (difference $< 10\%$). In another 10 types, the difference was between 17 to 50% of the total ascorbate content. In the remaining 3 fruits, the difference was greater than 60%.

For the vegetables studies, the difference in results obtained by the two methods was found to be higher. Only in 3 vegetables was the difference found to be $< 10\%$. Twelve of them gave a difference of between 13–50%, while for the remaining 9, the difference was greater than 50%.

Recovery values obtained using both methods are tabulated in Table 3. The mean recovery value obtained for the dye-titration method was significantly higher than that for the fluorometric method ($p < 0.01$). Standard deviations and coef-

TABLE 2
Vitamin C content of fresh vegetables (mg per 100 g edible portion)

	Dye-titration method (1)	Microfluorometric method (2)	Difference (2) – (1)
Four-angled bean *(Psophocarpus tetragonolobus)*	0.7 ± 0.2	11.9 ± 0.2	11.2
Lettuce *(Letuca sativa)*	0.9 ± 0.0	4.2 ± 0.1	3.3
Brinjal *(Solanum melongena)*	1.4 ± 0.0[a]	5.1 ± 0.2	3.7
Cucumber *(Cucumis sativus)*	1.4 ± 0.1	16.4 ± 0.7	15.0
Plantain flower *(Musa sapientum)*	1.5 ± 0.2[b]	13.2 ± 1.1	11.7
Carrot *(Daucas carota)*	2.4 ± 0.1[a]	4.5 ± 0.2	2.1
Angled Loofah *(Luffa acutangula)*	4.2 ± 0.1	9.2 ± 0.5	5.0
Small onion *(Allium fistulosum)*	5.4 ± 0.6[a]	6.2 ± 0.1	0.8
Winter gourd *(Benincasa hispida)*	6.2 ± 0.9[b]	15.1 ± 1.1[b]	8.9
Green bean sprout *(Phaseolus aureus)*	7.7 ± 0.1	13.2 ± 0.3	5.5
Yam bean *(Pachyrrhizus erosus)*	9.6 ± 0.8[b]	10.2 ± 1.5[b]	0.6
French bean *(Phaseolus vulgaris)*	10.2 ± 0.4	16.7 ± 0.8	6.5
Potato *(Solanum tuberosum)*	12.7 ± 0.7	24.9 ± 1.0	12.2
Chives, Chinese *(Allium odorrum)*	13.0 ± 1.0	15.4 ± 0.9	2.4
Ladies finger *(Hibiscus esculentus)*	14.0 ± 0.5	30.1 ± 3.2	16.1
String bean *(Vigna sinensis)*	17.2 ± 0.2	21.7 ± 1.1	4.6
Tomato *(Lycopersicum esculentum)*	17.8 ± 0.7[a]	19.0 ± 1.5	1.3
Garlic leaves *(Allium sativum)*	20.8 ± 2.5	28.6 ± 5.6	7.8
Cabbage *(Brassica oleracea)*	25.3 ± 1.9	38.8 ± 1.7	13.6
Spinach, red *(Amaranthus gangeticus sive tricolor)*	27.5 ± 1.5[a]	61.1 ± 4.7	33.6
Bitter gourd *(Momordica charantia)*	46.6 ± 3.9[b]	64.2 ± 5.6[b]	17.6
Mustard leaf, Chinese (*Brassica juncea*)	53.2 ± 6.2	50.8 ± 9.6	–2.4
Cauliflower *(Brassica oleracea)*	54.5 ± 5.3[b]	83.3 ± 5.8[b]	28.8
Tapioca leaves *(Manihot utilissima)*	77.2 ± 6.1[a]	1100.0 ± 14.9	22.8

ficients of variation for the former method were also smaller than those for the latter.

TABLE 3
Recovery Values

	Dye-titration method	Microfluorometric method
Number of determinations	26	25
Mean ± SD	96.5 ±4.7%	91.4 ±8.2%
Coefficient of variation	4.8	9.0
Range	84.6 – 108%	71.4 – 106%

Results of reproducibility studies are tabulated in Table 4. Neither of the two methods gave consistently higher values for coefficients of variation. Comparing the standard deviations, the observed F-ratio was calculated to be 1.07. The two methods did not give significantly different variances ($p < 0.05$).

Since the dye-titration method is based on the oxidation-reduction reaction, a number of other reducing substances in foods (besides ascorbic acid) could interfere with the determination. Many molecules (e.g., phenols, sulphydryls, and triose reductones) and ions (e.g., ferrous, cuprous, or sulphite) are able to reduce the DCIP dye (Pachla *et al.*, 1985), and therefore giving rise to falsely high titration results. Generally, interferences can be overcome by adjusting the pH and other reaction conditions so that most other materials react only very much more slowly than does ascorbate (Bender, 1982).

Another major practical problem associated with the titrimetic method of DCIP is the difficulty in ascertaining end-point when the food ex-

TABLE 4
Variation in methods for the determination of vitamin C

	Dye-titration method		Microfluorometric method	
	mean ± SD	CV[1]	mean ± SD	CV
Winter gourd	6.2 ± 0.9	14.4	15.1 ± 1.1	7.4
Yam bean	9.6 ± 0.8	8.6	10.2 ± 1.5	14.9
Bitter gourd	46.6 ± 3.9	8.3	64.2 ± 5.6	8.7
Cauliflower	54.5 ± 5.3	9.6	83.3 ± 5.8	6.9
Apple, red	2.2 ± 0.1	4.5	3.6 ± 0.4	10.4
Pineapple	14.3 ± 1.0	6.8	18.0 ± 1.1	6.2
Starfruit	25.4 ± 1.8	7.2	39.4 ± 2.5	6.4
Guava, big	98.4 ± 10.9	11.1	101.4 ± 8.4	8.3

Each value is the mean of 6 analyses
[1] Coefficient of variation

tracts are coloured, especially reddish-purplish colours. As indicated in Tables 1 and 2, this problem was encountered for several fruits and vegetables in this study. Even with the aid of ether to determine the end-point, unsatisfactory results have been encountered. Furthermore, with the additional ether extraction steps, the procedure became rather tedious.

The fluorometric method is a more lengthy procedure, and requires the availability of a fluorometer. It is, however, less susceptible to interference. The use of boric acid in sample blanks in order to take into consideration extraneous fluorescence under the same reaction conditions is an important step in this procedure. Borate inhibits the reaction of ascorbate with the diamine, but does not prevent the formation of fluorophore by other compounds.

CONCLUSION

The study has quantitated the differences in vitamin C values obtainable by the dye-titration and fluorometric methods for a number of fruits and vegetables. There is considerable variation in these differences, depending on the amount of DHAA present in the food.

It is clear that the use of the dye-titration method would result in underestimation of total vitamin C activity in a food. However, if only ascorbic acid values are required, the titrimetric procedure as described would give good results for most samples. The exception would be those that yield highly intense coloured extracts. Careful titration using ether extraction should be carried out for these foods. Attention should also be paid to ensure that no interfering substances are contributing to the titration results. In spite of these potential difficulties, the titration method has become widely used due to its convenience. Rapid determinations may be carried out with simple laboratory equipment. In the comparative studies reported by Wills *et al.* (1983) and Bradbury and Singh (1986), there was fairly good agreement in ascorbic acid levels estimated by the simple dye-titration method and the more sophisticated liquid chromatography procedure.

If a fluorometer was available, ascorbic acid plus DHAA values may be determined. It is, however, a more lengthy procedure, and greater care has to be given to the various steps involved. Reproducibility studies have to be carried out, and effort made to minimize variations. Recovery runs have to be undertaken frequently to monitor performance. From the nutritional point of view, total vitamin C values would be more useful.

Either method may be used, depending on the objectives and resources available to the analyst. The important point is that in reporting vitamin C values, what the analytical data represents must be clearly stated.

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