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A METHOD FOR THE ESTIMATION OF ASCORBIC ACID FROM PRESERVED FRUIT JUICES AND SQUASHES

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A titrimetric method based on the use of potassium ferricynide for the estimation of ascorbic acid in fruit juices and squashes containing metabisulphite or sodium benzoate as preservative has been described.

INTRODUCTION

Previously reported titrimetric methods include the use of 2,6-dichlorophenolindophenol [1] methyleneblue [2], *N*-bromosuccinimide [3], cupric ion [4] and other reagents [5] (e.g. 1,2-naphthoquinone-4-sulphonate, iodine, periodate and chloramin T) for the estimation of ascorbic acid. These methods lack specificity, especially in the estimation of ascorbic acid in fruit juices and squashes where potassium metabisulphite or sodium benzoate is present as the preservative.

Modifications have been proposed in the *N*-bromo succinimide method for the assay of ascorbic acid in the presence of potassium metabisulphite. Nazar *et al.* [6] have proposed the complexing of bisulphite with formaldehyde but the method has been reported [7] to be irreproducible due to the side reactions [7, 8] of formaldehyde with *N*-bromosuccinimide and ascorbic acid. Recently Sarwar *et al.* [7] have used acetone as the complexing agent for the suppression of interference due to metabisulphite but the utility of the method is limited as it is not applicable when sodium benzoate is present as a preservative.

As the fruit juices and squashes are commercial products and invariably contain potassium metabisulphite or sodium benzoate as preservative, therefore the estimation of ascorbic acid in these products is of vital importance. We describe here a simple and accurate titrimetric method for the estimation of ascorbic acid not only in solutions (with or without preservative) but also in preserved fruit juices and squashes. The method is based on the selective oxidation of ascorbic acid by ferricynide, followed by quantitative estimation to an equivalent amount of ferrocynide produced by the help of potassium dichromate using diphenylamine as indicator.

EXPERIMENTAL

All reagents used were of analytical grade.

i. 0.01 N solutions of potassium ferricynide and potassium dichromate were prepared by dissolving the requisite amounts of the reagents in distilled water.
ii. 2.5% (w/v) solution of diphenylamine in concentrated sulphuric acid was used as indicator.
iii. Ascorbic acid solution was prepared in distilled water and used immediately.
iv. 0.5% (w/v) aqueous solution of potassium metabisulphite or sodium benzoate was used as preservative.
v. 100-200 ml of the juices were obtained by squeezing the requisite amount of fruits while squashes were prepared by the standard commercial methods. 100 ml of the fruit juices or squashes was diluted with distilled water and the volume made up to 250 ml.

GENERAL PROCEDURE

Accurately measured volumes of ascorbic acid solution (5 to 10 ml), potassium ferricynide (0.01 N, 10 ml) and sulphuric acid (1:20 v/v, 20 ml) were taken in an 150 ml Erlenmeyer flask. The contents were thoroughly shaken for 2-3 min. at ambient temperature followed by the addition of 85% orthophosphoric acid (10 ml) and diphenylamine solution (2 drops). The mixture was titrated against standard potassium dichromate solution till the appearance of a blue colour.

Estimation of ascrobic acid in the presence of preservative. Titration was carried out as described above except that 1 ml. solution of the preservative per 10 ml. of the sample solution and sulphuric acid (1:20 v/v, 20 ml) were added before the addition of ferricynide solution.

Table 1. Estimation of ascorbic acid in neat solutions and in the presence of preservative (1 ml of preservative per 10 ml sample)

Ascorbic acid (mg/100 ml)	Neat solution	Error (∓%)	Presence of PMB*	Error (∓%)	Presence of SB*	Error (∓%)
1	1.01	1.00	1.01	1.00	1.01	1.00
2	1.99	0.50	2.01	0.50	1.98	1.00
4	4.01	0.25	4.04	1.00	4.02	0.50
6	6.02	0.33	6.05	0.83	6.04	0.66
8	7.99	0.12	8.04	0.50	8.06	0.75
10	10.01	0.10	10.05	0.50	10.03	0.30
(μg/100 ml)						
5	4.86	2.80	4.85	3.00	4.87	2.60
10	9.85	1.50	10.16	1.60	10.15	1.50
25	25.25	1.00	25.28	1.12	25.26	1.04
200	199.40	0.30	201.00	0.50	200.80	0.40
500	502.00	0.40	502.40	0.48	503.00	0.60
800	801.00	0.12	803.00	0.37	802.50	0.31
1000	999.00	0.10	1002.00	0.20	1002.00	0.20

*PMB = Potassium metabisulphite SB = Sodium benzoate.

Table 2. Estimation of ascorbic acid from preserved juices and squashes (1 ml of preservative per 10 ml of sample) (mg/10 ml)

Sample	Without preservative	Presence of *PMB	Error (∓%)	Presence of SB	Error (∓%)
Orange squash	3.50	3.49	0.28	3.48	0.57
Lemon squash	4.50	4.45	1.11	4.51	0.22
Mango squash	5.10	0.15	0.98	5.08	0.39
Orange juice	6.00	6.01	0.16	6.03	0.50
Kinnow juice *(Citrus aurantum)*	3.00	2.98	0.66	3.03	1.00

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*PMB = Potassium metabisulphite SB = Sodium benzoate

Table 3. Recovery of ascorbic acid added to preserved fruit juices and squashes

	Sample	Original centents mg%	Ascorbic acid added mg%	Found mg%	Recovery mg%	Error (∓%)
1.	Orange squash	35	10	45.10	10.10	1.00
2.	Lemon squash	45	10	54.80	9.80	2.00
3.	Mango squash	51	10	60.90	9.90	1.00
4.	Orange juice	60	25	85.20	25.20	0.80
5.	Kinnow juice *(Citrus aurantum)*	31	20	50.85	19.85	0.75
6.	Grape fruit juice	43	10	53.10	10.10	1.00

Calculation. Amount of ascorbic acid (mg) = V x C

V = Volume of 0.01 N potassium dichromate used.

C = 0.88 mg of ascorbic acid per ml. of the titrant used.

RESULTS AND DISCUSSION

In the present investigation ferricynide has been used as an oxidising agent in an acid medium and the amount of the ferrocynide produced is determined by the help of potassium dichromate. At pH less than 5, the ascorbic acid has been reported [9] to show lower oxidation potential (0.16 – .18 volts) as compared to bisulphite (0.9–1.1 volts). Since, in the present method, the pH of the reaction mixture has always remained below 5, the preferential oxidation of ascorbic acid has occurred in the presence of bisulphite.

This procedure supercedes the other titrimetric methods in sensitivity, precision and applicability. The results obtained (Table 1) show that amounts of ascorbic acid (with or without preservative) as low as 5 μg can be determined. Preservatives such as potassium metabisulphite or sodium benzoate pose no interferences and the method can be reliably used for the routine analysis of commercially available preserved fruit juices and squashes as shown by the results presented in Table 2. The recovery of ascorbic acid (Table 3) added to fruit juices and squashes show little interferences from sugars and coloured substances.

The additional advantage of the present method is that standard solutions can be stored for a longer period of time without affecting the method efficiency.

REFERENCES

1. (a) R.N. Chopra and A.C. Roy, Indian J. Med. Res., **24**, 239 (1936).
   (b) M. Freed, *Methods of Vitamin Assay* . (Interscience Publishers, New York, 1966).
2. H. Lund and H. Lieck. Nature (London **137**, 784 (1936).
3. (a) M.Z. Barakat, M.F. Abd el-Wahab and M.M.El-Sadr, Anal. Chem, **27**, 536 (1955).
   (b) D.F. Evered, Analyst, **85**, 55 (1960).
4. M.Z. Barakat, S.K. Shehale, N. Dar Wish and A. El-Zoheiry, Anal. Biochem, **53**, 245 (1973).
5. M. Hashmi, *Assay of Vitamins in Pharmaceutical Preparations* (John Wiley and sons, New York 1973).
6. M. Nazar, M.Y. Ikram-ul-Haq and A.F.M. Ehtesham uddin, Pakistan J. Sci. Ind. Res, **11**, 381 (1968).
7. M. Sarwar, Zafar Iqbal and Shaheen Zaidi, Mikrochim. Acta (Wien), 699 (1975).
8. (a) M. Yamaguchi and T. Adachi, Nippon Kagaku Zasshi, **79**, 487 (1958).
   (b) M. Yamaguchi, Nippon Kagaku Zasshi, **77**, 513 (1956).
9. J. Lindquist, Analyst, **100**, 339 (1975).