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Stable, Convenient-working Extractants for the Determination of L-ascorbic Acid in Citrus Extracts

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As an effort to find a more stable, convenient-working extractant than regularly used methaphosphoric acid for determination of L-ascorbic acid (AA) in citrus extracts, several extractants for assay of AA were examined. The extractants tested were (I) 15 g stick HPO_3+ 40 ml acetate+450 ml H_2O, (II) 0.5% stick HPO_3+0.2% Oxalate+H_2O, (III) 0.2% Oxalate+5% Acetate+H_2O, (IV) 0.2% Oxalate+ 10% Acetate+H_2O, (V) 7% Citrate+0.5% Malate+H_2O. Based on measurements of AA stored in the extractants and AA content in the juice of *Citrus limon* Burm. cv. Frost Nucellar Lisbon, the combinations of oxalate plus acetate resulted in more convenient, less expensive and more stable extractants.

INTRODUCTION

Chemical methods for determination of L-ascorbic acid (AA) are based upon its reducing properties. Among many available methods, the titrimetric method of Barakat *et al. (1973)* seems to be the simplest and most economical one. However, in addition to its limitations such as unsuitable for deep-colored extracts, metaphosphoric acid used slowly reacts with water to form a more acid orthophosphate (Bessey, 1938; Harris and Olliver, 1942), decreasing its capability for stabilizing AA. It has been reported that oxalic acid might be substituted for metaphosphoric acid (Ponting, 1943). From a practical point of view, weighing stick HPO_3 is not convenient. Furthermore, researchers working with fruit materials may have to take various samples and analyze AA along with other fruit qualities at the same time. Therefore, a stable extractant is definitely needed. The present paper reports some extractants and their capabilities after storage in stabilizing AA based on the method of Barakat et al. (1973) as an effort to find a more stable and more convenient-working extractant.

MATERIALS AND METHODS

Extractants tested were (I) Metaphosphoric-acetic acid (MPAA) of Barakat et al. (1973): 15 g stick HPO_3+40 ml acetate+450 ml H_2O, (II) 0.5% stick HPO_3+0.2% Oxalate+H_2O, (III) 0.2% Oxalate+5% Acetate+H_2O, (IV) 0.2% Oxalate+ 10% Acetate+ H_2O, (V) 7% Citrate+0.5% Malate+H_2O. The acid compositions in (II) and (III) were as individually tested by Ponting (1943), whereas those in (V) represent the highest ones

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found in acid citrus we have investigated. Water used was destilled-deionized one. Except the starch indicator, all chemicals were of the highest grades. Stick metaphosphoric acid, glacial acetic acid, citric acid monohydrate, DL-malic acid, KI and water-soluble starch were purchased from Wako Pure Chemical Industries, Ltd., Osaka, Japan. Oxalic acid dihydrate and $CuSO_4.5H_2O$ were purchased from Nacalai Tesque, Inc., Kyoto and L-ascorbic acid was from Katayama Chemical, Osaka, Japan.

Recovery test of AA

Thirty and fifty mg of AA were weighed and dissolved in the above extractants. Parts of them were let to remain in an unairconditioned-room temperature of 33-35°C during Summer 1994, stored in a refrigerator of 2°C, and in a freezer of -2°C for 1 and 2 weeks. A part of them was directly titrated (as a O-week storage). The frozen samples were slowly thawed to room temperature and quickly refrigerated while waiting for titration.

AA in lemon juice

Two fruits of 'Frost Nucellar Lisbon' lemon *(Citrus limon* Burm.) (at a silver stage) were quickly peeled and extracted with a hand-pressed juicer, and centrifuged at 2,500 rpm for 20 minutes at about 5°C. Ten ml of the supernatant juice was quickly and directly diluted with the above extractants to 100 ml, and subjected to treatments as in the recovery test of AA. Determination and calculation of AA followed Barakat *et al. (1973).* All samples were run in duplicate.

RESULTS AND DISCUSSION

The results of AA recovery test are summarized in Table 1. Except extractant V which is definitely not recommended, AA was quite well recovered from extractant I to IV up to one week in the refrigerator and two weeks in the freezer. The results also showed that extractant I to IV were similarly good for stabilizing AA, especially when the samples were frozen. The results were further confirmed with the juice sample (Table 2). It should be noted, however, extractant I was turned out to be less stable than extractant II to IV in stabilizing AA of juice sample. Gradual conversion of metaphosphoric acid into orthophosphoric acid (Bessey, 1938; Harris and Olliver, 1942) and a better stabilizing capability of oxalate than acetate (Ponting, 1943) might explain the inferior performance of extractant I. Comparing with the published data of the same cultivar (Sinclair, 1984), lower results in Table 2 might be due to differences in cultivation procedures, climatic conditions and maturation. However, although extractant II, III, and IV are comparably good, working with extractant III and IV should be more convenient, less expensive and more stable.

As shown here either extractant III or IV can be substituted for MPAA in the method of Barakat et al. (1973). By using this modified method, consequently colorless samples will give better results as the end of titration can be sharply recognized. Those who work with citrus fruits may find this modified method convenient and reliable since interfering substances are not present in appreciable amounts in citrus fruits (Harris and Olliver,

Table 1. L-ascorbic acid (AA) recovered from various extractants at various storage duration.

Extractant[x]	AA weighed (mg)	Storage (week)	Per cent recovery of AA (%)		
			Troom[y]	Trefrig.[y]	"freezer"
I	30	0[z]	98.49 [a]		
		1	72.81	98.60 [g]	104.56 [f]
		2	32.06	94.39 [e]	98.17 de
	50	0[z]	99.07 [b]		
		1	76.83	98.63 [g]	94.91 [cd]
		2	45.84	95.99 [f]	93.62 [c]
II	30	0[z]	100.28 [d]		
			74.75	100.52 [ijk]	99.90 [ef]
			39.46	98.38 [g]	100.14 [ef]
	50	0[z]	100.17 [d]		
			81.74	101.44 [ijk]	101.31 [ef]
		2	57.56	100.18 [hi]	101.65 [ef]
III	30	0[z]	101.26 [e]		
			52.45	101.70 [jk]	101.98 [ef]
		2	22.52	98.26 [g]	101.11 [ef]
	50	0[z]	99.57 [c]		
		1	75.85	101.82 [k]	101.91 [ef]
		2	52.92	98.95 [gh]	102.33 [ef]
IV	30	0[z]	104.36 [f]		
			66.14	104.36 [l]	104.36 [f]
		2	10.85	98.57 [g]	104.21 [f]
	50	0[z]	100.54 [d]		
			63.80	100.24 [hij]	99.85 [ef]
		2	23.58	95.92 [f]	100.06 [ef]
V	30	0[z]	98.71 [ab]		
		1	2.60	62.24 [c]	87.05 [b]
			0	16.59 [a]	81.92 [a]
	50	0[z]	99.01 [b]		
			28.05	83.56 [d]	87.66 [b]
			0	45.03 [b]	89.43 [b]

[x] I=15 g HPO_3+40 ml Acetate+450 ml H_2O; II=0.5% HPO_3+0.2% Oxalate+H_2O; III=0.2% Oxalate+5% Acetate+H_2O; IV=0.2% Oxalate+ 10% Acetate+H_2O; V=7% Citrate+0.5% Malate+H_2O.

[y] Troom=room temperature of 33-35 °C; Trefrig.=in the refrigerator of 2 °C; Tfreezer=in the freezer of-20 °C; the same letters in the same column were not significantly different at 5% level according to Duncan New Multiple Range Test.

[z] A direct titration at the room temperature.

Table 2. Ascorbic acid content in the juice of 'Frost Nucellar Lisbon' lemon detected by various extractants at various storage duration.

Extractant[w]	Storage (week)	Ascorbic acid content (mg/100 ml)		
		[T]room[)]	[T]refrig.[)]	[T]freezer[)]
I	0[x]	**24.64** [a]		
	1	*0*	17.16 [b]	22.88 [b]
	2	*0*	*20.95* [c]	*19.80* [a]
II	0[z]	29.75 [b]		
		0	**24.64** [d]	94.91 [cd]
	2	**0**	**27.35** [e]	**28.34** [c]
III	0[z]	**30.80** [b]		
		0	29.75 [cf]	29.48 [c]
	2	*0*	**28.60** [cf]	28.16 [c]
IV	0[z]	**30.89** [b]		
		0	30.36 [f]	30.80 [c]
	2	**0**	*29.31* [ef]	28.60 [c]
V	0[z]	29.22 [b]		
		0	0 [a]	19.80 [a]
	2	**0**	0 [a]	22.44 [ab]

[x,)] 'See notes in Table 1.

1942) and dehydroascorbic acid concentration is low (Sinclair, 1984). Moreover, various chemical analyses can be accomplished without interfering each other since samples for AA can be stored in a freezer up to two weeks (possibly longer) providing that extraction with the extractants is quickly done and other possible loses due to oxidation are prevented.

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