



インドフェノール滴定法，インドフェノール・キモレン法およびヒドラジン法による生馬鈴薯のビタミンC含有量の評価

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Estimation of Vitamin C Content in Raw Potatoes by Ind-Titrn, Ind-Xylene, and Hydrazine Method

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北川三郎, 渡部典子: インドフェノール滴定法, インドフェノール・キ
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Customarily, we put potatoes to a good use in school meals, so that it is necessary to evaluate the real vitamin C value to determine the ascorbic acid (AsA) content in raw potatoes.

Methods for the determination of AsA have been proposed for avoiding interference from other non-AsA. In fresh vegetables, especially in potatoes, the determination value for AsA is affected by the presence of various compounds, like plant pigments as flavones, reductones and so on.

Oxidation of AsA by 2, 6-dichlorophenolindophenol is found available for determining AsA. However, this method is not applicable for the analysis of products containing water- or acid-soluble pigments or reducing agents for AsA. These non-AsA react with the dye and influence the end point of titration. In many more pigment-containing, or turbid acid, extracts, this method is not entirely satisfactory for measuring the degree of reduction of the dye. Then it becomes possible to use a xylene extraction, eliminating interference from acid soluble pigments, because of the readily soluble in xylene.

The most widely used method for the determination of the total vitamin C is based upon Roe's procedure. Among the number of methods available, the oxidation-reducing and Roe's methods are widely used as analytical procedures in the measurement of vitamin C.

Potatoes are not rich in AsA, but we take so many potatoes that often provide a considerable proportion of AsA in the diet of school meals. However, their AsA contents are not held constant for the same species. The AsA level of raw potatoes from 4 to 50 mg/100 g is generally given in the literatures. A AsA level of 30 mg or over can only be found in fresh potatoes, whereas a level of 15 mg is given for them from Japanese Food Composition Tables¹⁾.

In this experiment, the contents of AsA in raw potatoes were investigated by the following methods; 2, 6-dichlorophenolindophenol visual titration method, Indophenol-xylene extract photometric method, and 2, 4-dinitrophenylhydrazine method. The AsA values of them are considered from the estimation of the applicable vitamin C level by comparison with the values of these methods.

Materials and Methods

The species, white potatoes, "Danshaku"; Hokkaido-grown potatoes at Kamikawa. After raw potatoes are cut and chopped, samples are treated very rapidly and blended with metaphosphoric acid as quickly as possible, preventing further oxidation of AsA. The samples are separated into three parts. The part containing the peels is referred to as the whole; the part of peeled potatoes, the edible; and the part of only peeled chops, the peel. The peels of potatoes are removed by the paring-knife and are cut into thin slices. The three parts are respectively used for determining their AsA by the following methods.

The 2, 6-dichlorophenolindophenol visual titration method (Ind-Titron method): this method is based upon the procedure of the Vitamin Assay by the Association of Vitamin Chemists²⁾.

The Indophenol-xylene extraction method (Ind-Xylene method): this method follows the same procedure as the photometric assay²⁾.

The 2, 4-dinitrophenylhydrazine method (Hyd-method): this method is an adaptation of the method described by Roe; it follows the same procedure²⁾. The instrument used for these methods is the photoelectric spectrophotometer, Shimazu Model QV-50.

Results

Calibration constant K for ascorbic acid by two methods

Ind-Xylene method

The preparation of the calibration chart for AsA followed the same procedure of the Vitamin Assay²⁾. The instrument was set at 100% transmittance with distilled water, the transmittance readings were converted to optical density at 500 m μ . Table 1 shows the determination of the instrument constant K for the standard AsA solution in each concentration. Our average K values are usually kept constant linearly, but are variable in lower concentration. It seems to be stable in the range of about 0.020 mg per ml to 0.030 mg per ml for determining

TABLE 1 Calibration constant K for ascorbic acid by Ind-Xylene method

Trial No.	Optical density							
	Ascorbic acid mg per ml							
	0.004	0.006	0.008	0.010	0.015	0.020	0.025	0.030
1	0.260	0.273	0.296	0.303	0.310	0.313	0.331	0.378
2	0.351	0.385	0.333	0.313	0.323	0.317	0.347	0.393
3	0.196	0.273	0.296	0.303	0.316	0.323	0.336	0.370
4	0.278	0.316	0.308	0.313	0.304	0.313	0.325	0.395
5	0.351	0.375	0.333	0.340	0.410	0.370	0.373	0.395
6	0.339	0.316	0.308	0.313	0.346	0.337	0.341	0.378
7	0.204	0.244	0.270	0.289	0.300	0.307	0.342	0.387
8	0.238	0.300	0.297	0.292	0.338	0.314	0.342	0.385
9	0.267	0.254	0.270	0.289	0.310	0.314	0.331	0.390
10	0.225	0.280	0.272	0.288	0.305	0.302	0.331	0.378
Av.	0.271	0.302	0.298	0.304	0.326	0.321	0.340	0.383
K*	1.476	1.987	2.685	3.289	4.601	6.231	7.353	7.833

* means extinction coefficient.

AsA. The dye solution must be checked with a definite concentration of the standard AsA solution in each assay.

Hyd-method

The preparation of the calibration chart followed the same procedure of the Vitamin Assay²⁾. As shown in fig. 1, the calibration chart for AsA was prepared by plotting per cent transmittance as ordinate and concentration of AsA (mg per ml) as abscissa on semilogarithmic paper. The plot of transmittance % against AsA mcg per ml was found to be linear. The optimal concentrations for determining AsA are given in a range from 4 mcg per ml to 12 mcg per ml according to the calibration curve for the standard AsA solution.

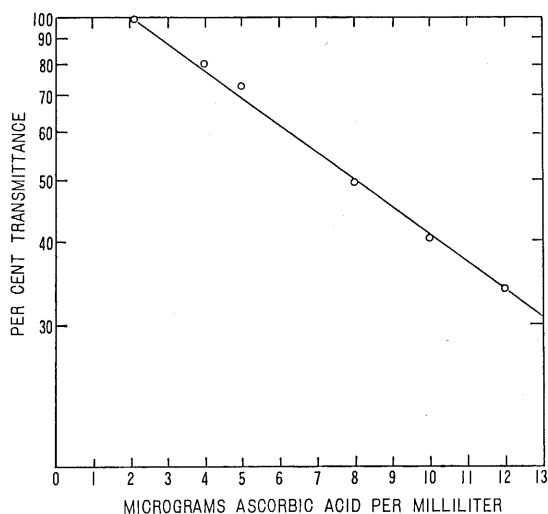


FIG. 1 Calibration chart for ascorbic acid

Ascorbic acid content of raw potatoes by Ind-Xylene method

The stability of the color in the xylene layer should be checked by preparing the total dye tubes at the beginning and at the end of a series of samples. In this method, the peroxide treatment is applied to correct the action of the non-AsA reducing materials present. This procedure by peroxide is described as treat-A. The formaldehyde treatment is also applied to correct a similar action. This procedure is also described as treat-B. Under our experimental conditions, these treatments were not necessary for our samples, but they were carried out in each assay to investigate the liable variation of the AsA values due to these correction treatments.

The AsA contents of each part in raw potatoes are shown in tables 2, 3 and 4 respectively. Data in these tables are summarized in the following table and involve a comparison of AsA values with the three parts in regard

TABLE 2 Ascorbic acid content of whole part in raw potatoes by Ind-Xylene method

Trial No.	Ascorbic acid mg per 100 g		
	Non-Treat	Treat-A	Treat-B
1	20.55	20.12	17.48
2	20.25	19.80	17.48
3	20.38	19.04	16.06
4	19.62	19.60	16.64
5	19.00	18.98	16.82
6	20.78	20.38	18.31
7	19.97	18.87	16.05
8	20.25	19.24	16.25
9	19.94	19.97	16.72
10	19.30	18.94	16.29
Av.	20.00	19.50	16.85

to these treatments.

TABLE 3 Ascorbic acid content of edible part in raw potatoes by Ind-Xylene method

Trial No.	Ascorbic acid mg per 100 g		
	Non-Treat	Treat-A	Treat-B
1	18.73	18.09	14.56
2	18.90	18.67	14.66
3	18.87	19.42	14.38
4	19.08	18.56	15.31
5	19.02	19.48	19.90
6	18.93	19.15	15.76
7	18.50	18.42	14.82
8	18.94	18.57	14.48
9	18.78	18.42	15.13
10	18.86	18.25	14.90
Av.	18.86	18.70	14.89

TABLE 4 Ascorbic acid content of peel part in raw potatoes by Ind-Xylene method

Trial No.	Ascorbic acid mg per 100 g		
	Non-Treat	Treat-A	Treat-B
1	3.34	3.06	1.24
2	3.18	2.66	1.38
3	3.52	3.11	1.17
4	3.00	3.03	0.55
5	3.73	2.85	1.16
6	3.18	2.69	1.27
7	2.98	2.77	1.22
Av.	3.28	2.87	1.29

Average AsA contents of each part in raw potatoes by treatments in Ind-Xylene method

Parts of potatoes	AsA mg per 100 g		
	Non-Treat	Treat-A	Treat-B
Whole	20.00	19.50	16.85
Edible	18.86	18.70	14.89
Peel	3.28	2.87	1.29

The AsA content of any part with the non-treat gives a relatively higher level compared with the treatments. These correction treatments in the AsA assay tend to show a slight decrease on the non-treat level. It is noteworthy that the value of the peel part indicates significant quantities on its AsA level. It also seems that this level depends on the influence of non-AsA reducing materials, especially on some plant pigments in the peel of raw potatoes. This point is the task of the next study if the considerable AsA level in the peel is a true vitamin C in raw potatoes.

The results in table 5 show the AsA values in each part of raw potatoes and their mean errors by these treatments. The variation of the values is found to be great from the values by the treat-B in each part. There is so considerable doubt as to whether the variation is due to samples or to these treatments.

TABLE 5 The standard deviation of the levels of ascorbic acid in raw potatoes

Part of Raw Potatoes	Ascorbic acid mg per 100 g		
	Non-Treat	Treat-A	Treat-B
Whole	22.16 ± 0.14	21.59 ± 0.06	19.78 ± 0.20
	20.00 ± 0.19	19.50 ± 0.12	16.85 ± 0.17
Edible	19.69 ± 0.06	19.21 ± 0.12	13.45 ± 0.17
	18.86 ± 0.04	18.70 ± 0.10	14.89 ± 0.09
Peel	3.28 ± 0.07	2.87 ± 0.05	1.29 ± 0.08

Ascorbic acid content of raw potatoes by Hydrazine method

The AsA content of raw potatoes was determined by the Hyd-method under the same conditions of the Ind-Xylene method. The procedure of the assay was described above.

As shown in fig. 1 and table 6, the stability of the calibration curve in the Hyd-method was in a marked contrast to the unstable liability of the calibration in the Ind-Xylene method.

As shown in tables 7, 8 and 9, the AsA contents of each part of raw potatoes are obtained from the same sample at two dilutions; 10 ml-, and 5 ml-diln. The AsA values at 10 ml-diln. were higher than those at 5 ml-diln. from the assay of each part, especially in the case of the whole part. The AsA values obtained from the assay of one sample at two dilns did not appreciably agree even with any part of raw potatoes. The same interferences are indicated from substances other than AsA. The AsA values of the peel by this method showed a considerable higher level than those by the Ind-Xylene method.

TABLE 6 Calibration of spectrophotometer for ascorbic acid by Hyd-method

Trial No.	Transmittance % for Ascorbic acid (ml per cgm)					
	2	4	5	8	10	12
1	105	89.0	80.0	67.0	57.0	44.0
2	105	90.5	84.0	68.0	57.0	44.0
3	105	91.0	83.0	67.5	56.5	44.0
4	105	90.0	83.0	64.0	58.0	44.0
5	101	88.0	84.5	66.0	56.0	45.0
6	105	87.0	82.0	66.0	55.0	45.0
7	103	90.0	84.5	67.0	56.0	45.0
8	102	87.0	80.0	67.0	54.0	43.5
9	101	87.0	79.0	58.0	54.0	42.0
10	105	88.5	82.0	61.0	51.5	45.0
Av.	104	88.8	82.2	60.1	55.4	44.2

TABLE 8 Ascorbic acid content of whole part in raw potatoes by Hyd-method

Trial No.	Ascorbic acid content of whole part				
	10 ml-dilut		5 ml-dilut		Average
	T %*	AsA mg %	T %*	AsA mg %	AsA mg %
1	48.0	16.8	70.0	10.2	13.5
2	49.2	16.6	71.0	9.8	13.2
3	48.8	16.4	70.0	10.2	13.3
4	49.0	16.6	70.0	10.2	13.4
5	48.0	16.8	69.0	10.4	13.6
6	48.0	16.8	70.0	10.2	13.5
7	48.0	16.8	70.0	10.2	13.5
8	48.0	16.8	70.0	10.2	13.5
9	48.2	16.8	71.2	10.0	13.4
10	49.0	16.6	69.2	10.4	13.4
Av.	48.0	16.8	70.0	10.2	13.4

* means per cent transmittance

TABLE 7 Ascorbic acid content of edible part in raw potatoes by Hyd-method

Trial No.	Ascorbic acid content of edible part				
	10 ml-dilut		5 ml-dilut		Average
	T %*	AsA mg %	T %*	AsA mg %	AsA mg %
1	54.0	14.8	76.0	8.8	11.8
2	53.0	15.0	78.5	8.2	11.6
3	56.5	13.8	72.0	9.8	11.8
4	55.5	14.4	75.0	9.0	11.7
5	52.0	15.4	78.0	8.2	11.8
6	57.8	13.6	70.0	10.2	11.9
7	58.5	13.0	69.0	10.4	11.7
8	58.0	13.4	69.0	10.4	11.9
9	57.0	13.8	71.0	10.0	11.9
10	57.8	13.6	70.0	10.2	11.9
Av.	56.0	14.1	72.9	9.5	11.8

* means per cent transmittance

TABLE 9 Ascorbic acid content of peel part in raw potatoes by Hyd-method

Trial No.	Ascorbic acid content of peel part				
	10 ml-dilut		5 ml-dilut		Average
	T %*	AsA mg %	T %*	AsA mg %	AsA mg %
1	79.0	8.2	86.0	6.6	7.4
2	88.0	8.0	82.0	7.6	7.8
3	78.5	8.2	82.0	7.6	7.9
4	79.0	8.2	82.5	7.2	7.7
5	77.0	8.6	82.0	7.6	8.1
6	76.0	8.8	82.5	7.2	8.0
7	81.0	7.8	83.5	7.2	7.5
8	79.0	8.2	81.5	7.6	7.9
Av.	78.7	8.3	82.8	7.3	7.8

* means per cent transmittance

Comparison of ascorbic acid contents in raw potatoes by Ind-Titrn, Ind-Xylene and Hydrazine methods

The AsA contents of each part in raw potatoes were respectively determined by the Ind-Titrn, Ind-Xylene, and Hyd-methods for the same samples. The AsA values in tables 10, 11 and 12 show the comparison between each part of raw potatoes by these three methods. As shown in tables 10 and 11, the AsA values of both parts, except the peel are given on similar levels by these methods. The AsA values of the peel, as shown in table 12, show a remarkably higher level only by the Hyd-method. It must be investigated further whether this high level involves the AsA content, and to what extent the true vitamin C exists in the peel of raw potatoes.

TABLE 10 Comparison of ascorbic acid contents of edible part in raw potatoes by Ind-titrn, Ind-Xylene and Hyd-method

Trial No.	AsA content of of edible part mg %		
	Ind-Titrn	Ind-Xylene	Hyd
1	15.70	16.29	16.00
2	15.70	16.65	16.40
3	15.70	16.29	16.00
4	15.70	15.83	16.00
5	15.70	16.20	16.00
6		16.65	16.00
7		15.38	16.60
8		15.74	16.40
9		16.11	
Av.	15.70	16.12	16.20

TABLE 11 Comparison of ascorbic acid contents of whole part in raw potatoes by Ind-titrn, Ind-Xylene and Hyd-method

Trial No.	AsA content of of whole part mg %		
	Ind-Titrn	Ind-Xylene	Hyd
1	17.0	19.0	18.0
2	17.0	18.7	17.0
3	17.0	18.5	18.6
4	17.0	18.0	17.0
5	17.0	18.9	16.6
6		18.0	17.4
7		18.1	17.4
8		18.1	17.0
Av.	17.0	18.4	17.4

TABLE 12 Comparison of ascorbic acid contents of peel part in raw potatoes by Ind-titrn, Ind-Xylene and Hyd-method

Trial No.	AsA content of peel part mg %		
	Ind-Xylene	Ind-Titrn	Hyd
1	3.1	2.9	12.2
2	3.1	3.9	13.2
3	3.1	3.0	11.6
4	3.1	3.9	11.4
5	3.1	4.6	12.8
6		3.5	12.4
7		4.1	11.6
8		6.9	11.8
Av.	3.1	4.1	12.1

Discussion

As pointed out by Akiji Fujita, *et al.*³⁾, the AsA values by the Hyd-method for foods have been given at a rather higher level than those of the Japanese Food Composition Tables¹⁾. Their report indicate that many foodstuffs for animal and vegetable origins show remarkable AsA values by the Hyd-method, but the TLC values were negligible.

According to the report by R. Zacharias, *et al.*⁵⁾, the AsA values by 10 analytical methods were discussed more fully for determining AsA in raw potatoes. They suggested that variable AsA values were obtained by comparing the direct assay with the indirect assay of the thin-layer chromatographic procedure. Their reports suggest that many alternative methods or many techniques of separation and purification give rise to some loss of AsA through long or tedious

procedures. In spite of the development of newer techniques some reports point out that it is very difficult to determine the existence of the true vitamin C in raw potatoes.

The AsA values in raw potatoes are apt to variate through many alternative methods. However, it seems that the AsA values assayed by the Ind-Titrn method, out of many alternative methods, are considered to be the real vitamin C content of raw potatoes.

Summary

A practical application of the general routine methods: 2,6-dichlorophenolindophenol visual titration method, Indophenol-Xylene extract photometric method, and 2,4-dinitrophenylhydrazine method were investigated for the determination of the AsA content in raw potatoes. The whole and the edible parts of raw potatoes showed a similar AsA value by these three methods, except the peel part by the Hydrazine method.

This study includes the investigation of possible variations of the AsA values of raw potatoes by the main routine method. The AsA values are liable to vary with the troublesome procedures of the alternative methods.

Considering many conditions, the 2,6-dichlorophenolindophenol visual titration method seems to reveal the real vitamin C content for AsA determination in raw potatoes.

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