Austin Journal of Nutrition and Food Sciences

Open Access

Austin Publishing Group

Research Article

Application of Vitamin C Measurement Procedure using 4-Hydroxy-2, 2, 6, 6-Tetramethylpiperidine-1-Oxyl and O-Phenylenediamine Dihydrochloride for Various Fruits

Kiuchi S[1], Ihara H[1]*, Nemoto I[1], Hirano M[1], Watanabe S[1], Nishiguchi Y[2], Namba S[3] and Hashizume N[4]

[1]Faculty of Risk and Crisis Management, Chiba Institute of Science, Japan

[2]Department of Pharmaceutical Practice, Toho University, Japan

[3]Department of Medical Technology, Tsukuba International University, Japan

[4]Department of Health and Nutrition, University of Human Arts and Sciences, Japan

***Corresponding author:** Ihara H, Faculty of Risk and Crisis Management, Chiba Institute of Science, Japan

Received: November 23, 2017; **Accepted:** December 22, 2017; **Published:** December 29, 2017

Abstract

Application of a measurement procedure for total Ascorbic Acid (AsA) content (i.e., the sum of AsA and Dehydro Ascorbic Acid (DAsA)) in various fruits using 4-hydroxy-2, 2, 6, 6-tetramethylpiperidine-1-oxyl and o-phenylenediamine dihydrochloride was introduced. Total AsA and DAsA contents in 13 fruits (i.e., kiwifruit, pineapple, mango, tomato, grapefruit, blueberry, banana, watermelon, plum, peach, melon, apple and pear) were measured, and the observed total AsA content in those fruits was significantly correlated with that reported in the "Standard Tables of Food Composition in Japan".

Keywords: Nutrition; Ascorbic acid; Vitamin C; Fruits; 4-Hydroxy-2, 2, 6, 6-tetramethylpiperidine-1-oxyl (TEMPO)

Abbreviation

AA: Ascorbic Acid; DHAA: Dehydroascorbic Acid; SVCT1: Sodium-dependent Vitamin C Transporter Type 1; GLUT1: Glucose Transporter 1; TEMPO: 4-Hydroxy-2, 2, 6, 6-tetramethylpiperidine-1-oxyl; OPDA: o-Phenylenediamine Dihydrochloride; MPA: MetaphosphoricAcid; HPLC: High-performance Liquid Chromatography; ECD: Electrochemical Detection; DNPH: 2, 4-Dinitrophenylhydrazine; GLO: L-GulonolactoneOxidase; RDA: Recommended Dietary Allowance

Introduction

We previously developed an automated method for measuring total ascorbic acid concentration (i.e., the sum of ascorbic acid: AsA and dehydro ascorbic acid: DAsA) in serum using 4-hydroxy-2, 2, 6, 6-Tetramethylpiperidine-1-Oxyl (TEMPO) and O-Phenylenediamine Dihydrochloride (OPDA) [1]. OPDA reacts exclusively with DAsA, and the AsA in specimens was first oxidized to DAsA by the addition of TEMPO. DAsA originally present in the specimens and that derived from As A were both condensed with OPDA to form a fluorescent product measurable by spectrophotometry at 340 nm. Good agreement had been shown in total AsA concentrations in serum between measurement by the TEMPO method and that by High-Performance Liquid Chromatography (HPLC) with Electro Chemical Detection (ECD) [TEMPO = 0.99 (HPLC) + 0.1, r = 0.981]. Laboratories without access to HPLC could measure serum total AsA concentration using the TEMPO method.

Previously, we applied the automated TEMPO method for total AsA and DAsA concentrations in urine specimens [2]. Furthermore, we reported that urinary excretion of DAsA in healthy adults varied from 0.9 to 13.8 mg/L. Here, we introduce the possibility of application of the TEMPO method [3] for measurement of total AsA and DAsA contents in various fruits. The present method is a manual procedure, and is thus suitable for laboratories without access to an automated analyzer.

Sample treatment: Ten volumes of 4.0% Meta Phosphoric Acid (MPA) were added to one part flesh of the fruits (some contained the peel) or squeezed juice of the fruits (w/v). After homogenization, samples were centrifuged at 6,200 rpm (2,000 × g) for 5 min to spin down the pellet and obtain a clear supernatant. The supernatant was used for the following analysis.

Materials and Methods

Reagent

TEMPO solution was prepared by dissolving 20 mg of TEMPO (Sigma-Aldrich Co. LCC., St. Louis, MO, USA) in 100mL of 0.067 mol/L phosphate buffer, pH 6.4. OPDA solution was prepared by dissolving 5 mg of OPDA (Wako Pure Chemical Industries, Ltd., Osaka, Japan) in 10mL of the same buffer, followed by storage in a refrigerator (4°C) in the dark. MPA solution (4%) was prepared by dissolving 4 g of potassium metaphosphate (Wako Pure Chemical Industries) in 100 Ml of distilled water. A standard solution was prepared by dissolving 10 mg of DAsA (Wako Pure Chemical Industries) in 100mL of distilled water.

Assay procedure

An aliquot (0.125mL) of the above supernatant ("measurement") or standard solution ("calibration") was mixed with 1.0 mL of TEMPO solution and incubated at 37°C for 5 min. Next, 0.425 mL of OPDA solution was added to the mixture, and the absorbance (A340) of the reaction mixture was measured using a Hitachi 7012 spectrophotometer (Hitachi High-Technologies Corporation, Tokyo, Japan) for 15 min. AsA content (mg/L) in the supernatant was obtained from the A 340 of "measurement" as compared with that

Citation: Kiuchi S, Ihara H, Nemoto I, Hirano M, Watanabe S, Nishiguchi Y, et al. Application of Vitamin C Measurement Procedure using 4-Hydroxy-2, 2, 6, 6-Tetramethylpiperidine-1-Oxyl and O-Phenylenediamine Dihydrochloride for Various Fruits. Austin J Nutri Food Sci. 2017; 5(3): 1095.

Table 1: AsA and DAsA contents in various fruits.

	Our analysis			STFCJ
	Total AA (mg/100 g)	DHAA (mg/100 g)	DHAA/Total AA ratio (%)	Total AA (mg/100 g)
Apple	18.1	6.3	34.8	6
Banana	10.0	9.4	94.0	16
Dragon fruit	3.4	2.9	85.3	7
Grapefruit	40.6	9.6	23.6	36
Japanese lemon	53.4	8.3	15.5	40
Kiwifruit	101.6	13.0	12.8	69
Lemon	52.7	9.4	17.8	50
Mango	18.6	3.2	17.2	20
Oleaster	8.1	7.2	88.9	5
Pineapple	59.6	10.8	18.1	27
Satsuma mandarin	46.9	7.2	15.4	32
Strawberry	78.3	11.3	14.4	62

AsA: Ascorbic Acid; DAsA: Dehydro Ascorbic Acid; STFCJ: Standard Tables of Food Composition in Japan (2015).

of "calibration", and AsA content in fruits was expressed in terms of mg AsA per 100 g of fruit. For the measurement of DAsA, TEMPO solution was replaced by 0.067 mol/L phosphate buffer, pH 6.4, followed by incubation and addition of OPDA solution in the same manner as with the AsA measurement in the absence of TEMPO. The assay range was linear from 0.0 mg/L up to 100 mg/L for DAsA content, but the Limit of Quantitation (LOQ) was 1.0 mg/L. Despite being a manual procedure, within-day variation for AsA content (10.0±0.2mg/L) was found to have a Coefficient of Variation (CV) of 2.0%. Between-day variation for the same samples over ten successive days exhibited a CV of 4.7%. Meanwhile, pressed apple juice spiked with 10.0 mg/L AsA showed a recovery of 13.1 mg/L.

Total AsA and DAsA contents in the various fruits were assayed (Table 1). Although not the same sample was assayed, total AsA content in these fruits (except for melon) was significantly correlated with those reported in the "Standard Tables of Food Composition in Japan: STFCJ [4]" [TEMPO = 1.08 (STFCJ) + 0.04, r = 0.970]. However, conflicting results were observed with regard to DAsA content between our study and previous reports. In our study, DAsA and AsA contents [(DAsA, mg/100g)/ (total AsA, mg/100 g)] were 7.8/8.4 (93%) in banana and 4.0/4.3 (92%) in apple. In contrast, Mazurek et al. [5] reported these respective contents as 2.24/15.42 (14.5%) in banana, while Roe et al. [6] reported 0.0/4.5 (0.0%) in apple; all of the samples were purchased from a city market. However, Wills et al. reported that DAsA content was not present in fresh banana fruits assayed following purchase from local retail markets in Australia [7].

AsA content in fruits following harvest was decreased with a simultaneous increase in DAsA over time [5]. Finally, DAsA is irreversibly converted to 2, 3-diketogulonic acid, which does not react with OPDA. The degradation of AsA to DAsA, and then to 2, 3-diketogulonic acid could be the reason for the differences between our results and the previous reports. Because the various fruits assayed in this study were purchased from a grocery store, we were unable to adjust the post-harvest analysis dates to correspond to the previous reports. The discrepancy between the current AsA result in melon and that in STFCJ remains to be further investigated, by measuring the same specimens simultaneously by the TEMPO method and HPLC, since there are different kinds of melon in Japan. Moreover, we are planning to investigate endogenous substances (i.e., sugar, acid, vitamin, and carotenoid) present in fruits and their possible interference with the TEMPO method.

Conclusion

Total AA content (i.e., the sum of AA and DHAA) in fruits could be easily determined by a manual procedure with the use of TEMPO-mediated oxidation of AA and the condensation reaction between DHAA and OPDA. However, the accurate determination of DHAA content remains to be further investigated, since DHAA was artificially produced from AA during the storage of fruits post-harvest. In the context of AA degradation to DHAA during storage, total AA represents a practical measure of the actual vitamin C content in fruits.

Acknowledgment

We deeply thank Ayaka Sasamoto, Akane Adachi, and Takanori Ishii, Chiba Institute of Science for their help in interpreting the significance of the results of this study. This study was supported by the Research Foundation of the Chiba Institute of Science.

References

1. Ihara H, Matsumoto N, Shino Y, Aoki Y, Hashizume N, Nanba S, et al. An automated assay for measuring serum ascorbic acid with use of 4-hydroxy-2,2,6,6-tetramethylpiperidinyloxy, free radical and σ-phenylenediamine. Clin Chim Acta. 2000; 301: 193-204.

2. Ihara H, Shino Y, Hashizume N, Moto M, Ikarashi H, Horie H, et al. Determination of dehydroascorbic acid in human urine. J Anal Bio-Sci. 2006; 29: 447-452.

3. Ihara H, Kiuchi S, Kobayashi T, Yanagida R, Niida M, Harada K, et al. Simple and rapid determination of vitamin C content in various fruits using 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl and o-phenylenediamine dihydrochlorides. Int J Anal Bio-Sci. 2017; 5: 66-70.

4. Office for Resources, Policy Division Science and Technology Policy Bureau (Japan). Standard Tables of Food Composition in Japan-2015, 17th revised edn.

5. Mazurek A, Pankiewicz U. Changes of dehydroascorbic acid content in relation to total content of vitamin C in selected fruits and vegetables. Acta Sci Pol, Hortorum Cultus. 2012; 11: 169-177.

6. Roe JH, Oesterling MJ. The determination of dehydroascorbic acid and ascorbic acid in plant tissues by the 2, 4-dinitrophenylhydrazine method. J Biol Chem. 1994; 152: 511-517.

7. Wills RBH, Wimalasiri P, Greenfield H. Dehydroascorbic acid levels in fresh fruit and vegetables in relation to total vitamin C activity. J Agric Food Chem. 1984; 32: 836-838.

Citation: Kiuchi S, Ihara H, Nemoto I, Hirano M, Watanabe S, Nishiguchi Y, et al. Application of Vitamin C Measurement Procedure using 4-Hydroxy-2, 2, 6, 6-Tetramethylpiperidine-1-Oxyl and O-Phenylenediamine Dihydrochloride for Various Fruits. Austin J Nutri Food Sci. 2017; 5(3): 1095.