Ascorbic Acid Determination in Vegetables and Fruits: Comparison of Colorimetry with High Performance Liquid Chromatography

Hiroko Seki* and Yuta Nakanishi

Department of Life Science, Faculty of Agriculture, Tamagawa University, Tokyo, Japan

*Correspondence to:
Hiroko Seki
Department of Life Science
Faculty of Agriculture, Tamagawa University
Tokyo, Japan
Tel: +81-42-739-8269
E-mail: sekihiroko@agr.tamagawa.ac.jp

Received: January 02, 2020
Accepted: February 17, 2020
Published: February 20, 2020

Citation: Seki H, Nakanishi Y. 2020. Ascorbic Acid Determination in Vegetables and Fruits: Comparison of Colorimetry with High Performance Liquid Chromatography. J Food Chem Nanotechnol 6(1): 28-32.

Copyright: © 2020 Seki and Nakanishi. This is an Open Access article distributed under the terms of the Creative Commons Attribution 4.0 International License (CC-BY) (http://creativecommons.org/licenses/by/4.0/) which permits commercial use, including reproduction, adaptation, and distribution of the article provided the original author and source are credited.

Published by United Scientific Group

Abstract
Ascorbic Acid (AsA) is included in various fruits and vegetables and its quantity correlates with quality after harvest. The quantity of AsA is usually evaluated by colorimetry but as this technique uses reduction of iron, it is easy for colorimetry to be influenced by reducing agents such as AsA. In this study, we compare colorimetry with high performance liquid chromatography (HPLC) in measurement of AsA in fruits and vegetables. We also evaluated the correlation between colorimetry and antioxidant and iron reduction ability. As a result, the quantity of AsA measured by colorimetry was higher than that with HPLC for reduction materials except for AsA. There was poor correlation between colorimetry and antioxidant ability but good correlation between colorimetry and iron reduction ability. We conclude that colorimetry is not suitable for accurate evaluation of the quantity of AsA in vegetables and fruits but it is suitable for evaluation of iron reduction.

Keywords
Ascorbic Acid, HPLC, Antioxidant ability, Iron reduction ability

Abbreviations
FRAP: Ferric Reducing Antioxidant Power; HPLC: High Performance Liquid chromatography; AsA: Ascorbic Acid; DPPH: 2,2-Diphenyl-1-Picrylhydrazyl; TPTZ: 2,4,6-Tri(2-Pyridyl)-1,3,5-Triazine

Introduction
The consumption of the species defined as functional foods in the medical terminology due to their outstanding phytochemical properties is growing owing to the increasing public awareness of the issue [1, 2]. The importance of berry fruits in a healthy and balanced diet is being increasingly emphasized by dieticians and health care professionals because of their various, rich, and effective phytochemical content [3]. Vitamin C (Ascorbic Acid, AsA) in green vegetables decreases with time [4] but it is used for quality evaluation [5]. Therefore, the accurate measurement of the quantity of AsA in vegetables and fruit is needed. At present colorimetry, titrimetry, enzymatic methods, or HPLC are used [6]. The HPLC method can measure AsA most accurately, but is expensive. There are two methods of colorimetry: (1) AsA reacts with 2, 4-dinitrophenyl-hydrazine directly; (2) Fe³⁺ is reduced to Fe²⁺ by AsA and Fe²⁺ reacts with a color coupler and color appears. The hydrazine method has a high specificity but is slow [7]. The iron reduction method is easy and quick but is affected by other reduction materials and so is more often used. There are three methods of iron reduction colorimetric
measurement of AsA, namely, α, α’-dipyridyl method [7], o-phenanthroline method [8], and ferrozine method [9]. All these methods are often used in determination of AsA in body fluids or tissues in animals [10] but less commonly in fruits and vegetables [6].

Colorimetry of AsA uses the reduction capability of AsA. Therefore other reduction materials present can affect colorimetry results strongly. At present, the determination of the reduction ability of food is often assessed by the ferric reducing antioxidant power (FRAP) method [11]. In addition, it is important to evaluate the antioxidant ability of vegetables and fruits. Colorimetry can also be used to evaluate antioxidant ability in fruits and vegetables using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, an easy and stable method [12]. As colorimetry of AsA using reduction of iron is part of both FRAP and DPPH methods [12], the colorimetry of AsA is more likely to reflect both reduction ability and the antioxidant ability. Therefore, in our study, we compare the quantity of AsA in various fruits and vegetables as measured by colorimetry with that measured by HPLC and evaluate whether we can use colorimetry to evaluate the iron reduction ability and antioxidant ability of various vegetables and fruits.

Materials and Methods

Sample preparation

We purchased oranges, pineapples, lemons, bananas, broccoli, grapefruits, mandarin oranges, and green peppers at the stores near the university. We used strawberries, kiwis, oranges, ponkans, Chinese cabbages, Jabuticabas, raspberries, mangos, apples, passion fruits, artichokes, green soybeans, and tomatoes which were harvested in our university. AsA was extracted by placing 5 g of tissues from fruits and vegetables in 25 mL of 5 % trichloracetic acid; the mixture was shaken vigorously. The mixture was centrifuged (11,509 ×g, 10 min, 5 °C) and the supernatant was used as sample.

Selection of the methods used and comparison of the quantity of AsA measured by the colorimetry and HPLC method in fruit and vegetables

We compared linearity of the 0-50 mg/L standard curve of AsA by the α, α’-dipyridyl method [10], the o-phenanthroline method [8] and the ferrozine method [9]. In the α,α’-dipyridyl method, the reaction mixture consisted of 150 µL of sample, 10 µL of phosphorus acid, 80 µL of 1% dipyridyl solution, 10 µL of 3% ferric chloride 10 µL to sample 150 µL. The reaction mixture was incubated at 30 °C for 15 minutes and measured at 520 nm. In the o-phenanthroline method, the reaction mixture consisted of 250 µL of sample, 10 µL of 3 % ferric chloride, 50 µL of ammonium solution, 50 µL of acetic acid buffer solution (pH 4), and 480 µL of water. The reaction mixture was incubated at room temperature for 10 minutes and measured at 546 nm. We measured some samples by the best of these colorimetry methods (i.e., with the best linearity of standard curve and measuring range) and by HPLC and compared the results of the two techniques. The HPLC details were: pump, LC-10AD (Shimazu, Kyoto, Japan); column, COSMOSIL(R) 5C18-MS-II Packed Column 4.6 mm.ID x150 mm (NACALAI TESQUE, INC, Kyoto, Japan); mobile phase, 2% NaHPO₄ (pH 2.8); flow rate, 0.7 mL/min; temperature, 40 °C; detector, Shimazu SPD-10A; wavelength, 250 nm.

Comparison of the quantity of AsA measured by colorimetry with antioxidant ability via the DPPH method

We used the DPPH method to measure antioxidant ability [13]. To make DPPH solution, 8 mg of DPPH was dissolved in 50 mL of ethanol and 50 mL of water was added. 3.6 mL of DPPH solution was added to 0.4 mL of sample and incubated at room temperature for 30 minutes and measured at 520 nm. We used the blank 50% of ethanol instead of sample and calculated DPPH radical scavenging ability. We compared the quantity of AsA by colorimetry with antioxidant ability by the DPPH method in samples made as above.

Comparison of the quantity of AsA measured by the colorimetry method with the iron reduction ability

We used the FRAP method to measure iron reduction ability [14]. To make 2, 4, 6-Tris(2-pyridyl)-1,3,5-triazine (TPTZ) solution, 31 mg of TPTZ was dissolved in 10 mL of 40 mM hydrochloric acid. FRAP reagent mixture is obtained by mixing acetic acid buffer solution (pH 3.6) 10 with TPTZ solution 1 with 20 mmol/L FeCl₃ 1 in a mixing ratio. We added 1.0 mL of FRAP reagent mixture to 100 µL of sample and incubated at 37 °C for 4 minutes and measured at 570 nm. We showed Fe²⁺ production (µmol/L) using FeSO₄·7H₂O as a control and compared the quantity of AsA measured by colorimetry method with iron reduction ability by the FRAP method in samples made as above.

Statistical analysis

To compare the content of AsA in vegetables and fruits determined using colorimetry and HPLC, T-tests were used for pairwise comparisons. P < 0.05 was considered statistically significant. The relationship between colorimetry and antioxidant ability as well as that between colorimetry and iron reduction ability were evaluated via correlation.

Results and Discussion

Selection of the methods used and comparison of the quantity of AsA measured by the colorimetry method and the HPLC method in fruit and vegetables

Each correlation and standard curve in the α,α’-dipyridyl, o-phenanthroline, and ferrozine methods are shown in table 1. From Table1, the standard curves and correlation coefficients were: $y = 0.027x + 0.083$ and $R^2 = 0.99$ for the α,α’-dipyridyl method, $y = 0.019x + 0.16$ and $R^2 = 0.90$ for the
o-phenanthroline method; and \( y = 0.031x + 0.092 \) and \( R^2 = 1.0 \) for the ferrozine method. Thus the ferrozine method showed the best correlation and was used in subsequent comparisons in our study.

The quantities of AsA in fruit and vegetables measured by the ferrozine method and by the HPLC method are shown in Table 2. The quantities of AsA by the ferrozine method (0.70-1.0 mg/g) were higher than by HPLC (0.010-0.59 mg/g) in all examined sample \((p<0.05)\). The largest difference between the methods was seen in raspberries (0.92 mg/g), while the smallest difference was in oranges (0.023 mg/g). The correlation of the quantities of AsA measured by the two methods was high \((R^2 = 0.92)\) (Figure 3).

### Table 1: Correlation and standard curve.

| Method         | \( R^2 \)  | Standard curve |
|----------------|-----------|----------------|
| \( \alpha', \alpha'' \)-dipyridyl | 0.99     | \( y = 0.027x + 0.083 \) |
| o-phenanthroline | 0.90    | \( y = 0.019x + 0.16 \) |
| Ferrozine      | 1.00     | \( y = 0.031x + 0.092 \) |

Comparison of the quantity of AsA measured by colorimetry with antioxidant ability in DPPH method

The quantities of AsA by the ferrozine method and antioxidant ability by the DPPH method in fruits and vegetables are shown in Table 3. The quantities of AsA measured by the ferrozine method were between 0.058 and 0.62 mg/g, with the highest seen in grapefruits (0.62 mg/g) and the lowest in broccoli (0.058 mg/g). Antioxidant ability measured by the DPPH method was between 25% and 51%, highest in pineapple (51%) and lowest in broccoli (25%). It has been reported that pineapple has several beneficial properties, including antioxidant ability [20]. Furthermore, it has higher antioxidant ability than papaya, blueberry, and olive [21]. In this study, pineapple exhibited the highest antioxidant ability. Thus, fruit and vegetables that have a lot of AsA also have high antioxidant ability but with poor correlation \((R^2 = 0.49)\) (Figure 1). DPPH radical scavenging ability is often assessed in various fruits and vegetables and the main contributing components to DPPH depend on the kind of fruits and vegetables. It was reported that main contributing components are polyphenol in rosemary [22], persimmon leaves [19], pears [23], apples, Japanese pears, peaches (white or yellow pulp), nectarines, Japanese plums, prunes, and grapes [24] and ellagic acid in strawberry [25] and melanoidin in miso [26]. AsA hardly contributes DPPH radical scavenging ability as we confirmed and so colorimetry is not suitable for evaluation of antioxidant ability.

### Table 2: Quantity of AsA in fruit and vegetables measured by the ferrozine method and by the HPLC method.

| Fruit       | Ferrozine (mg/g) | HPLC (mg/g) |
|-------------|-----------------|-------------|
| Japanese oranges | 0.31 ± 0.042   | 0.19 ± 0.022 |
| Oranges     | 0.070 ± 0.0091  | 0.050 ± 0.0066 |
| Ponkans     | 0.37 ± 0.00075  | 0.26 ± 0.0014 |
| Raspberries | 0.94 ± 0.013    | 0.020 ± 0.000017 |
| Jabuticasbas | 0.70 ± 0.060    | -            |
| Mandarin oranges | 0.30 ± 0.0013  | 0.082 ± 0.0032 |
| Tomatoes    | 0.14 ± 0.077    | 0.023 ± 0.0046 |
| Bananas     | 0.66 ± 0.029    | 0.0059 ± 0.00033 |
| Broccolis   | 0.058 ± 0.0049  | 0.013 ± 0.00097 |
| Kiwis       | 0.49 ± 0.0020   | 0.29 ± 0.016 |
| Chinese cabbages | 0.10 ± 0.0010 | 0.045 ± 0.00019 |
| Green peppers | 0.15 ± 0.0020  | 0.0068 ± 0.0012 |
| Lemons      | 0.47 ± 0.0087   | 0.28 ± 0.015 |
| Grapefruits | 0.62 ± 0.033    | 0.27 ± 0.052 |
| Strawberries | 0.80 ± 0.076    | 0.58 ± 0.0015 |
| Pineapples  | 1.0 ± 0.049     | 0.59 ± 0.026 |

Values represent the mean of ten independent determinations \((n = 10)\). Standard deviations are given in ± values.

Our results suggest that as the colorimetry method uses strong iron reduction ability, we can use that method when a sample has little reducing material except for AsA [15]. Many fruits and vegetables have reducing materials other than AsA but the quantity of AsA in orange, lemon, pepper and tomato is measured using 2, 2'-bipyridyl [16]. On the other hand, as the reducing materials affect the measurement of AsA in tomato, AsA was measured using HPLC [17]. The quantity of AsA by the ferrozine method was higher than that by HPLC in fresh and freezing vegetables, and this may be caused by polyphenol in fruits and vegetables [13]. Fruits and vegetables have much more polyphenol than AsA, plums have 167 - 250 mg/100 mL of polyphenol and 2.3-4.0 mg/100 mL of AsA [18], and persimmon leaves have 6.3×10^2 mg/100 g of polyphenol and 87 mg/100 g of AsA [19].

### Table 3: Quantities of AsA by the ferrozine method and antioxidant ability by the DPPH method in fruits and vegetables.

| Fruit       | Ferrozine (mg/g) | DPPH Radical scavenging ability % |
|-------------|-----------------|-----------------------------------|
| Japanese oranges | 0.31 ± 0.042   | 35 ± 2.6 |
| Oranges     | 0.40 ± 0.036   | 34 ± 0.58 |
| Ppainapples | 1.0 ± 0.18     | 51 ± 0.31 |
| Tomatoes   | 0.14 ± 0.077   | 41 ± 1.5 |
| Bananas   | 0.066 ± 0.029  | 29 ± 12 |
| Broccolis | 0.058 ± 0.0048 | 25 ± 15 |
| Green peppers | 0.15 ± 0.0020 | 46 ± 1.7 |
| Lemons     | 0.47 ± 0.0087  | 41 ± 1.9 |
| Grapefruits | 0.62 ± 0.033   | 40 ± 3.8 |

Values represent the mean of five independent determinations \((n = 5)\). Standard deviations are given in ± values.
Ascorbic Acid Determination in Vegetables and Fruits: Comparison of Colorimetry with High Performance Liquid Chromatography

Seki and Nakanishi.

and the iron reduction ability measured by the FRAP method in fruits and vegetables are shown in Table 4. The quantities of AsA were between 0.14 and 0.95 mg/g, with the highest in strawberries (0.95 mg/g) and the lowest in bayberries (0.14 mg/g). The iron reduction ability by the FRAP method was between $1.8 \times 10^2$ and $3.1 \times 10^3$ µmol/L, with the highest in strawberries ($3.1 \times 10^3$ µmol/L) and the lowest in mangoes ($1.8 \times 10^2$ µmol/L). While determining the iron reduction ability of 35 Ugandan fruits and vegetables, it was reported that mangoes had relatively higher values than other fruits and vegetables [27]. Iron reduction ability depends on each fruits and vegetables. There was high correlation between quantity of AsA by colorimetry and iron reduction ability ($R^2 = 0.85$) (Figure 2).

The FRAP method is often used in evaluation of iron reduction ability in various food; strawberry purees [28], avocado, pineapple, banana, papaya, passion fruit, watermelon and melon’s different parts (pulp, seed, raw peel and dried peels) [29], some beverages, chocolates, nuts, and seeds [30]. The quantity of Fe²⁺ reduced from Fe³⁺ by samples are measured in the FRAP method. This is the same principal as used in the ferrozine method, but iron reduction ability evaluated using ferrozine method has not been reported in food samples. As the ferrozine method is used in measuring iron reduction ability in the process of birch and pine wood degradation [31] and in dry beans [32], colorimetry is suitable for evaluation of iron reduction ability.

**Conclusion**

In this study, we evaluated the colorimetry method for AsA measurement in fruits and vegetables. In colorimetry, some reduction components affect AsA measurement in fruits and vegetables and differ from results obtained using HPLC. Therefore, we conclude that colorimetry is not suitable for AsA measurement in fruits and vegetables. The correlation between the ferrozine method and antioxidant ability is low but between the ferrozine method and iron reduction ability is high, so we conclude that colorimetry can evaluate iron reduction ability in fruit and vegetables.

**Acknowledgment**

The authors wish to thank Ms. Miki Katsumata from Food Science Hall at Tamagawa University.

**References**

1. Güneşli M, Korkmaz N, Okatan V. 2019. Polyphenol content and antioxidant capacity of berries: a review. *International Journal of Agriculture Forestry and Life Sciences* 3(2): 350–361.
2. Eskmez I, Polat M, Korkmaz N, Mertoğlu K. 2019. Investigation of some blackberry cultivars in terms of phenological, yield and fruit characteristics. *International Journal of Agriculture Forestry and Life Sciences* 3(2): 233-238.

3. Okatan V. 2018. Phenolic compounds and phytochemicals in fruits of black mulberry (*Morus nigra L.*) genotypes from the Aegean region in Turkey. *Polia Horticulturae* 30(1): 93-101.

4. Nogaki Y, Yoshida K. 1996. Contents and changes of ascorbic acid, reducing sugar and another several ingredients in some vegetables and fruits during storage at domestic refrigerator. *Annals of the Institute of Nutrition Sciences Kagawa Nutrition University* 4: 55-62.

5. Barrett DM, Beadlife JC, Sheveln R. 2010. Color, flavor, texture, and nutritional quality of fresh-cut fruits and vegetables: desirable levels, instrumental and sensory measurement, and the effects of processing. *Crit Rev Food Sci Nutr* 50(5): 369-389. https://doi.org/10.1080/10408391003626322

6. Iijima T, Baba O, Kawata T, Ueno T, Tsukoro T, et al. 1989. Comparison of total AA values in food by 2,4-dinitrophenylhydrazide method, α,α’-dipryridyl method and high-performance liquid chromatography. *Vitamins*—*Journal of the Vitamin Society of Japan* 63(10): 497-502.

7. Okamura M. 1982. Determination of total ascorbic acid in foods by α,α’-dipryridyl method. *Journal of Japanese Society of Food and Nutrition* 35(3): 223-227.

8. Lau OW, Luk SF. 1987. Spectrophotometric determination of ascorbic acid in canned fruit juices, cordials, and soft drinks with iron(III) and 1,10-phenanthroline as reagents. *J Assoc Off Anal Chem* 70(3): 518-520.

9. Iobe K, Yoshikawa H, Ishibashi Y, Iwata H. 1989. Indirect spectrophotometric determination of trace amounts of silicon in pure iron using iron(II)-ferrozine complex. *Bunseki Kagaku* 38(8): 373-377. https://doi.org/10.2116/bunseki Kagaku.38.8_373

10. Murata A, Ishimatsu H, Uchi Y, Kang YC, Kato F. 1986. Determination of vitamin C by the α,α’-dipryridyl method. *Bulletin of the Faculty of Agriculture Saga University* 61: 9-19.

11. Wada M, Kuroda N, Nakashima K. 2016. Analysis of ingredients and assessments of the functionality in functional foods and supplements. *Bunseki Kagaku* 65(6): 301-308. https://doi.org/10.2116/bunseki Kagaku.65.301

12. Suzuka M, Tanaka Y, Kato M, Sago T. 2016. Biological antioxidant potential of cherry. *Bulletin of Yamagata Prefectural Yonezawa University of Nutrition Sciences* 3: 7-10.

13. Araki H, Yamaguchi N, Shinohara N, Watanebe S. 2014. DPPH-radical scavenging activities and polyphenol components for domestic black tea. *Memoirs of Tokyo Seiei College* 6: 1-10.

14. Meguro S. 2016. Studies on the constituents relate antioxidant and α-glucosidase inhibitory activities in extract of the seed and roots of perennial buckwheat. *Ibaraki Christian University Library* 50: 245-249.

15. The Japanese society for food science and technology. 1996. Shinshokuhinbunsekibou, Kourin, Tokyo, 447.

16. Balogh T, Szarka A, Szakra A. 2015. A comparative study: methods for the determination of ascorbic acid in small and middle sized food analytic laboratories. *Acta Alimentaria* 45(3): 354-362. https://doi.org/10.1556/AAlim.2015.0017

17. Miki N. 1981. High performance liquid chromatographic determination of ascorbic acid in tomato products. *Nippon Shokuhin Kogyo Gakkaishi* 28(5): 264-268. https://doi.org/10.3136/nskk1962.28.5_264

18. Komiyama Y, Harakawa M, Tsuji M. 1979. Polyphenol contents and enzymatic browning of plums harvested in Japan. *Nippon Shokuhin Kogyo Gakkaishi* 26(8): 325-330. https://doi.org/10.3136/nskk1962.26.8_325

19. Sonohara N, Izumi K. 1991. The relationship between vitamin C and polyphenol content in persimmon leaves. *Journal of Japanese Society of Nutrition and Food Science* 44(3): 213-219. https://doi.org/10.4327/juns.44.213

20. Hossain MA, Rahman SMM. 2011. Total phenolics, flavonoids and antioxidant activity of tropical fruit pineapple. *Food Res Int* 44(3): 672-676. https://doi.org/10.1016/j.foodres.2010.11.036

21. Moraes CT, Hermes VS, Oliveira RA, Flores SH. 2016. Evaluation of bioactive compounds, chemical and technological properties of fruits byproducts powder. *J Food Sci Technol* 53(11): 4067-4075. https://doi.org/10.1007/s13197-016-2413-7

22. Fujie A, Kuhota M, Umemura Y, Oka K. 2001. Vitamin C content, DPPH radical-scavenging activity and polyphenol content in fresh herbs. *Journal of Food Process Technology* 154(1): 50-54.

23. Araki S, Kudoh Y, Ueno H. 2008. Elucidation of health function on fruits juice extract. *Reports of Kumamoto Industrial Research Institute* 47: 36-41.

24. Ohike N, Kawamata K. 2012. The effects of various blanching treatments on fruit polyphenols. *The Journal of Japanese Society of Nutrition and Dietetics* 70(3): 207-212. https://doi.org/10.5264/eiyogakuzashi.70.207

25. Stangeland T, Renberg SF, Lye CA. 2009. Total antioxidant activity in 35 Ugandan fruits and vegetables. *Food Chem* 113(1): 85-91. https://doi.org/10.1016/j.foodchem.2008.07.026

26. Sone K, Okimura M, Kitari E, Kimura T. 2017. Characteristics of new strawberry cultivar “Oishi Berry” with high ascorbic acid content and high antioxidative effect. *Bulletin of the National Agricultural Research Center for Kyushu Okinawa Region* 66: 65-86.

27. Shimohashi A, Terada K. 2003. Radical-scavenging ability of fruit and effect for radical-scavenging ability by cooking and brewing in food. *The Faculty Journal of Komazawa Women’s Junior College* 36: 1-6.

28. Auby K, Auby K, Wrolstad RE, Ekeberg D, Skrede G. 2007. Polyphenol composition and antioxidant activity in strawberry puree; impact of achen level and storage. *J Agric Food Chem* 55(13): 5156-5166. https://doi.org/10.1021/jf070467a

29. Morais DR, Rotta EM, Sarge SC, Schmidt EM, Bonafe EG, et al. 2015. Antioxidant activity, phenolics and UPLC-ESI(-)-MS of extracts from different tropical fruits parts and processed peels. *Food Res Int* 77(P3): 392-399. https://doi.org/10.1016/j.foodres.2015.08.036

30. Zajko ME, Witkowska AM. 2014. Antioxidant potential and polyphenol content of beverages, chocolates, nuts, and seeds. *Int J Food Prop* 17(1-3): 86-92. https://doi.org/10.1080/10942912.2011.614984

31. Goodell B, Daniel G, Jellison J, Qian Y. 2006. Iron-reducing capacity for resistance to iron deficiency chlorosis in dry bean using iron byproducts powder. *J Food Sci Technol* 53(11): 4067-4075. https://doi.org/10.1007/s13197-016-2413-7

32. Ellsworth JW, Jolley VD, Nuland DS, Blaylock AD. 1997. Screening for resistance to iron deficiency chlorosis in dry bean using iron reduction capacity. *J Plant Nutr* 20(11): 1489-1502. https://doi.org/10.1080/01904169709365351