International Food Research Journal 18(3): 1017-1020 (2011)

Measurement of Ascorbic acid in Australian native plants

*Sommano, S., Caffin, N., McDonald, J. and Kerven, G.

School of Agriculture and Food Sciences, The University of Queensland, Brisbane,QLD, 4067, Australia

Abstract: Ascorbic acid is one of the compounds found in a number of commercially important native plants fruits e.g. Kakadu plum, wild lime and bush tomato. High performance liquid chromatography (HPLC) was used to determine ascorbic acid in these native plant fruits. Ascorbic acid degradation of both standards and plant extracts was observed during HPLC sequence runs. These losses were considerable even though factors such as light, temperature and water activity, which accelerate the loss of ascorbic acid, were eliminated. Several concentrations of sodium metabisulphite were added to both standards and plant extracts to evaluate the effect on the rate of ascorbic acid degradation. A concentration of 500 µg/mL was the most effective but did not eliminate the problem. To correct for any loss still occurring, the rate constant k for ascorbic acid degradation was calculated and used to extrapolate back to the original ascorbic acid concentration. The k value was also found to vary for the different plants studied. For example the k value without added sodium metabisulphite for Kakadu plum, wild lime and Kakadu plum intermediate raw material were 0.00532, 0.02710 and 0.04429 respectively. With the addition of 500 µg/mL sodium metabisulphite the k value decreased to 0.00005, 0.00915 and 0.00586 respectively.

Keywords: Bush food, high-performance liquid chromatography (HPLC), dissolved oxygen, sodium metabisulphite

Introduction

Ascorbic acid (AA) is water soluble vitamin which also known to be one of the more labile vitamins. Fruits and vegetables are the primary dietary source of ascorbic acid. In the past ascorbic acid was named to be anti-scorbutic factor because many studies illustrated its function to prevent scurvy in animals (Asard *et al*., 2004). Recently, the role of ascorbic acid as an *in vivo* antioxidant has obtained much attention. As a function of free-radical scavenger, ascorbic acid can react against *in vivo* peroxidations, including quenching of singlet oxygen species.

Kakadu plum (*Terminalia* spp.) is one of the famous native fruits found in the Northern Territory and Western Australia (Ahmed and Johnson, 2000). It is pale green olive-sized fruit with a stone that clings to the fruit flesh as in a mango. It also has been accepted that Kakadu plum have a high vitamin C content which ranges from 406–5320 mg/100 g edible portion (Brand Miller *et al*., 1993) and as such the fruit has been recommended for further marketing studies and the optimisation of vitamin C production.

Wild lime (*Microcitrus* spp.) is newly named due to their very small juvenile leaves and their minute flowers (Birmingham, 1998). The fruit is round to oblate in shape and approximately 2 cm in diameter, weighing from 1-3 g. The skin is light yellow-green on maturity and contains a large oil gland. The natural distribution of this specie is the semi-arid regions of eastern Australia, from Rockhamton to Longreach in Queensland, south to Dubbo in central New South Wales and west to Quorm, in the flinders ranges of South Australia. Wild lime contains relatively high amount of ascorbic acid (0-82 mg/100 g edible portion) portion (Brand Miller *et al*., 1993).

Oxygen, temperature, light, metal catalysts, pH and the presence of ascorbic acid oxidase lead to the degradation of ascorbic acid. At low pH, ascorbic acid is stable in the fully protonate from. Maximal stability usually performs at pH about 4.

Metal catalysts can increase degradation rate compare to uncatalyzed oxidation. The loss of vitamin C while cooking depends on the degree of heating and how much surface area exposed to the oxygen. However, in the low water activity condition, the crystalline L-ascorbic acid remains highly stable even in the presence of oxygen.

Champion *et al*. (2004) stated that ascorbic acid is oxidized when reacting with oxygen in the presence of air or with oxidant substance or with particular enzyme. Jung *et al*. (1995) studied the effect of singlet oxygen quencher on the destruction of ascorbic acid. They found that the oxidation of ascorbic acid was greatly reduced by the addition of 0.2 mM sodium azide even with the acceleration of ribloflavin. The result shown that 0.2 mM sodium azide inhibited almost 81% of photooxidation after 12 min light storage. Sulpher dioxide and it's satls (sodium and potassium salts) are used in the production of wine. Sulpher dioxide (SO_2) is added in to a bottle of wine

*Corresponding author.
Email: *s.sommano1@uq.edu.au*

in according to inhibit microorganisms and bind with the dissolve oxygen. Excess amount of dissolved oxygen combines directly with ethanol and higher alcohol to form aldehydes which is accumulated and gives wine a flat and stale aroma and flavour (Rotter, 2001).

High performance liquid chromatography (HPLC) was used to determine ascorbic acid in these native plant fruits. The problem due to the use of a sequence HPLC run is that ascorbic acid still degrades even with the temperature and light control. For example, when 60 samples (including replications) are running on HPLC in sequence and the retention time to make a complete elution is set at 30 min, the ascorbic acid content of the last sample may decrease to almost half of the first one. However there is no reported work examining this problem of loss of ascorbic acid during HPLC sequence runs.

Sodium metabisulphite ($Na_2S_2O_5$) does not interfere with ascorbic acid elution but is able to retard the oxidation of ascorbic acid. Since each plant has a different matrix, ascorbic acid might react with some compound in the matrix to make it less or more stable.

This study determines the effect of an oxygen quencher added to native food samples containing ascorbic acid. Sodium metabisulphite was chosen because it does not interfere with ascorbic acid elution but is able to retard the oxidation of ascorbic acid. The rate of degradation of ascorbic acid in each type of raw material was also studied. Since each sample has a different food matrix, ascorbic acid may react with some compound in the matrix to make it less or more stable.

Material and Methods

Raw materials

Kakadu plum which was supplied by Robins Foods , Braeside VIC , Australia were thinly sliced and placed in the plastic trays. The fruits were prefrozen overnight before transferring to freeze drier. As well as wild lime (Robins Foods , Braeside VIC , Australia), the whole fruits were halved and placed in the plastic trays. Dried fruits were grinded to powder, stored in the containers in – 80°C freezer.

Kakadu plum filtrate which was used as an intermediate material for commercial sauces. It was prepared by boiling 12 kg of the plum in 150 kg of water for 30 min. The stone of the fruit could be removed from the fresh in this stage. The filtrate was stored in cool room (4°C) prior to the extraction.

The amount of oxygen quencher (sodium metabisulphite) on standard stability test

The stock standard 1000 µg/mL of ascorbic acid (Sigma-Aldrich, NSW, Australia) was prepared by dissolving 100 mg of ascorbic acid in 100 mL volume metric flask with nitrogen spiked deionize water. Working standards were also prepared by diluting down the stock standard 0.5 mL to 10 mL volumetric flask. Sodium metabisulphite (Sigma-Aldrich, NSW, Australia) at concentrations of 0, 5.0 and 10 mg/100 mL were added in the working standards. Each of the standards was transferred to three vials and the ascorbic acid contents were detected by HPLC at time 0, 24 and 48 hr.

The test was also confirmed by using raw material sample (Kakadu plum filtrate). Approximately 15 g of the filtrate was mixed with 10 mL of 0.05 N phosphoric acid (H_3PO_4) on a rotary shaker for min. The mixture was then centrifuged at 5,000 rpm for 15 min at 4ºC. The effect of $Na_2S_2O_5$ was then tested on the supernatant (ascorbic acid extract).

The amount of sodium metabisulphite was chosen regarding to the ability to delay the degradation of ascorbic acid.

Ascorbic acid stability test on the raw material samples

The dried fruits (0.5 and 1.0 g for Kakadu plum and wild lime respectively) were extracted with 15 mL of extracting solution.

The mixture was then mixed for 30 min at high speed in a rotary shaker and centrifuged at 5,000 rpm for 15 min. The supernatant was transferred to 100 mL volumetric flask. The first sample was diluted without the addition of sodium metabisulphite. The second one was with the addition of x µg/mL of sodium metabisulphite (the amount was chosen according to the previous standard test). Each samples was spiked with 50 µg/mL of ascorbic acid,purified by passing through a C_{18} cartridge, preconditioned by flushing with methanol followed by deionize water, and a 0.45-µm Milipore filter. The ascorbic acid was detected at three time interval 0, 5, 20 hrs. Taking into account that each replicated sample took 30 min cycle to run on HPLC, four replications of each of the samples were , thear for, analysed each days.

HPLC analysis

The prevail C_{18} column (150 x 4.6 mm) was used with 25 mM KH_2PO_4 pH 2.25 as mobile phase (as per procedure developed at Analytical services, The University of Queensland, QLD, Australia). Ascorbic acid standards were prepared at the concentrations of 0, 250, 500 and 1,000 µg/mL. The detection of the ascorbic acid was performed at 235 nm using UV

detector.

Results and Discussion

The amount of singlet oxygen quencher (sodium metabisulphite) on standard stability test

The results (Table 1 and 2) showed that sodium metabisulphite could beneficially retard the degradation of ascorbic acid by removing the oxygen. The following equation explains how sodium metabisulphit (sulpher dioxide salt) quenches with oxygen (Rotter 2001):

$$SO_3^{2-} + O_2 \rightarrow 2SO_4^{2-}$$

At 48 hr and the presence of sodium metabisulphite, % loss decreased from about 95% to 20% (Table 1). Addition of 500 µg/mL sodium metabisulphite into the standard solution was more effective inminimizing the degradation than 1000 µg/mL. Similar result was obtained with plant material samples (Table 1). Therefore, sodium metabisulphite at concentration of 500 µg/mL was chosen for the subsequence tests.

Table 1. The effect of sodium metabisulphite ($Na_2S_2O_5$) on stability of standard ascorbic acid (50 µg/mL) and fruit material (Kakadu plum filtrate)

1) Ascorbic acid standard 50 µg/mL			
		$[Na_2S_2O_5]$	
Time (hr)	0	500 µg/mL	100 µg/mL
0	51.5± 0.1	51.1± 0.05	51.3± 0.07
18	32.1 ± 0.2	46.8 ± 0.1	45.9 ± 0.3
24	25.7 ± 0.4	45.8 ± 0.1	44.4 ± 0.3
48	3.2 ± 0.33	40.7 ± 0.3	37.6 ± 1.06
% loss	93.69%	20.41%	26.76%
2) Kakadu plum filtrate extracts			
		$[Na_2S_2O_5]$	
Time (hr)	0	500 µg/mL	100 µg/mL
0	10.5± 0.2	11.2 ± 0.3	10.0 ± 0.1
24	1.9 ± 0.6	9.2 ± 0.3	8.04 ± 0.4
48	1.4 ± 0.1	7.4 ± 0.4	6.2 ± 0.6
% loss	87.14%	33.69%	37.91%

Values are mean of triplicate samples ± SD.

Ascorbic acid stability test on the raw material samples

The result demonstrated that the % loss of ascorbic acid weredifferent for the three sample tested. Kakadu plum (dried fruit) exhibited the lowest % loss (13%). Kakadu plum filtrate, nonetheless, gave the highest % loss (60%) (Table 2). This may be explained by the fact that ascorbic acid is more stable in the sample with lower aw (Uddin *et al*., 2001).

Table 2. Loss (%) of ascorbic acid in dried Kakadu plum, dried wild lime and Kakadu plum filtrate with and without the addition of sodium metabisulphite

Time (min)	Dried Kakaduplum		Dried wild lime		Kakadu plum filtrate	
		with 500 µg/mL $Na_2S_2O_5$		with 500 µg/mL $Na_2S_2O_5$		with 500 µg/mL $Na_2S_2O_5$
0	861.5 ±96	661.9 ±135	106.9 ±13.5	118.6 ±15.3	86.1 ±9.6	110.3 ±16.1
5	842.4 ±86.150	661.2 ±141.3	96.2 ±13.0	113.6 ±14.6	73.0 ±7.2	106.8 ±15.5
20	774.6 ±86.0	661.2 ±141.4	62.2 ±11.3	98.8 ±9.8	35.5 ±5.6	98.1 ±14.4
% loss	13.6	0.1	41.8	16.7	58.8	11.1

Values are mean of triplicate samples ± SD.

The result in Table 3 also supported the use of singlet oxygen quencher. Using 500 µg/mL sodium metabisulphite in dried Kakadu plum extract decreased the loss from 13% to 0.1% in 20 hrs and more than haft of the % loss decreased in wild lime. Even in the highly water containing sample (Kakadu plum filtrate), the loss reduced from about 60% to 10%. Some of the standard deviation values are quite high but they are consistent across time for each sample. The method may need some attention to improve accuracy.

Table 3. Rate constants (calculated) and half-lives of the reaction

Samples	Rate Constant k ($hour^{-1}$)	R^2	Half-life (hours)
Dried Kakadu plum	0.00532	0.999	130.3
DKP + 500 µg/mL $Na_2S_2O_5$	0.00005	-0.374	14508.3
Dried wild lime	0.02710	1	25.6
DWL + 500 µg/mL $Na_2S_2O_5$	0.00915	0.999	75.7
Kakadu plum filtrate	0.04429	0.998	15.6
KPF + 500 µg/mL $Na_2S_2O_5$	0.00586	0.999	118.2

Result shows good suits in most cases ($R^2 > 0.998$).

Figure 2 represents the relation between ascorbic acid and time of measurement for dried Kakadu plum. Graphs demonstrate the first order relationship and thus the equation for first–order kinetics upon integration of a general reaction rate expression for degradation kinetic (Nisha *et al*., 2004) can be expressed as:

$$\ln c/c_0 = -kt$$

To gain advantage from this relationship, the rate constant k and half life for each sample were calculated in order to extrapolate back to the initial

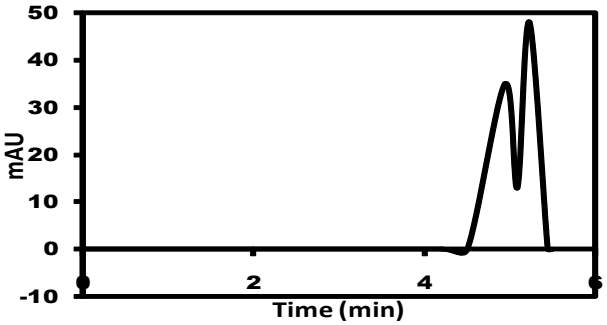

Figure 1. HPLC Chromatogram of stand ascorbic acid (RT= 4.9 min) on C_{18} column with H_2SO_4 mobile phase (see material and methods)

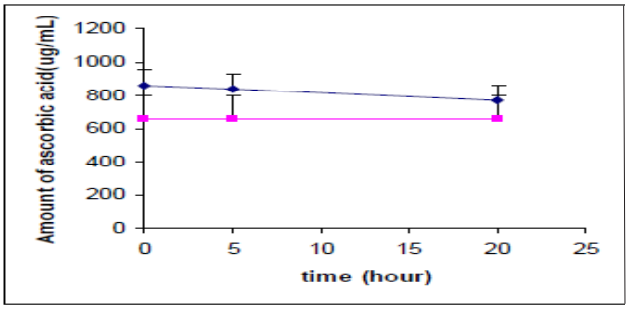

Figure 2. Correlation curve between amount of ascorbic acid and time (hr) of Kakadu plum. ◊-◊ correlation curve of dried Kakadu plum (R^2 = 0.999) ○-○ correlation curve of dried Kakadu plum plus 500 µg/mL sodium metabisulphite

amount of ascorbic acid since there was a long gap between the first and the last samples on HPLC run. The half-lives of dried Kakadu plum, dried wild lime and Kakadu plum filtrate increased by approximately 100, 3, 10 folds respectively (Table 3). These were greater than other food sources such as the half life of ascorbic acid was 34.1 hours at 30°C in dried kiwi fruits (a_w 0.8) (Uddin *et al*., 2001) and 55 hours at 35°C, in intermediate moist apples (a_w 0.83) (Singh *et al*., 1983).

Conclusion

The use of sodium metabisulphite as a singlet oxygen quencher is very useful in retarding the rate of ascorbic acid degradation. The factors effecting degradation are oxygen, light, temperature and a_w. However, during HPLC sequence runs where all of those factors are reduced or eliminated, there are still some losses. The food matrix could be considered one of the factors which might affect ascorbic acid degradation. This study found that the ascorbic acid in different type of fruits showed different rates of degradation. Thus, they were studied individually. Moreover, since the degradation kinetics can be expressed as a first order reaction, the rate constant k can be calculated for each sample. Since loss of ascorbic acid was still observed during long HPLC sequence runs, the k values can be used to extrapolate back to the initial ascorbic acid concentration in the samples.

Acknowledgements

This work was a part of the research project founded by RIRDC, Australia. The author would like to thank Mr. Graham Kervin, school of Lands, Crop and Food Sciences, The University of Queensland, Australia for his grateful experience on HPLC.

References

Ahmed, A. and Johnson, KA. 2000. Horticultural development of Australian native edible plants. Australian Journal of Botany 48: 417-426.

Asard, H., May J.M. and Smirnoff, N. Vitamin C functions and biochemistry in animals and plants, Bios Scientific Publishers, New York , 2004.

Birmingham, E. 1998. Australian native citrus:wild limes form the rainforest to the desert. The Australian New Crops Newsletter (10).

Brand Miller, J., James, K. W. and Maggiore, P. M. A. 1993. Tables of composition of Australian aboriginal foods, Canberra, A.C.T: Aboriginal Studies Press.

Champion, D., Simatos, D., Kalogianni, E.P. Cayot, P. and Le Meste, M. 2004. Ascorbic acid oxidation in sucrose aqueous model systems at subzero temperature. Journal of Agricultural and Food Chemistry 52: 3399-3404.

Jung, M. Y., Kim, S.K. and Kim, S.Y. 1995. Riboflavin-sensitized photooxidation of ascorbic acid: Kinetics and amino acid effects. Food Chemistry S3: 397-403.

Nisha, P., Singhal ,RS. and Pandit, AB. 2004. A study on degradation kinetics of ascorbic acid in amla (*Phyllanthus emblica* L.) during cooking." International Journal of Food science and Nutrition 55(5): 415-422.

Rotter, B. 2001. Sulpher Dioxide. [online] Available: *http://www.angelfire.com/space/wino/SO2.html.,* [Accessed 9th October 2010].

Singh, R.K., Lund, D.B. and Buelow, F.H. (1983) Storage stability of intermediate moisture apples: kinetics of quality change. Journal of Food Science 48: 939-944.

Uddin, M. S., Hawlander, M. N. A. and Liwen, Z. 2001. Kinetics of ascorbic acid degradation in dried kiwifruits during storage. Drying Technology 19(2): 437-446.