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# Determination of Ascorbic Acid in Omani Citrus Fruits

S.M.Z. Al-Kindy, A.O. Abdulnour and Rahma S. Al-Siyabi

*Department of Chemistry, College of Science, Sultan Qaboos University, P.O.Box 36, Al-Khod 123, Muscat, Sultanate of Oman.*

## تحديد كمية حامض الأسكوربيك في الموالح العُمانية

سلمى الكندي ، وعبدالرحمن عبد عبد النور ، ورحمة السيابي

خلاصة تمت هذه الدراسة على الموالح العُمانية في سلطنة عُمان لتحديد كمية حامض الأسكوربيك (فيتامين ج) الموجود بها . وقد كان ذلك بواسطة اكسدة حامض الأسكوربيك بواسطة ن-بروموسكسنيميد وبواسطة *HPLC* ليعض العينات للتاكد من دقة الطريقة المستعملة كما تم تحديد كمية النقصان لحامض الأسكوربيك عند حفظ عصير هذه الموالح تحت درجات حرارة مختلفة اوضحت الدراسة ان كمية فيتامين ج الموجود في الموالح العُمانية تماثل تلك المعترف بها والمعروفة عالميا اوضحت الدراسة كذلك ان انسب درجة حرارة لحفظ عصير الموالح هي درجة حرارة صفر مئوية وان العصير المحفوظ عند درجة حرارة الغرفة (او في الثلاجات المنزلية) يفقد كثيراً من فيتامين ج بمرور الوقت

ABSTRACT: Omani citrus fruits were analysed for their Vitamin C (ascorbic acid AA) content by a titration method involving N-bromosuccinimide (NBS) as an oxidising agent and by high performance liquid chromatography. Vitamin C content was also determined after storage at different temperatures. Vitamin C contents of the fruits were found to lie within the values reported in the literature.

Citrus is a genus of about sixteen species of which ten are commercially grown for their edible fruits. Some of the citrus plants grown locally in Oman include lime (Citrus aurantifolia), sweet orange (Citrus sinensis), sour orange (Citrus aurantium L), mandarin (Citrus nobilis), lemon (Citrus limon) and grapefruit (Citrus paradis).

The composition of citrus fruits varies with cultivar, climate, rootstock and cultural practices (Albrigo and Davies, 1994). Most citrus fruits, like other fruits, are primarily made up of water, but also contain over 400 other constituents including moderate levels of carbohydrates, organic acids, amino acids, ascorbic acid (vitamin C) and minerals (Albrigo and Davies, 1994). Much of the increase in orange juice consumption is linked to the potential health-related benefits of the juice (Nagay and Attaway, 1980). Consumers of the 1990's are interested in low fat, high mineral and vitamin C sources of food.

Vitamin C is the most important water soluble vitamin in citrus fruits (Ting and Rouseff, 1981). It is one of the simplest vitamins being a lactone of a sugar. It is a strong reducing agent, readily losing hydrogen to form dehydroascorbic acid (Lehninger, 1975). Ascorbic Acid (AA) is required in the diet of the following few vertebrates: monkeys, guinea pigs, fruit bats, humans and some fishes. Some insects and other invertebrates also require AA but most higher plants and animals can synthesis it from glucose and other precursors using L-gulonalactose oxidase enzyme (Emsley, 1995).

Prolonged lack of Vitamin C in the diet of humans causes the well known disease, scurvy, while less severe deficiency produces alteration in connective tissue structure and may cause decreased resistance to infections (Lehninger, 1975). Humans need AA to make steroids.

Vitamin C is one of the antioxidants that eliminates peroxides and free radicals and hence reduces the damage from free radicals to the cells, such as premature ageing, heart diseases, cataracts, arthritis and cancer (Emsley, 1995). Scurvy can be prevented by as little as 20 mg of AA per day, but there is evidence that as much as 60 mg per day is required for complete normal physiological function and well being (Tietz, 1986).

In this work the amount of vitamin C in Omani citrus fruits was determined. Vitamin C has previously been determined in citrus fruits but not from Oman. It has long been known that the nutrient content of food varies from sample to sample and from source to source. Furthermore, the amount of vitamin C in citrus fruit is known to be affected by nutrition and fertilisation practices, with the fruit quality being greatly influenced by the amount of N, P and K (Embleton *et al*, 1978). This effect varies from species to species, with sweet oranges showing a greater response to N and P than lemons (Embleton *et al*, 1978). As the amount of N increases,

fruit size, juice content and ascorbic acid level decrease (Davies and Albrigo 1994). The stability of AA in citrus fruit juice stored at different temperatures was also investigated. The storage at room temperature was included for comparison with that at refrigerator temperature which is the normal domestic condition for storage.

### Experimental Procedure and Materials Used

Citrus fruits locally grown were purchased from the Public Authority for Agricultural products in Ghala. All chemicals used were of Analar grade and were obtained from BDH Chemicals Ltd., Poole, England. HPLC grade methanol was obtained form Fluka, England.

INSTRUMENTATION: High Performance Liquid Chromatography (HPLC) system consisted of a Pye Unicam Pu 4015 pump (Philips) with a Rheodyne Injector (Berkeley California) fitted with a 25 µl sample loop. Separation was carried out on a reversed phase column (10 µm , 250 x 4.6 mm i.d.), using 60: 40% MeOH/$H_2O$ as eluent at a flowrate of 1.0 ml/min. A Pye Unicam (Pu 4025, Philips) variable wavelength UV detector was used. Chromatograms were recorded with a Pu 4810 computing (Philips) integrator.

### Method

SAMPLE PREPARATION: Fresh juice (200-250 ml) was squeezed from the fresh fruit. Oxalic acid (1.2 g) was added followed by sufficient filter aid to form a thin paste. The paste was then filtered using a Buchner funnel to obtain a clear juice.

### Determination of Ascorbic Acid

TITRIMETRIC METHOD: Aliquots of fruit juice (5.00 ml) were transferred into 250 ml Erlenmeyer flasks and were titrated against standard NBS-solution (Kennedy 1984) to a blue starch-iodine complex. The analysis was repeated ten times and the mean value calculated. A blank titration was carried out and was corrected for in the final volume.

HPLC METHOD: Pure ascorbic acid standards (20-100 ppm) were injected into the column. The mobile phase (60: 40% MeOH/$H_2O$) was degassed using a vacuum and then passed through the column for at least 60 minutes to equilibriate the column prior to injection. Detection was carried out using 265 nm wavelength. 25 µl of the sample were injected in all cases. The juice samples prepared as described above were filtered with a 0.45 µm millipore filter and injected on to the column as described above for the standards.

STABILITY OF VITAMIN C UNDER DIFFERENT STORAGE CONDITIONS: The stability of vitamin C was determined by storing the fresh juice at ambient temperature on the laboratory bench, in a refrigerator (10◦C) and in a freezer (0◦C). Samples were analysed after 24 hours, one week and three weeks. In all cases the juice was stored without adding oxalic acid.

### Results and Discussion

The concentration of AA in Omani citrus fruits is relatively high especially in oranges, citrus s.p (an un-identified citrus species) and lemons (Table 1). There was no significant difference in AA obtained by the two methods (e.g. 46.14 mg/100 ml for citrus sp. juice, 32.22 mg/100 ml for lime juice (titrimetric method) and 47.00 mg/100 ml for citrus sp. juice, 31.60 mg/100 ml lime (HPLC method), respectively. The concentration of AA in mandarin is close to that reported by Sawamera *et al.* (1990) using HPLC with UV detection. Vitamin C content obtained for oranges (43 mg/100 ml) and for lemon (39 mg/100 ml) lies within the range reported by Friedrich (1988), of 40-60 mg/100 ml for oranges and 40-80 mg/100 ml for lemon, respectively. All the values reported have an error of less than 1.0 % with the coefficient of variation ranging from 0.66% for oranges and 0.78% for lemon.

The vitamin C content reported here is for samples purchased by the housewife for consumption, and no attempt was made to determine AA from citrus fruits grown in various regions of the Sultanate; hence factors such as pre or post harvest, choice of cultivars, cultural practices, storage conditions and growing location were not taken into consideration, although such factors are known to play a major role in vitamin C content. Vanderslice *et al.* (1990) reported a large difference in vitamin C content from California navel orange purchased from different supermarkets. This reflects the importance

TABLE 1

*Ascorbic acid concentration in citrus fruits.*

| Fruits | Ascorbic Acid Conc. (mg/100 ml) |
|---|---|
| Citrus sp. Juice | 46 |
| Orange | 43 |
| Lemon | 39 |
| Grapefruit | 36 |
| Mandarin | 34 |
| Lime | 32 |

TABLE 2

*Ascorbic acid content after one week.*

| Fruit | Orange | Lemon | Grapefruit | Mandarin | Lime |
|---|---|---|---|---|---|
| Ascorbic acid mg/100 ml at 0° | 41.0 | 37.0 | 32.0 | 33.0 | 29.0 |
| Ascorbic acid at room temp. (mg/100 ml) | 38.0 | 32.0 | 29.0 | 30.5 | 25.0 |

TABLE 3

*Ascorbic acid content after 24 hours.*

| Fruit | Orange | Lemon | Grapefruit | Mandarin | Lime |
|---|---|---|---|---|---|
| Ascorbic acid at 0°C (mg/100 ml) | 43.0 | 38.0 | 33.0 | 33.8 | 29.5 |
| Ascorbic acid at room temp. (mg/100 ml) | 38.0 | 35.0 | 31.0 | 32.5 | 28.0 |

of studying vitamin C content as a function of environmental and storage conditions. (Mullin *et al.* 1991).

The Determination of AA concentration after 24 hours at 0∘C and at room temperature revealed that the best storage conditions for citrus fruit juice is at 0∘C (Tables 2 and 3). If the fresh juice is left at room temperature for 1 day it loses between 11-13% of its vitamin C content. This is a typical result as vitamin C is known to undergo air oxidation to dehydroascorbic acid at room temperature.

Studies on the effect of time and temperature revealed that the loss of AA stored in the freezer compartment for a week was only 4-6%, while a higher loss of about 17-20% was obtained for the juice stored at room temperature (Tables 2 and 3). Similar results were obtained by Maini and Attri (1995) for salt-treated lemon juice stored at room temperature. These results substantiate the findings of Aminuddin *et al.* (1982), Heide (1960), and Seur and Rushing (1965) who reported an increase in the loss of AA in citrus fruits with an increase in storage time and temperature. Watada (1987) reported a rapid loss of AA content at higher storage temperature for leafy vegetables. For green beans the loss was as much as 40% after a week at a storage temperature of 10∘C. The loss in vitamin C for fruits was not as high as that of leafy tissues (Watada, 1987) which is in agreement with the results reported here. The results obtained for the determination of vitamin C for the lemon juice stored in a refrigerator (normal domestic storage conditions) showed a continuous decrease in ascorbic acid

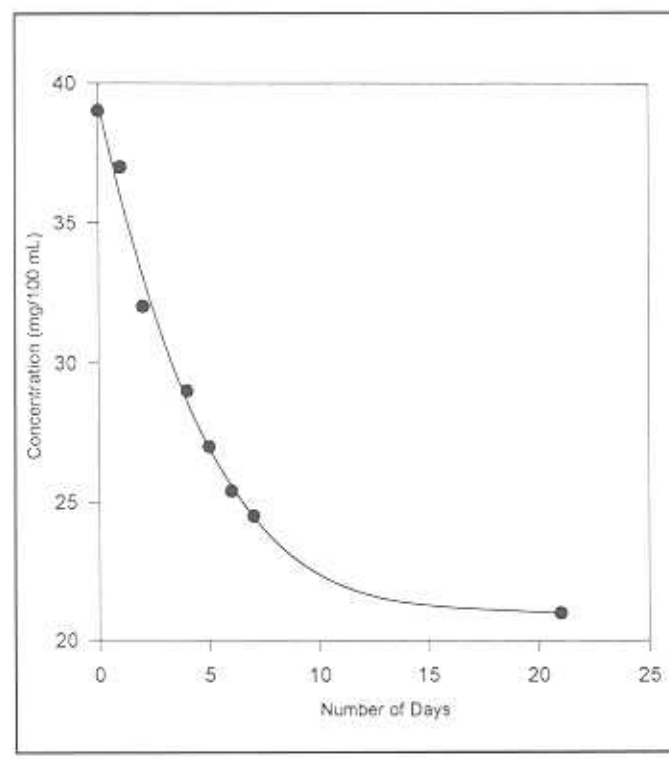


**Figure 1.** Stability of AA in refrigerator.

content with time for a week with the loss amounting to about 46% for the juice stored for 20 days in the refrigerator (Figure 1).

This study shows that vitamin C content of citrus fruits locally grown in Oman lies within the range reported in the literature.

## References


AMINUDDIN M, KHAN W. H. and S PARVEEN M. S., 1982, J. Sci. Res. **XI** 15-20.

DAVIES F. S. and ALBRIGO G. L., 1994, *Citrus Redwood Books*, Trawbridge Wilshire.

EMSLEY J., 1995, Chem Brit **31**, 946.

EMBLETON T. W., JONES, W. W., PALLARES C. and PLATT R. G., 1978, Effect of Fertilisation on Citrus on Fruit Quality and Ground Water Nitrate-Pollution Potential. *Proceedings of the International Society of Citriculture* **2**, 280-285.

FRIEDRICH, 1988. *VITAMINS*, Walter de Gruyler Berlin. New York **5**, 978-985.

HEIDE J. L., 1960, *Nutrition Evaluation of Food Processing*. John Wiley and Sons Inc. N.Y. 153.

KENNEDY J. H., 1984, *Analytical Chemistry Practise*, Harcut Brace, Jovanovich Florida, U.S.A. 94-95.

LEHNINGER A. L., 1975, *Biochemistry*, Worth Publisher N. York. 350.

MAINI S. B. and Attri B. L., 1995, *Indian Journal of Hort.* **52**, 24-26.

MULLIN W. J., JUI, P. Y NADEAU L. and SAMYL T. G., 1991, Con. Inst. Food Sci. Technolo. T. 24, 169-171.

NAGAY S. and ATTAWAY J. A., (eds) 1980, Citrus Nutrition and Quality. *American Chemical Society Washington D.C.* 225-271.

SAWAMERA M. OOISHI S. and ZHONG-FUL., 1990, *J. Sci Food Agri* **53**, 279-281.

SEUR V. J. and RUSHING N. B., 1965, *Food Sci.* **30**, 178.

TING S. V. and ROUSEFF, R. L., 1981, Vitamins in Citrus Juice Products. *4th International Congress of Citriculture* **2**, Tokyo, Japan. 872-877.

TIETZ WIN, 1986, *Textbook of Clinical Chemistry Practice*, W. B. Sanders Philadelphia, U.S.A. 959.

VANDERSLICE J. T., HIGGS D. J., HEYES J. M. and G. BLACK., 1990, *J Food Compo Anal* **3**, 105-118.

WATADA A. E., 1987, *Post Harvest Physiology of Vegetables*. Marcel Dekker N.Y. U.S.A. 455-466.



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