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# Effect of Leaf Maturity and Sampling Time on Leaf Ascorbic Acid Content and Other Quality Parameters of Spinach (*Spinacea oleracea*)

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*ABSTRACT. Several quality determining compounds in Spinach foliage and sampling times (cv. King of Denmark) were tested at different stages of leaf maturity, within 24 hours. Under glass house conditions in summer 1993 ascorbic acid and total sugar contents varied significantly with leaf maturity and sampling time. Significant variations in dry matter, oxalic acid, $Cl_2$ and $PO_4$ contents were observed with leaf maturity. The highest amounts of ascorbic acid and leaf dry matter were recorded in the 5th week after planting (WAP) while total sugar content was highest in the 4th WAP. Evening sampling indicated higher quantities of ascorbic acid, dry matter and total sugar content than in morning sampling. Ascorbic acid was positively correlated with dry matter content and oxalic acid, but was negatively associated with total sugar content. Evening sampling at the 5th WAP could be identified as the best harvesting time, with respect to high composition of vitamin C in spinach leaves.*

## INTRODUCTION

Spinach (*Spinacea oleracea*) is a widely grown vegetable in many countries of the temperate region, and is mainly consumed without processing or cooking. This species is a good source of vitamins and minerals essential for human nutrition. Wills *et. al.*, (1984) reported that a 100 g portion of edible spinach contains 55 mg of vitamin C, 210 mg of carotene, 110 mg of Ca, 3.9 mg of Fe and 85 mg of Mg.

Most quality parameters of vegetables are influenced by various environmental factors. Among these, the effect of temperature on the

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ascorbic acid content is frequently mentioned. Reuther and Nauer (1972), Mudambi and Rajagopal (1977), and Ting (1977) have shown a negative relationship between fruit vitamin C contents and temperature. High oxalate levels in spinach have been reported under long day conditions by Sengbusch *et. al.*, (1965).

Ontogeny or the growth stage has been identified as another major factor affecting plant chemical composition in numerous ways. There are contradictory reports on the effects of the growth stage on oxalate contents of spinach leaves (Sengbusch *et. al.*, 1965; Sing and Saxena, 1972), as ascorbic acid in food is reduced under unfavourable environments. In this regard, knowledge on the relationship of ascorbic acid with other biochemical compounds is extremely important.

The L-sorbase pathway indicates D-glucose as the precursor of ascorbic acid synthesis in plants (Reichstein *et. al.*, 1934). In addition, oxalic acid and L-xylose are the ultimate stable products in the biodegradation pathway of ascorbic acid (Cantarow and Scheparts, 1963). In this study, the compositional variations of spinach (with special emphasis on ascorbic acid) with respect to leaf maturity and time of sampling were determined, with the objective of obtaining maximum leaf quality at harvesting.

## MATERIALS AND METHODS

The experiment was conducted under temperature controlled glass house conditions in the early summer of 1993. Average day and night temperatures were 29.5°C and 20°C, respectively. The spinach variety, King of Denmark, was cultivated in 20 l PVC troughs, which contained complete nutrient solutions. For germination, seeds were imbibibed in water for 12 hours, transferred into petri dishes and incubated for 10 days at 20°C. The seedlings at two cotyledon-leaf stage were transferred onto floating wire-nets in mild nutrient solutions, and managed for another 10 days, before being transplanted into troughs containing strong nutrient solutions. Solution culture was continued with constant aeration and frequent replacement of the solution as described by Weerakkody, (1994).

Sampling was done at three stages of leaf maturity-namely: 4, 5 and 6 weeks after first transplanting (WAP), at morning (0800 h) and evening (1700 h). The weather was fairly favourable at sampling, except in the case of the 5th week which was cloudy. Morning temperature was always 2-3°C higher than evening temperature. The spinach foliage was used as samples, excluding the 2 cm long stem base and leaves smaller than 3 cm in length. Nine randomly selected plants were used for the preparation of fresh and dry samples. These were utilized for subsequent analysis.

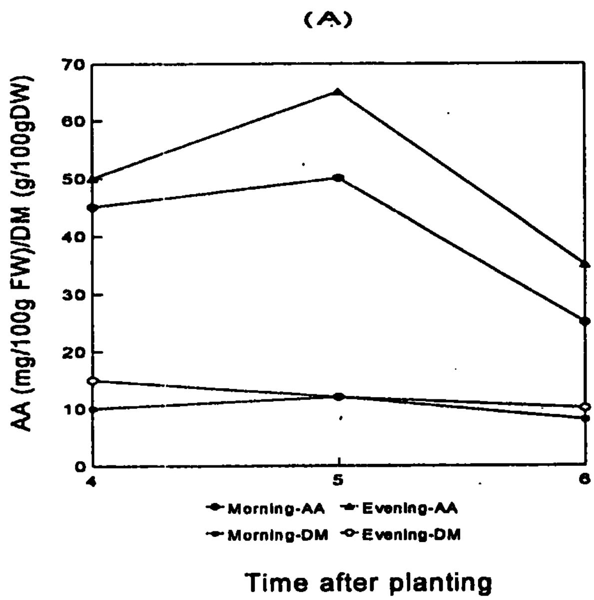
For the HPLC analysis of ascorbic acid, 50 μl aliquots of fresh sample extracts in 7% metaphosphoric acid were passed through a TSK-ODS80 column at a speed of 1 ml/min using a Shimadzu C-R6A chromatograph; where the mobile phase was 2% ammonium phosphate at 2.6-2.8 pH. The UV detection was made at a 250 nm wave length. Total sugar was determined in fresh (frozen) plant samples, using the Anthrone method (Yoshida *et. al.*, 1976) with D-glucose as the standard. Anion contents were determined by ion chromatography. In this analysis, aliquots of 100 μl extracts were injected and passed through TSK gel (IC-Anion-PW), at a rate of 1.2 ml/min.; where the mobile phase was a 2.7% borate buffer.

The experiment was designed as a two factor factorial in a completely randomized design, with three replicates in which the factors were stages of maturity and sampling time. Means of main effects and the interactions were evaluated by the Duncan's New Multiple Range Test using Mstat C computer program (Freed and Eisensmith, 1989).

## RESULTS AND DISCUSSION

The plant ascorbic acid content significantly increased from the 4th and 5th weeks after planting (WAP) and then rapidly decreased at the 6th WAP. There was always a higher ascorbic acid content in the evening samples than in the morning samples (Figure 1A). Mean ascorbic acid content (43.91 mg/100 g) was higher than the maximum ascorbic acid content reported from field samplings (Weerakkody, 1994). The changes in dry matter content with leaf maturity was similar to the variation of ascorbic acid, although the differences between two sampling times were not significant (Figure 1A). The dry matter content had a significant positive correlation ($r^2$=0.88) with the ascorbic acid content.

The reduction in total sugar content from the 4th to 6th WAP was highly significant, and higher amounts of total sugar were observed in the evenings (Figure 1B). The interaction between leaf maturity and time of sampling on total sugar content was also highly significant.



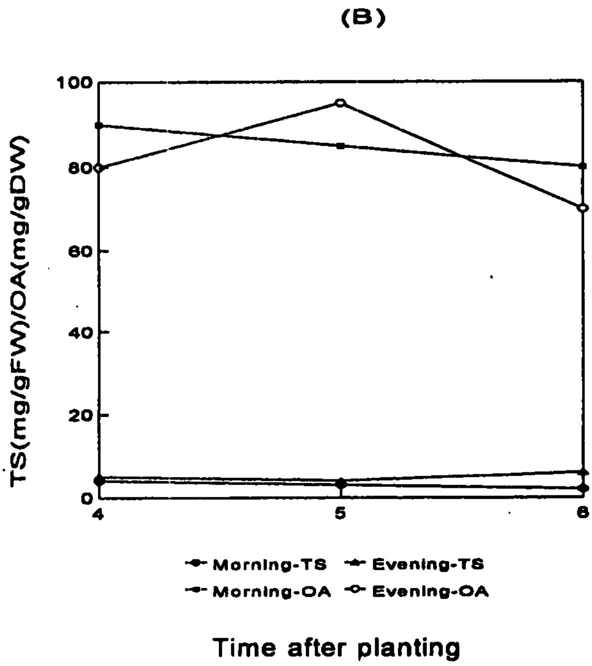




Figure 1. Variation in Ascorbic acid(AA), Dry matter (DM), Total sugar (TS) and Oxalic acid (OA) contents with leaf maturity and sampling time.

There was a significant reduction in the plant oxalic acid content at 6 weeks after planting (Figure 1B). This phenomenon was significantly correlated with ascorbic acid and dry matter contents ($r^2=0.84$ and 0.96 respectively). Significant weekly differences were observed in chloride and phosphate contents (Table 1). These did not show significant differences between morning and evening samplings.

**Table 1. Anion contents of Spinach at different stages of leaf maturity and sampling time of day (mg/g DW).**

| Weeks After Planting | Chloride | | Phosphate | | Sulphate | | Nitrate | |
|---|---|---|---|---|---|---|---|---|
| | M | E | M | E | M | E | M | E |
| 4 | 22.4$^a$ | 25.0 | 12.6$^b$ | 10.0 | 7.7 | 7.6 | 1.5 | 1.8 |
| 5 | 18.9$^b$ | 18.4 | 25.8$^a$ | 21.4 | 7.8 | 11.1 | 1.6 | 1.5 |
| 6 | 18.9$^b$ | 18.8 | 25.7$^a$ | 16.9 | 8.3 | 9.6 | 1.7 | 1.7 |
| Mean | 20.1 | 20.1 | 21.6 | 16.1 | 7.9 | 9.4 | 1.6 | 1.7 |
| LSD | 3.80 | | 2.53 | | 3.51 | | 0.125 | |

M : Morning E : Evening

The mean values of dry matter (1.6 mg/g) and sugar (80.91 mg/g) contents matched with the average values obtained under field conditions. The mean oxalic acid content was marginally higher than average values of field planted spinach, while nitrate contents were well below the values reported under field conditions.

The variations reported in different quality parameters with leaf maturity can be considered as an effect of various biochemical changes associated with plant growth. A common trend was observed in terms of sampling time on the ascorbic acid, dry matter and total sugar contents. The values were higher in the evenings. As suggested by Reuther and Nauer, (1972) and Ting, (1977) solar radiation and low temperature could cause differences in ascorbic acid contents between mornings and evenings. Since sugar and dry matter contents are directly affected by the photosynthesis, the effect of light period (day/night) could be considered the main cause for the higher values of these two parameters in the evenings. The drastic drop reported in the sugar content at the 5th week-evening sampling (under cloudy weather) supports this interpretation. With respect to effects of sampling time on oxalic acid content, the concentration at the 5th WAP; was different to the pattern observed in the 4th and 6th WAP causing difficulties in relating it to the biodegradation of ascorbic acid.

Since soluble carbohydrates amount for a substantial proportion of the dry matter content of leaf, the positive correlation between dry matter and ascorbic acid contents can be related to the need of carbohydrates for the biosynthesis of ascorbic acid (Reichstein *et. al.*, 1934). However, the total sugar content is different to that of ascorbic acid and dry matter. However, the low value of total sugar observed at the 5th WAP, under cloudy weather conditions, limits the possibility of making further interpretations. Although oxalic acid is a major end product of the biodegradation of ascorbic acid, oxalic acid does not have a negative correlation to ascorbic acid with respect to leaf maturity. This suggests a further break down or a transformation into water insoluble forms, such as, calcium oxalate in plants. Ultimately, leaf maturity and the sampling time of day appear to be important factors affecting plant ascorbic acid, dry matter and total sugar contents of spinach.

There may be several reasons for the differences observed in the chemical composition with change in experimental conditions. In the case of ascorbic acid, the higher values may be associated with the modifications made in the sample analytical procedure, omitting the pre-storage period applied in earlier experiments (Weerakkody, 1994). In the case of nitrate content, it is difficult to identify factors that caused this favourable reduction obtained under nutrient culture conditions.

## CONCLUSIONS

As a measure of assuring high quality of harvested products, the selection of an appropriate harvesting time, with respect to growth stage and time of day, appears to be very important. Spinach had the highest ascorbic acid leads, dry matter content and yield at the 5th week after transplanting in nutrient cultures. In addition, evening sampling always provided higher nutrients and yields than morning sampling. The weather conditions at harvesting, for instance, temperature and solar radiation could influence these parameters. However, these inferences have to be tested with field-grown spinach.

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