<Original>Changes of Ascorbic Acid Contents in Various Market Forms of Spinach (Spinacea oleracea L.) during Postharvest Storage in Light and Dark Conditions

Sci. Rep. Grad. sch. Agric. & Biol. Sci., Osaka Pref. Univ. **55** : 1~6. (2003). Original

Changes of Ascorbic Acid Contents in Various Market Forms of Spinach (*Spinacea oleracea* L.) during Postharvest Storage in Light and Dark Conditions

Merry Evelyn A. TOLEDO, Yoshinori UEDA, and Takashi SHIROSAKI

(Laboratory of Postharvest Physiology and Quality Control, Graduate School of Agriculture and Biological Sciences, Osaka Prefecture University, 1-1 Gakuen-cho, Sakai, 599-8531, Osaka, Japan)

Abstract

Changes in ascorbic acid contents in various market forms of spinach (*Spinacia oleracea* L.) during postharvest storage in light (20-25 μmole m^{-2} s^{-1}) and dark conditions were investigated. In the leaves of whole spinach plants, the decline in ascorbic acid contents, specifically in mature leaves which comprise the bulk of the plants, was effectively minimized with the provision of supplemental lighting. Supplemental lighting has likewise been demonstrated to positively affect the ascorbic acid status of leaves from spinach plants with trimmed off parts and those without roots, and in detached spinach leaves. Light then seems to be an effective environmental factor in ascorbic acid maintenance of spinach leaves during the postharvest period.

Key Words: ascorbic acid, dark, light, postharvest storage, spinach, *Spinacia oleracea* L.

Introduction

Spinach (*Spinacia oleracea* L.) is a cool season annual herb that belongs to the goosefoot family (Chenopodiaceae). This popular horticultural crop may be stored and/or marketed in a variety of ways as bunched or unbunched, with or without roots or as a fresh cut product. Likewise, it may be prepared and consumed by a variety of ways as fresh (salads) or processed dishes (baked boiled, sautéed, etc.). It is low in calories and contains appreciable amounts of vitamins A and C and minerals especially iron. However, it is a highly perishable vegetable and various postharvest environmental conditions affect its quality and marketability.

Ascorbic acid (vitamin C) is one of the most important quality attributes in many horticultural crops and has many biological roles in the human body. The content of ascorbic acid in fruit and vegetables can be influenced by various factors such as genotypic characteristics, cultural practices, harvesting methods and postharvest handling practices. During postharvest handling and storage, many factors affect the ascorbic acid contents of many horticultural produce (Lee and Kader, 2000). Lee and Kader (2000) reported that rapid loss of ascorbic acid, especially in leafy vegetables, may be caused by water loss, bruising and other mechanical injuries, and by trimming and cutting.

Light is an environmental factor whose effect has been extensively studied in many field experiments. During the postharvest period, it has also been reported by Hosoda et al. (1981a, b) that light at relatively low intensities, positively affects the ascorbic acid contents of komatsuna (*Brassica campestris* L.) leaves. However, during the postharvest period, the effect of light, especially that of low intensity, on most horticultural produce having various market forms has not yet been clearly elucidated nor conclusively established.

This study then aims to investigate the effect of low intensity light (20-25 μmole m-2 s-1) and darkness on the changes in ascorbic acid (AsA) contents in the leaves of various market forms

of spinach during the postharvest period.

Materials and Methods

Plant material and storage methods. Spinach (*Spinacia oleracea* L.) plants were purchased from a local grocery store close to the University and immediately brought to the laboratory. Unless otherwise stated, winter-harvested spinach was used as the experimental material.

Experiment 1. Spinach plants were washed in running water and their roots were wrapped with water-soaked cotton. Plants were then arranged individually in slanting position in trays without binding stocks and stored under continuous white fluorescent light (20-25 μmole m^{-2} s^{-1}; measured at the top of the plant) or dark condition at 8℃. A high humidity refrigerator, able to maintain relative humidity at 95-98%, was used as the storage facility. Trays were covered with polypropylene plastic to minimize desiccation and atomized water was applied everyday for light-stored spinach and every other day for dark-stored spinach for a period of 23 days. For light storage, trays were wrapped with reflective aluminum foil so as to facilitate exposure of lower stem parts to light. Spinach plants stored in light and dark conditions were removed at scheduled sampling days. During sampling, composite leaf tissues from 3 to 4 young (fully emerged but not yet fully expanded) leaves or mature leaves were collected. After collecting leaf disks for chlorophyll analysis, the leaves were cut into small pieces (approximately 0.5 to 1 cm^2), pre-weighed, frozen in liquid nitrogen and stored at -75℃ ready for AsA analysis. Data was gathered from 3 sets of plants. All measurements were repeated twice.

Experiment 2. During postharvest handling, storage and marketing of spinach, detachment or breaking off of leaves occur. A second experiment was then set-up to represent this situation. Early summer-harvested plants were used as experimental materials. In the set-up, plants were divided into 2 groups. The first group, had their young leaves detached while the second group had their mature leaves detached from the plant. Then, the roots were wrapped in water-soaked cotton. Plants were then subjected to the same storage conditions, sampling procedures and data gathering strategies as the preceding activity.

Experiment 3. Spinach plants grown in hydroponic media and harvested mechanically are usually sold in market shelves without roots. A third experiment was then designed to determine the changes in AsA content of mature spinach leaves from plants with and without roots. The same experimental procedure as previous was followed, except that roots were no longer covered with water-soaked cotton and plants were only stored for 4 days.

Experiment 4. This experiment was conducted to measure AsA contents in detached mature leaves during storage with the aim of elucidating the effect of light on fresh-cut or minimally processed spinach leaves. Leaves were cut into halves (along the midrib) using a surgical blade. One half of each leaf was preweighed, cut into small pieces (approximately 0.5 to 1 cm^2), frozen in liquid nitrogen and stored at -75℃. The other half was also preweighed, floated on distilled water with 0.1% chloramphenicol and stored in light (as stated above for whole spinach plants) and dark conditions at 8℃ for 4 days. After storage, leaves were cut into small pieces and treated the same way as their half counterparts. Frozen samples were then analyzed for AsA. Data were gathered from duplicate measurements of 5 leaves.

Visual quality evaluation. Visual quality in terms of leaf color and state of leaves was assessed by a panel of evaluators composed of 10 faculty and students of the College of Agriculture, Osaka Prefecture University. Evaluation was done at every sampling day starting from the harvest day. Leaf color was scored on a 5-1 scale with reference points of 5, green; 4, dull green; 3, 75% green (25% yellow); 2, 50% green (50% yellow); 1, 25% green (75% yellow). Similarly, state of leaves was scored on a 5-1 scale with reference points of 5, very fresh; 4, fresh; 3, partly wilted; 2, wilted (outer leaves wilted); 1, very wilted (inner and outer leaves wilted). The score of 3 was regarded as the limit of marketability. Visual quality evaluation of spinach was only done during the first experiment i.e. whole spinach plants.

Chlorophyll content determination. Leaf disks (5.5 mm in diameter) were punched along

the periphery of the leaf (5 disks per leaf) using a metal cylindrical borer and the chlorophyll extracted using *N*-*N*-dimethylformamide. The absorbance of the extracts was measured at 647 and 664 nm and total chlorophyll was estimated according to Moran (1982). As with visual quality evaluation, chlorophyll content was measured only in the first experiment. Results were expressed as mg chlorophyll per 100 g of fresh weight.

Ascorbic acid assay. Frozen leaf tissues were ground in ice-cold mortar and pestle containing 5% metaphosphoric acid at 4 times the tissue weight. After the tissue was well homogenized, distilled water was then added at 5 times the tissue weight. The homogenate was then filtered and clear supernatant was collected for AsA analysis. Total AsA and dehydroascorbic acid (DHA) was analyzed following the 2,4-Dinitrophenylhydrazine Method of Roe et al. (1948) using L-AsA (Wako Pure Chemicals, Ind.) as standard. Reduced AsA was calculated from the difference between total AsA and DHA. Results were expressed as mg AsA per 100 g of fresh weight.

Results and Discussion

Changes in the visual quality, chlorophyll and ascorbic acid contents of leaves from whole spinach plants. During storage of spinach plants, visual quality (Fig. 1) and chlorophyll contents (Fig. 2) were observed to be declining as storage time progressed. However, marked differences were not demonstrated between light and dark storage in terms of both parameters until the onset of leaf yellowing i.e. the 14th day of storage. This would seem to indicate that the physical appearance of spinach leaves during storage was not affected by either the presence or absence of light. But, alterations of the AsA contents in the leaves, more specifically in mature leaves, were already observed just after the first few day of storage (Fig. 3A). AsA contents in the light-stored mature leaves were relatively higher compared

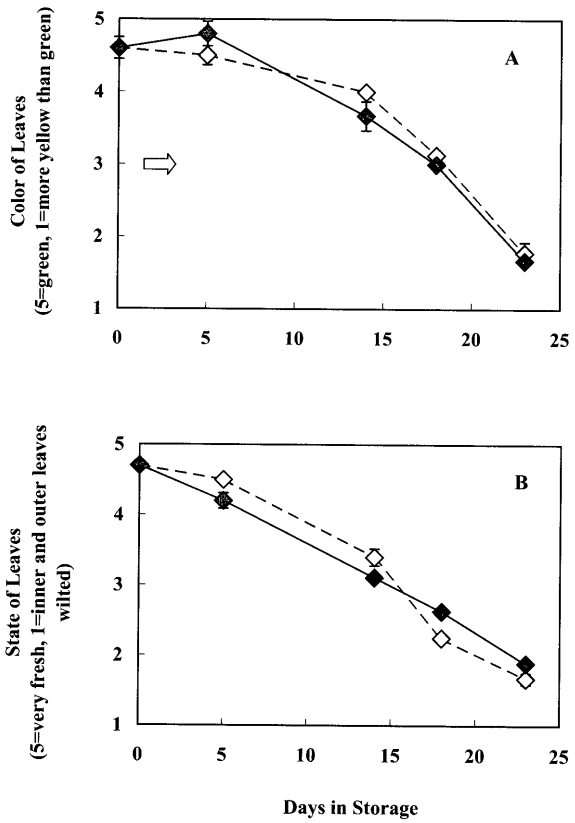

Fig. 1. Visual quality scores of whole spinach plants in terms of leaf color (A) and state of leaves (B) during storage in light and dark conditions at 8℃ for 23 days.

Blank triangles, light-stored leaves; black triangles, dark-stored leaves.

Mean ± SE of 10 replications. Arrow denotes the limit of marketability.

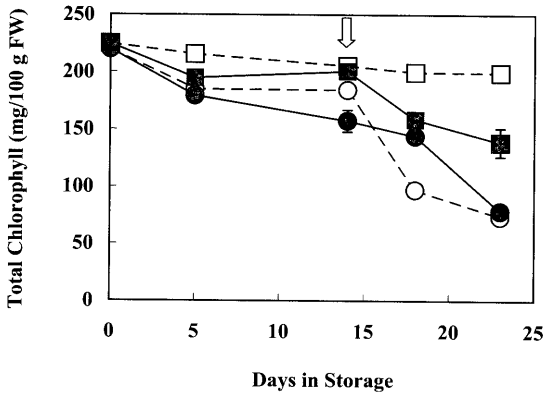

Fig. 2. Changes in total chlorophyll contents of spinach leaves from whole spinach plants during storage in light and dark conditions at 8° C for 23 days.

Blank squares, young leaves in light; black squares, young leaves in darkness; blank circles, mature leaves in light; black circles, mature leaves in darkness.

Mean ± SE of 3 replications. Arrow denotes the onset of yellowing at the upper leafy part of outermost leaves which has been observed in 25% of all stored plants. FW, fresh weight.

to their dark-stored counterparts from the 5th day of storage up until the onset of leaf yellowing. Afterwards, changes in the AsA contents did not seem to vary between the leaves stored in the light and dark conditions. Meanwhile, the AsA contents in young spinach leaves in both light and dark conditions were essentially maintained up until the 18th day of storage. Results seem to suggest that if spinach is immediately sold after harvest, supplemental lighting may not necessarily have an impact on AsA levels. However, during the cool season, when spinach are sometimes placed in cold storage for several days prior to market showcasing due to abundant supply, supplemental lighting may then become a very effective method in maintaining AsA contents especially in mature leaves which comprises the bulk of the spinach plant.

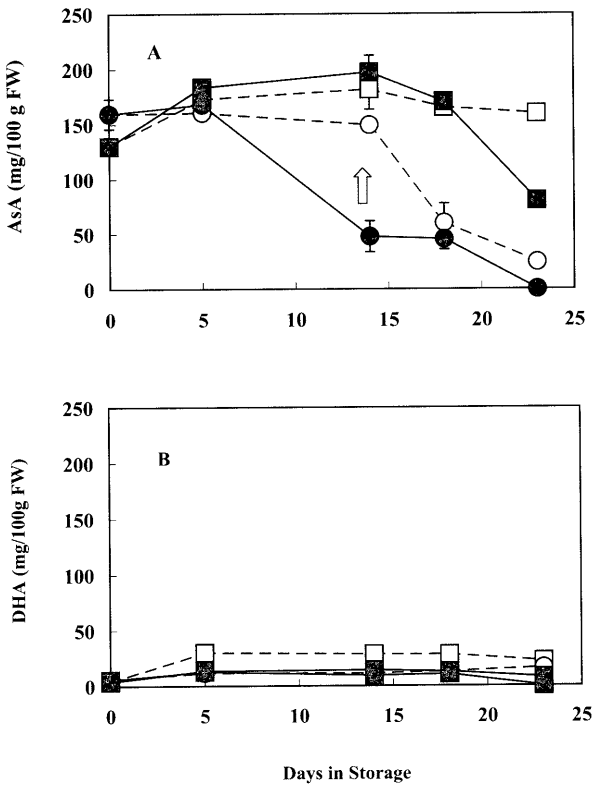

Fig. 3. Changes in ascorbic acid (A) and dehydroascorbic acid (B) contents in spinach leaves from whole plants during during storage in at 8℃ in light and dark conditions.

Blank squares, young leaves in light, black squares, young leaves in darkness; blank circles, mature leaves in light, black circles, mature leaves in darkness.

Means ± SE of 3 replications Arrow denotes the onset of yellowing at the upper leafy part of outermost leaves which has been observed in 25% of all stored plants. FW, fresh weight

Meanwhile, the DHA contents all leaf samples were kept at minimum levels all throughout the course of storage (Fig. 3B). This would indicate that an effective AsA recycling system is present in the spinach leaves and that light and dark treatments do not necessarily pose an adverse effect on such system. Moreover, low levels of DHA in the leaf samples could indicate that the metabolic conversion of AsA into oxalic acid (Yang and Leowus, 1975; Fujii et al., 1993) is neither increased nor decreased in the presence or absence of light.

Changes of the ascorbic acid contents in: (a) leaves from plants with trimmed off parts (b) leaves from plants without roots, and (c) detached leaves. During the postharvest period, AsA content was significantly affected by physical alterations done on many horticultural crops. These would include trimming, cutting and processing (Lee and Kader, 2000). Trimming of outer leaves and of the core and associated inner leaves of Chinese cabbage has been reported by Klieber and Franklin (2000) to pose a greater effect on the reduction of AsA than storage at 4℃ for 11 days. In the dark-stored leaves of spinach plants with trimmed off parts i.e. either the young or mature leaves were detached, AsA was also observed to rapidly decline even during the early days of storage (Fig. 4A). This negative effect however, was not observed when leaves were provided with supplemental lighting. In fact, it is interesting to note that, the impact of light on AsA contents is more readily observed in the leaves from plants with trimmed off parts than in the leaves from plants which have been carefully stored whole (refer to preceding text). Since trimming off or breaking off of fragile spinach leaves is not unusual during postharvest handling, plants with trimmed off leaves would therefore be a more representing form of spinach during market showcasing. Results of the AsA contents of leaves from plants with trimmed off parts may then indicate that light could still be very effective in supporting the AsA status in the leaves of plants which are immediately stored right after harvest.

In the leaves of spinach plants without roots,

AsA contents were found to be similar to the leaves from plants with roots (Fig. 5). However, higher AsA levels were observed in the light-stored than in the dark-stored leaves. Results indicate that root removal in spinach plants prior to postharvest storage would not pose any effect on AsA contents and regardless of root removal, lighting would still be an effective method to maintain AsA during the postharvest period.

The provision of supplemental light has also been observed to positively affect AsA contents in detached spinach leaves. Fig. 6 demonstrated that light-stored leaves showed slower decline in AsA contents compared to the dark-stored leaves and that their total AsA status (reduced AsA + DHA) was even higher than their pre-stored counterparts. However, the relatively higher DHA contents in the light-stored leaves may also indicate that light seems to cause some degree of stress to the leaves. Nevertheless, the higher DHA levels may not seem to be all together disadvantageous since DHA can easily be converted into AsA in the human body (Lee and Kader, 2000). These results may then find possible practical applications in improving the AsA status of fresh-cut or minimally processed vegetables.

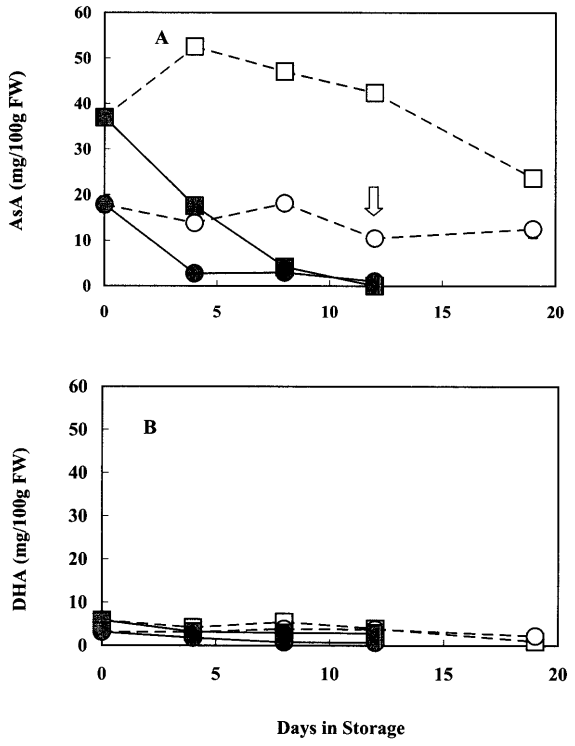

Fig. 4. Changes in ascorbic acid (A) and dehydroascorbic acid (B) contents in spinach leaves from plants with trimmed off leaves during storage in at 8℃ in light and dark conditions.

Blank squares, young leaves in light; black squares, young leaves in darkness; blank circles, mature leaves in light; black circles, mature leaves in darkness.

Means ± SE of 3 replications. Arrow denotes the onset of yellowing at the upper leafy part of outermost leaves which has been observed in 25% of all stored plants. FW, fresh weight.

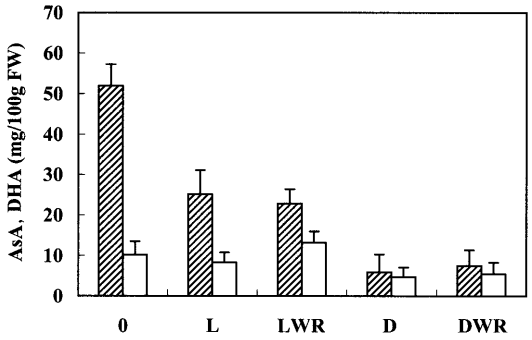

Fig. 5. Change in ascorbic acid (shaded bars) and dehydroascorbic acid (blank bars) contents of mature spinach leaves from plants with and without roots after storage in light and dark conditions at 8℃ for 4 days.

0, 0 day storage; L, leaves in light with roots; LWR, leaves in light without roots; D, leaves in darkness with roots; DWR, leaves in darkness without roots. Means ± SE of 3 replications.

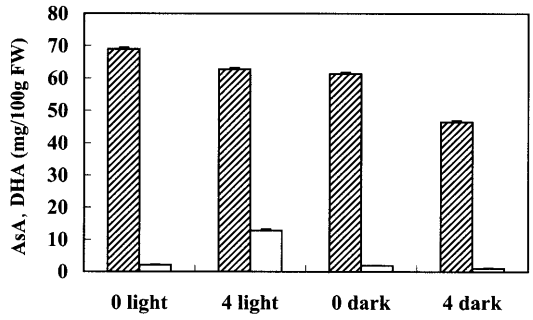

Fig. 6. Change in ascorbic acid (shaded bars) and dehydroascorbic acid (blank bars) contents of detached mature spinach leaves stored in light and dark conditions at 8° C for 4 days.

0 light, 0 day storage in light; 4 light, 4th day of storage in light; 0 dark, 0 day of storage in darkness; 4 dark, 4th day of storage in darkness. Means ± SE of 5 replications.

Supplemental lighting during the postharvest period has primarily been provided to enhance consumer appeal and improve the aesthetic value of vegetables. The present study has demonstrated that aside from these functions, low intensity light (20-25 μmole m^{-2} s^{-1}) could also be an important exogenous factor in improving the AsA status in the different storage and showcasing forms of spinach.

References

Fujii, N., Watanabe, M., Watanabe, Y., and Shimada, N. 1993. Rate of oxalate biosynthesis from glycolate and ascorbic-acid in spinach leaves. Soil Sci. Plant Nutr., **39**, 627-634.

Hosoda, H., Nawa, Y., and Kurogi, M. 1981a. Effect of light on postharvest quality in vegetables. Part 1. Changes in chemical components in detached komatsuna leaves during storage. Rept. Natl. Food Res. Inst., **38**, 33-39. (in Japanese with English abstract).

Hosoda, H., Nawa, Y., and Kurogi, M. 1981b. Effect of light on postharvest quality in vegetables. Part 2. Changes in chemical components in attached komatsuna leaves during storage. Rept. Natl. Food Res. Inst., **38**, 40-45. (in Japanese with English abstract).

Klieber, A. and Franklin, B. 2000. Ascorbic acid content of minimally processed Chinese cabbage. Acta Hortic., **518**, 201-204.

Lee, S.K. and Kader, A.A. 2000. Preharvest and postharvest factors influencing vitamin C content of horticultural crops. Postharvest Biol. Technol., **20**, 207-220.

Moran, R. 1982. Formulae for determination of chlorophyllous pigments extracted with *N,N*-dimethylformamide. Plant Physiol., **69**, 1376-1381.

Roe, J.H., Mills, M.B., Oesterling, M.J., and Damron, C.M. 1948. The determination of diketo-*l*-gulonic acid, dehydro-*l*-ascorbic acid, and *l*-ascorbic acid in the same tissue extract by the 2,4-dinitrophenylhydrazine method. J. Biol. Chem., **174**, 201-208.

Yang, J.C. and Loewus, F.A. 1975. Metabolic conversion of L-ascorbic acid to oxalic acid in oxalate-accumulating plants. Plant Physiol., **56**, 283-285.

(Received Dec. 18, 2002; Accepted Jan. 8, 2003)