Effects of Storage Temperature on the Keeping Quality of Malabar Spinach (*Basella alba* L.)

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The influence of storage temperature on freshness, ascorbic acid content, respiration rate, ion leakage from leaf tissue discs and morphological changes of malabar spinach (*Basella alba* L.) were investigated. At 25°C storage, yellowing of the leaves was mainly responsible for the quality deterioration and lost 50~60% in ascorbic acid content after 4~6 days. Those stored at 0 and 5°C, however, were still green and lost 26~36% after 12~14 days. Lower temperatures were effective in decreasing the yellowing, ascorbic acid loss and the respiration rate. However, upon transfer to 25°C, the CO₂ production increased rapidly and significantly higher increases were observed from those stored at 0 and 5°C than those stored at 10 or 15°C. This respiratory stimulation was positively correlated with the increase in the rate of ion leakage at 0 and 5°C. Chilling injury was observed in storage at 0 or 5°C and was manifested as pitting and softening (loss of turgidity) of leaves. Stomata were the only openings on the epidermis of the leaves and frequencies were greater on the abaxial surface than on the adaxial surface. Cracking of the tissue near the stomata and exudates outside the stomata were observed after 12~14 days of storage at 0 and 5°C.

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Malabar spinach, tsurumurasaki in Japanese is a green leafy vegetable of tropical origin which contains more vitamin A, C and calcium than spinach and chinese cabbage¹⁾. Young leaves are occasionally harvested and cut into pieces and utilized like spinach. Recently, malabar spinach has been grown in Shikoku Island (Japan) starting in April and harvested from July to October. However, the keeping quality of malabar spinach is not well known yet.

Fresh fruits and vegetables as living tissues are subject to continual changes after harvest. Such changes cannot be stopped but can be controlled within certain limits by using various postharvest procedures. Temperature management is the most important tool to extend the shelf life and maintain the quality of fresh fruits and vegetables. Above freezing point, for non-chilling-

sensitive commodities, every increase in temperature of 10°C accelerates deterioration and increases the rate of loss in nutritional quality by 2 to 3 fold²⁾. Many plants indigenous to the tropics and subtropics and some tissues from plants of temperate origin are injured by exposure to non-freezing temperatures below 12°C^{3)~5)}. This physiological disorder is referred to as chilling injury (CI) and has been of great concern for many years with harvested plant parts. HIRATA *et al.*⁶⁾ reported that CI in malabar spinach developed in storage at 1°C and was manifested as pitting and softening of the leaves. The ascorbic acid content decreased rapidly at low temperature occurring CI^{6),7)}. Plants, depending on their genetic origin and general physiological status, differ in chilling sensitivity. Although storage time and shelf life of some crops can be prolonged by

lowering the storage temperature, CI is induced with chilling sensitive commodities, which influences the quality of stored products. Many physiological responses to various stimuli in plant tissues have been ascribed to changes in membrane permeability. CI has been shown to increase membrane permeability, as measured by solute leakage and ion uptake, in tissues from various chilling sensitive species^{4),8)}.

Our objective was to clarify the postharvest characteristics of malabar spinach. The influence of storage temperature on freshness, ascorbic acid content, respiration rate, ion leakage and morphological changes were determined.

Materials and Methods

1. Plant material

About 25~30 cm malabar spinach shoots were obtained from a grower in Takamatsu, Kagawa, Japan. The sample shoots were harvested early in the morning and brought immediately to the Laboratory. The samples which were free from defects were selected and used in the experiment. Malabar spinach were put in polyethylene bags and stored at 0, 5, 10, 15 and 25°C, taken out at 4, 7, 10 and 14 d and then transferred to 25°C for 2 days.

2. Respiration rate measurement

About 100 to 600 g of malabar spinach were placed in a 6 liter glass jar and placed at different storage conditions. To measure production of CO₂, malabar spinach leaves were enclosed in glass jars at different temperatures for an appropriate length of time for CO₂ to accumulate to measurable levels (about 1 h). Five-milliliter triplicate samples were taken from the headspace of the sealed jars through the septum and were injected into a Shimadzu GC-8AIT gas chromatography (GC) equipped with a thermal conductivity detector (TCD) with helium as carrier gas at a flow rate of 20 ℓ /min. The results were expressed as $\text{m}\ell$ CO₂ $\text{kg}^{-1}\text{h}^{-1}$. After the designated exposure period, the subsamples of malabar spinach were taken out from the different storage conditions and placed at 25°C. After 2 days,

respiration rate of the transferred sample was measured.

3. Ascorbic acid analysis

L-Ascorbic acid (AsA) was determined by the 2,4-dinitrophenylhydrazine method described by JOSEPH *et al*⁹⁾. Five g flesh from malabar spinach leaves was grinded in a mortar and pestle with 1 g sea sand, added with 20 $\text{m}\ell$ 5% metaphosphoric acid and 25 $\text{m}\ell$ deionized water. The homogenate was centrifuged at 1,000 rpm for 10 min. The supernatant was used for ascorbic acid measurement. Color intensity of osazone derived from dehydroascorbic acid was measured at 530 nm by using a double beam spectrophotometer UVIDE C-320, Japan. The amount of AsA as reduced ascorbic acid was calculated by subtracting the amount of dehydroascorbic acid from that of the total ascorbic acid.

4. Assessment of Ion Leakage

A technique for assessing ion leakage described by FURMANSKI and BUESCHER¹⁰⁾ was used with some modifications. Leaf tissue discs were prepared by slicing cylinders using a cork borer (16 mm diameter). After slicing, the discs were washed 3 times with distilled water and immersed in a 100 $\text{m}\ell$ of 0.4 M mannitol solution. Beakers with the samples were put in double shakers with agitation at 100 cycles min^{-1} for 5 h at 25°C. Ion leakage was determined with a conductivity bridge (cell constant of. 940) after 1 and 5 h of incubation. The conductance was again determined after boiling the sample for 5 min and cooled to 30°C before measurement. The results were expressed as percentage (%) total ion leakage, i. e., initial reading/final reading $\times$ 100.

5. Scanning Electron Microscope

Leaf tissue discs were fixed in 4% glutaldehyde in 0.2 M phosphate buffer at pH 7 for 24 h at 5°C. The discs were rinsed 3 times with the same phosphate buffer with an interval of 16 h and then post-fixed with 1.6% osmium tetroxide for 3 h at 5°C. After 3 times washing with the same buffer with an interval of 8 h, the leaf discs were then dehydrated in an ethanol series at 30, 50, 70% for 30 min each ; 85 and 95% for 60 min each and

100% for 24 h. After the ethanol series, 50 and 100% iso-amyl acetate was used for 12 h each. The leaf discs were then dried using a Hitachi HCP-1 critical point drier in liquid carbon dioxide and mounted in an aluminum stubs (SEM cells) with a drop of silver paste. The SEM cells with the sample were coated with gold palladium in an ion sputter coater (RMC-Eiko Corp). The coated leaf discs were observed in a Hitachi S-800 scanning electron microscope at 8 kV accelerating voltage.

6. Statistics

Determinations of the effects of the different storage temperatures on the respiration rate, ion leakage and the loss of the ascorbic acid content were conducted in triplicate and analyzed statistically using a completely randomized design (CRD). Treatment means were compared using the least significant difference test.

Results and Discussion

1. Respiration rate

Changes in respiration rate of malabar spinach during storage at each temperature and after transfer to 25°C are shown in Fig. 1. The respiration rate of malabar spinach stored at 25°C was higher than that at the other storage temperatures on the day of harvest, but the difference became smaller as the storage progressed. However, upon transfer of malabar spinach to 25°C, CO₂ production increased rapidly especially after storage at 0 and 5°C with those harvested both in July and October. The increase of CO₂ production was greater with malabar spinach harvested in October than in July. The stimulation appeared after 4 days and was more apparent with longer storage. A similar result on cucumber leaves was reported by LYONS⁴⁾ and suggested that the stimulated production at warm temperature after transfer from various chilling temperature might be due to the accumulation of certain metabolic intermediates during the exposure to chilling. This respiratory stimulation upon transfer has been observed in many plant species¹¹⁾, but with chilling-sensitive species it is

Fig. 1 Changes in respiration rate of malabar spinach harvested in July and October, held at different storage temperatures and transferred to 25°C for 2 days

greatly amplified and can be used as an index of the severity of CI in those tissues. CI of malabar spinach was manifested as pitting and softening (loss of turgidity) of leaves. Malabar spinach exposed for 7 d at 0°C or 10 d at 5°C appeared to be the maximum storage days at each temperature before the occurrence of chilling injury symptoms. The development of CI was parallel to an increase in respiration with a longer exposure at chilling temperature.

2. Ascorbic acid content

Fig. 2 shows the percent loss of AsA content of malabar spinach harvested in July and October, held at various temperatures, and then transfer to 25°C for 2 days. The loss of AsA was relatively higher with malabar spinach harvested in July than in October and was more pronounced at 15 or 25°C than at 0, 5 or 10°C. At 25°C storage, the ascorbic acid loss was about 50~60% after 4~6 days, while those stored at 0 or 5°C had a loss of about 20~36% within 12~14 days of storage.

Broken lines represents transfer to 25°C. Vertical bars represent LSD (0.05). Initial AsA content, 164.86 and 171.72 mg% for July and October, respectively.

—△— 0°C —●— 5°C —○— 10°C —■— 15°C —□— 25°C

Fig. 2 Loss of ascorbic acid content (%) of malabar spinach harvested in July and October, held at different storage temperatures and transferred to 25°C for 2 days

YAMAUCHI¹²⁾ reported that yellowing of spinach leaves was associated with an increase in peroxidase enzyme activity which caused the decrease in AsA. The decrease of AsA in yellowing spinach stored at 1°C was found to be the interaction of ascorbate oxidase and peroxidase enzymes¹²⁾. Moreover, HAARD¹³⁾ reported that ascorbate oxidase and peroxidase activities increased with the development of CI. After transfer from storage at 10 or 15°C for 4 d to 25°C for 2 days, the AsA loss was relatively higher than those stored at 0 or 5°C. However, after transfer from 7 d storage at 5 to 25°C, a significantly higher loss was observed with pitting and wilting of the leaf tissue. In addition, malabar spinach stored at 0 or 5°C for 10 or 14 d, deteriorated so rapidly upon transferred to 25°C where AsA was unmeasurable. Leaf tissue which was severely injured by exposure to low temperatures may be largely the result of

autolysis of the cells¹⁴⁾. However, with continuous storage at 0°C for 14 d had the lowest ascorbic acid loss compared with those stored at 5, 10 or 15°C. The loss was relatively higher in July than in October harvest. In spinach harvested in autumn, ascorbic acid losses were higher compared to those harvested in spring and winter¹⁵⁾, which was in agreement with SCOTT and KRAMER¹⁶⁾ on beans, peas, spinach and asparagus in which the magnitude of loss varies depending on the product and the harvest date.

3. Rate of ion leakage

Discs of malabar spinach leaf tissue held at different temperatures resulted in a progressive increase in ion leakage with a decrease in temperature (Fig. 3). The chilling temperature at 0 or 5°C caused a steady increase in the rate of ion leakage with time. The results were comparable with the reports of WRIGHT and SIMON¹⁷⁾ and WRIGHT¹⁸⁾ on sweet potato and leaves of

Broken lines represent transfer to 25°C. Vertical bars represent LSD (0.05).

—△— 0°C —●— 5°C —○— 10°C —■— 15°C —□— 25°C

Fig. 3 Changes in the rate of ion leakage (%) from leaf discs of malabar spinach harvested in July and October, held at different storage temperatures and transferred to 25°C for 2 days

Fig. 4A and 4B Illustrate the stomates in the adaxial (A) and abaxial (B) side of malabar spinach leaves before storage treatment

Fig. 4C and 4D Stomata at varying degrees of openings after 7 d storage at 0°C (C) and 10 d storage at 5°C (D)

Fig. 4E and 4F Cracking of the tissues of malabar spinach leaves stored at 0°C for 12 d (E) and 5°C (F) for 14 d

Fig. 4G and 4H Exudates outside the stomatal openings of malabar spinach leaves stored after 10 d at 0°C (G) and 5°C (H) and transferred to 25°C for 2 days

cucumber and beans in which there was a rapid increase in ion leakage upon exposure at chilling temperatures. The predominantly increased leakage from malabar spinach tissue stored at 0 and 5°C may result from an impairment of cell permeability. LEVITT¹⁹⁾ suggested that CI may have been caused by a change in cell permeability. In addition, YAMAWAKI *et al.*²⁰⁾ reported that there was a continuous increase of ion leakage before the occurrence of CI. Ion leakage may serve as an index of susceptibility to CI. On the other hand, ion leakage from the leaf discs stored at 10 or 15°C remained at a low level throughout the storage period. The data after transfer to 25°C from chilling temperature at 0 or 5°C for 7, 10 and 14 d were not available since malabar spinach deteriorated rapidly with severe breakdown of the tissue.

4. Scanning Electron Microscope (SEM) photomicrograph

Surface features of malabar spinach as viewed by SEM includes stomata, exudates and cracking of tissues (Fig. 4). Fig. 4A and 4B illustrate the stomata in the adaxial and abaxial surfaces of the leaves of malabar spinach before the storage treatments. Stomatal frequencies were greater on the abaxial surface than on the adaxial surface. Stomata were the only natural openings observed in the epidermis because no trichomes or other natural openings were seen. Stomata were open to varying degrees after 7 and 10 days storage at different temperatures (Fig. 4C~4E). KAYS²¹⁾ reported that stomatal closure represents a means of conserving water within the tissue. When plant parts are detached at harvest from the parent plant, the supply of water from the root system is eliminated and stomata would decrease their aperture opening markedly. Since stomata were the only natural openings observed in malabar spinach leaves, they should be important in regulating moisture loss. On the other hand, LYONS⁴⁾ reported that stomata in some instances were unable to close at chilling temperature and the ability of leaves to transport water was impaired.

Cracking of the tissues were noticed from malabar spinach stored at 0°C for 12 days and 5°C for 14 days (Fig 4E~4F). The injury may be due to inability of the relatively inflexible metabolic cells to function at low temperatures. When they were stored for 12 days (10 d storage+2 d at 25°C) at 0 or 5°C, the tissues near the stomata began to crack and some exudates were formed outside the stomatal openings probably due to some leakage (Fig. 4G~4H). The stomata became disorganized and disintegrated and subsequently large exudates around the stomata were observed. The cracking of the tissue was more serious at 0°C, whereas at 5 and 10°C exudates outside the stomata were rampant. MOLINE²²⁾ described that if the temperature of the chilling sensitive plant is lowered to the critical temperature (below 10°C), membrane lipids may solidify, contract and break, thus destroying selective membrane permeability.

In conclusion, low temperatures at 0 and 5°C were effective in decreasing the respiration rate and ascorbic acid in storage of malabar spinach. Malabar spinach, however, is susceptible to chilling injury and can be kept fresh for a maximum of 7 d at 0°C. At 25°C, yellowing of the leaves was mainly responsible for quality deterioration in 4 to 6 days. As observed with SEM, stomata were the only openings on the epidermes of the leaves.

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- ツルムラサキの鮮度保持に及ぼす貯蔵温度の影響**

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- ツルムラサキ (*Basella alba* L.) の鮮度保持, アスコルビン酸含量, 呼吸速度, イオン漏出や形態学的変化に及ぼす貯蔵温度の影響について検討した。貯蔵温度25℃での品質の低下は葉の黄化が主な原因で, 貯蔵4~6日後に約50~60%のアスコルビン酸含量の減少が認められた。0℃と5℃では貯蔵12~14日後でも葉は緑色のままでアスコルビン酸の約26~36%を消失した。低温は黄化, アスコルビン酸の損失や呼吸速度を減らすのに効果的だった。しかしながら, 25℃に2日間移動すると炭酸ガス生成は急速に増大し, 10℃あるいは15℃よりも0℃と5℃で貯蔵した葉は有意に多くの増加が認められた。この呼吸の増大は, 0℃と5℃でのイオン漏出速度の増加と関係していた。低温障害は0℃と5℃貯蔵で認められ, その傷害は葉のピッチングや萎凋(膨圧ロス)であった。気孔は葉の表皮における唯一の開口部で, その頻度は向軸表面より背軸表面のほうが多かった。気孔近辺の組織の割れや気孔外部の浸出物は, 0℃と5℃で貯蔵12~14日後に観察された。

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