Effects of pH Level of L-Ascorbic Acid Solution and Soaking at Various Growth Stages on the Leaf Exogenous Ascorbic Acid Content of Lettuce and Japanese Bunching Onion

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Lettuce (*Lactuca sativa* L.) and Japanese bunching onion (*Allium fistulosum* L.) roots were soaked in L-ascorbic acid solutions ($1\,500\text{ mg L}^{-1}$) of various pH values for 12 h and changes in leaf ascorbic acid contents (the total content of L-ascorbic acid and dehydro-ascorbic acid) were analyzed. The ascorbic acid content in the leaves increased with a decrease in the pH value of the treatment solutions. In the treatment of the lowest pH value (pH 3.1), the ascorbic acid content in the lettuce leaves was increased from the initial value of $26 \pm 8\text{ mg}$ (per 100 g of fresh weight—the same will apply hereinafter) to $191 \pm 21\text{ mg}$, while that in the bunching onion leaves was increased from the initial value of $34 \pm 8\text{ mg}$ to $183 \pm 17\text{ mg}$. In the case of lettuce, the water absorbing ability of the roots worsened as the plants growth proceeded and thus the increased rate of the ascorbic acid content in the leaves was lowered simultaneously. These results suggest that lettuce and bunching onion absorb ascorbic acid well when the treatment solution has a pH value of 3.1. It also indicates that lettuce accumulates ascorbic acid in the leaf at a high level when young plants are treated.

INTRODUCTION

With consumers' knowledge of nutritional science, there arises an increased demand for vegetables which are excellent not only in appearance but also in taste and nutritional value. From a nutritional viewpoint, the main role of vegetables is to supply vitamins and minerals. Among vitamins, ascorbic acid (ascorbic acid refers to both L-ascorbic acid and dehydro-ascorbic acid) is one of the most important components in the evaluation of the quality of vegetables. The ascorbic acid content varies widely, even in the same species, depending on the fertilization and weather conditions (Aoyama, 1978; Shinohara, 1981) and cultivation time (Russel *et al.*, 1983). It is, therefore, doubtful whether vegetables contain enough vitamin C, compared with the "Standard Tables of Food Composition" (Kagawa, 1995), throughout the year. If vitamin C-enriched vegetables could be constantly supplied, it would be a significant contribution to the maintenance of consumers' health.

In earlier studies with the hydroponic production of leaf lettuce, we replaced the common culture solution by a solution containing only sodium L-ascorbate at a high concentration ($1\,000$ or $2\,000\text{ mg L}^{-1}$) immediately before harvest (Inoue *et al.*, 1994, 1995). These studies confirmed that L-ascorbic acid was absorbed via the roots. Thus, we proposed that leaf lettuce rich in exogeneous ascorbic acid could be produced by soaking the roots in a high concentration of L-ascorbic acid solution. It was also proved that, when a solution of sodium

L-ascorbate and a solution of L-ascorbic acid of the same concentration ($1\,500\text{ mg L}^{-1}$) were used, the latter was more effectively absorbed than the former (Inoue *et al.*, 1996). The initial solution pH values of the former and latter solutions were respectively, 7.2 and 3.1. Based on these results, we assumed that the efficiency of absorption of the ascorbic acid might be directly attributable to the pH value of the ascorbic acid solution. It is necessary from the practical viewpoint to examine the most suitable pH of the ascorbic acid solution for efficient introduction of ascorbic acid to plants. Therefore, the effect of the L-ascorbic acid solution pH on ascorbic acid uptake was examined in detail. We also studied the optimum growth stage in lettuce which would result in the greatest increase in the ascorbic acid content in leaves.

MATERIALS AND METHODS

Lettuce (cv. Red Fire) and bunching onion (cv. Hakata Kuronegi) were sown on an urethane substrate ($300\times 600\times 20\text{ mm}$) which could be cut into cubes (a piece size was $20\times 20\times 30\text{ mm}$, Shinwa Co.). After germination, the seedlings were transplanted into a culture bed ($1\,000\times 400\times 12\text{ mm}$ stylofoam covering; provided with 8 holes at a diameter of about 15 mm). Subsequently, they were grown by the Nutrient Film Technique for 32 d (lettuce) or 42 d (bunching onion) in a greenhouse. Twelve days after transplanting, the seedlings were thinned into one plant (lettuce) or three plants (bunching onion) per hole. Otsuka Hausu Baiyoeiki (Otsuka Chemical Co.) was used as the culture solution. The concentration of the macro nutrients was as follows (mg L^{-1}); 173N, 35P, 198K, 109Ca and 30Mg. The cultivation was performed by an intermittent supply method wherein the culture solution was supplied for 5 min every 15 min and, the pH of the culture solution was adjusted daily to 5.5 with hydrochloric acid or sodium hydroxide solution. The lettuce plants were used for Experiments 1 and 2. The bunching onion plants were used for Experiment 1.

Experiment 1: Effects of pH value of treatment solution on ascorbic acid content in leaves.

Four solutions of L-ascorbic acid (AsA) were prepared: (1) AsA solution without pH adjusted (resulting in pH 3.1), (2) AsA solutions regulated to pH 4.0 and (3) pH 5.0 with 2.0 M sodium hydroxide, and (4) prepared by dissolving sodium L-ascorbate (resulted in pH 7.2). These solutions were all maintained at an AsA concentration of $1\,500\text{ mg L}^{-1}$. This concentration level was selected since soaking at $2\,000\text{ mg L}^{-1}$ in earlier studies caused obvious wilting of lettuce leaves and soaking at $1\,000\text{ mg L}^{-1}$ caused no damage, but only a slight increase in ascorbic acid content (Inoue *et al.*, 1996). These ascorbic acid solutions were kept in 200 mL Erlenmeyer flasks covered with aluminum foil. The lettuce or bunching onion was taken from the hydroponic system and a number of plants per hole (lettuce=1, bunching onion=3) were inserted into the flask with sufficiently submerged roots in the ascorbic acid solution and then allowed to stand in a growth chamber (temperature: 27°C , relative humidity: 40–50%, PPFD: $380\text{ }\mu\text{mol m}^{-2}\text{ s}^{-1}$) for 12 h.

Experiment 2: Effects of soaking at various growth stages on ascorbic acid content in lettuce leaves.

The growth stages were classified based upon the plant fresh weight. Plants weighing 15 to 25 g (the early stage), 45 to 60 g (the medium stage) and 80 to 100 g (the latter stage) were sampled from the hydroponic system and used for the treatment. The ascorbic acid solution was prepared by dissolving AsA to provide a concentration of $1\,500\text{ mg L}^{-1}$ at pH 3.1. Other procedures or methods were described in Experiment 1.

Experiments 1 and 2 were replicated four times. After the soaking treatment, the plants were removed from the flasks. The amount of the residual ascorbic acid solution and the plant fresh weight were measured. The lettuce plants were divided into three parts: leaves,

stems and roots. The stems were omitted because there was not enough mass to analyze ascorbic acid. The bunching onion plants were divided into two parts: shoots and roots. The roots were rinsed with distilled water for a few minutes in order to remove the ascorbic acid attached to the root surface. Five grams of these fine pieces of plant tissue were homogenized in a mortar with 5 mL of metaphosphoric acid solution (10%) or of mixture solution of metaphosphoric acid (10%) and thiourea (4%) and filtrated through a filter paper (No. 6; ADVANTEC TOYO Co.). Dehydro-ascorbic acid (DHA) and AsA concentration in the filtrate and the residual ascorbic acid solution were measured by the 2,4-dinitrophenylhydrazine method (Roe and Oesterling, 1944) after proper dilution. The inorganic components in the pieces and the residual ascorbic acid solutions were determined by atomic absorption spectrometry after being digested by nitric and sulfuric acid mixture. Immediately before treatment, the ascorbic acid content in lettuce or bunching onion plants was measured as the ascorbic acid content in the control.

RESULTS AND DISCUSSION

Experiment 1. The plant fresh weights before and after soaking, pH values and mineral concentration of the residual ascorbic acid solutions are presented in Table 1. During the soaking period, neither of the vegetables showed any abnormal appearance in the leaves, while the root surface color turned light yellow. The fresh weight ratios after soaking exceeded 95% and no wilting was observed. The pH value of the residual ascorbic acid solution increased in the treatment with lower pH solution, while it decreased in the treatment with a high pH solution. Potassium and calcium, which were scarcely contained at the initiation (initial value of K and Ca concentration were approximately 0.85 mg L^{-1} and 0.23 mg L^{-1} , respectively) tended to increase in the residual ascorbic acid solution with a lower initial pH value. It has been demonstrated that K leaks from roots to a medium of low pH value (Marschner *et al.*, 1966). Since K and Ca were also observed in the residual solution of sodium L-ascorbate, ascorbic acid itself was considered to be a cause of some damage to the root cell membranes.

Figure 1 shows the ascorbic acid (AsA + DHA) concentration, the AsA concentration in the residual ascorbic acid solutions and amount of water absorbed per plant (lettuce) or per three plants (bunching onion). In both lettuce and bunching onion, no large differences were observed in water absorption among pH treatments. However, the ascorbic acid concentration in the residual ascorbic acid solutions increased in the higher initial pH value, which

Table 1 Fresh weight of lettuce and bunching onion, and final pH and mineral concentrations of the residual ascorbic acid solutions after soaking the roots for 12 h.

Plant	Treatment (initial pH)	Fresh weight (g)		Ratio (%) ^z	Residual ascorbic acid solution		
		Before soaking	After soaking		Final pH	Mineral concn. (mg L^{-1})	
Lettuce	pH 3.1	102.4	96.9	96 a ^y	3.5	15.6 a	4.1 a
	pH 4.0	98.2	93.3	95 a	4.6	8.0 b	3.0 b
	pH 5.0	95.7	91.8	95 a	6.1	9.0 b	2.8 b
	pH 7.2	96.7	92.1	95 a	6.4	7.8 b	2.3 b
Bunching onion	pH 3.1	23.3	22.1	95 a	3.4	5.6 a	2.2 a
	pH 4.0	23.6	23.2	99 b	4.3	4.5 b	1.8 ab
	pH 5.0	24.9	24.2	97 ab	5.5	3.9 b	1.7 ab
	pH 7.2	24.0	23.1	96 a	6.2	3.9 b	1.5 b

^z (Fresh weight after soaking/fresh weight before soaking) $\times 100$.

^y Mean separation within columns by Duncan's multiple range test, $p \leq 0.05$.

Fig. 1 The ascorbic acid concentrations in the residual solutions after soaking the roots of lettuce and bunching onion for 12 h. A water absorption of lettuce and bunching onion indicates the amount per one plant and three plants, respectively. Vertical bars indicate the standard deviation, bars smaller than the symbols are not shown. AsA and DHA in the figure indicate L-ascorbic acid and dehydroxy-ascorbic acid, respectively.

showed a less absorbance of ascorbic acid from the given solutions at a higher pH condition in the solution. In all the pH treatments, the AsA concentration of bunching onion exceeded that of lettuce. In other words, the solution after soaking of lettuce contained higher DHA concentration than that of bunching onion.

The ascorbic acid (AsA+DHA) contents and AsA contents in lettuce and bunching onion are presented in Figs. 2 and 3. In the case of lettuce, the ascorbic acid content in the leaves tended to increase in lower initial pH value of the treated solution. In particular, a marked increase was observed in the treatment of pH 3.1. The AsA content in the leaves showed a similar tendency. The ascorbic acid content in the roots also increased in the lower pH solution, since the DHA content was increased and the AsA content was stable. In the bunching onion, the ascorbic acid content in the leaves also increased in the lower pH solution and a remarkable increase was observed in the treatment of pH 3.1. Since only sodium ascorbate is contained in the ascorbic acid solution of pH 7.2, ascorbic acid should exist mostly in the form of ascorbate ions. While ascorbic acid should exist, ascorbic acid molecules form mostly in the treatment of pH 3.1. Molecular ascorbic acid became dominant in the lower pH solution. Therefore, it is assumed that both of these leaf vegetables may be able to absorb ascorbic acid molecules better than ascorbate ions.

In all pH treatments, the DHA content in lettuce exceeded that in bunching onion. Compared with the case of bunching onion, lettuce showed a high DHA concentration in the residual ascorbic acid solution (Fig. 1) and a large DHA content in the roots (Fig. 2). Since the ascorbic acid solution contained only AsA at the initiation of the soaking treatment, it was assumed that the enzyme, ascorbate oxidase, acted strongly in the lettuce. It has been reported that DHA is still effective in the physiological activity of vitamin C (Erdman and Klein, 1982; Russel *et al.*, 1983). Furthermore, it is considered that DHA has substantially the same activity as AsA in physiological activity (Pelletier and Brassard, 1977; Mokady *et al.*, 1984).

Fig. 2 Effect of pH value of L-ascorbic acid solution on the ascorbic acid content in lettuce after soaking the roots for 12 h. The ascorbic acid content in the control indicates the value immediately before soaking. Vertical bars indicate the standard deviation, bars smaller than the symbols are not shown. AsA and DHA in the figure indicate L-ascorbic acid and dehydroxy-ascorbic acid, respectively.

Fig. 3 Effect of pH value of L-ascorbic acid solution on the ascorbic acid content in bunching onion after soaking the roots for 12 h. The ascorbic acid content in the control indicates the value immediately before soaking. Vertical bars indicate the standard deviation, bars smaller than the symbols are not shown. AsA and DHA in the figure indicate L-ascorbic acid and dehydroxy-ascorbic acid, respectively.

Therefore, in this paper, the quantitative evaluation of the nutritional effect of vitamin C is made on the sum of AsA and DHA. Due to the soaking treatment, the leaf ascorbic acid contents in the treatment of pH 3.1 were 191 ± 21 mg (lettuce) and 183 ± 17 mg (bunching onion) each per 100 g of fresh weight, thus about 7.3 times and 5.4 times as much as the initial

Table 2 The ascorbic acid concentration in the residual ascorbic acid solutions, the water absorption ability and the ascorbic acid content in leaves and roots after soaking lettuce roots at various growth stage for 12 h.

Growth stage ^z	Ascorbic acid concn. in the residual solution (mg L ⁻¹)		Absorption ability ^x	Ascorbic acid content (mg/100 g • fresh weight)			
				Leaves		Roots	
	AsA + DHA ^y	AsA		AsA + DHA	AsA	AsA + DHA	AsA
Early stage	1 650 a ^w	1 216 a	10.3 a	204.4 a	125.9 a	92.5 a	34.9 a
Medium stage	1 609 a	1 148 a	9.2 b	189.2 a	128.8 a	88.4 a	27.9 b
Latter stage	1 560 a	1 017 b	6.8 c	151.6 b	91.6 b	40.5 b	14.4 c

^z The growth stage was classified depending on the fresh weight, that is, plant weight at the early, medium and latter stage are 15 to 25 g, 45 to 65 g and 80 to 100 g, respectively.

^y AsA and DHA indicate L-ascorbic acid and dehydroxy-ascorbic acid, respectively.

^x The water absorption ability per gram fresh weight of roots.

^w Mean separatin within columns by Duncan's multiple range test, $p \leq 0.05$.

values, respectively. These values were much higher than these observed in Brussels sprouts (150 mg/100 g fresh weight) which contain vitamin C in an extremely large amount (Kagawa, 1995).

Experiment 2. Table 2 shows the ascorbic acid concentration in the residual treatment solutions, the water absorption ability per gram fresh weight of roots and the ascorbic acid content of leaves and roots after soaking lettuce at various growth stages. At each growth stage, the ascorbic acid (AsA+DHA) concentration in the residual solution exceeded the initial value (1 500 mg L⁻¹). In contrast, the water absorption ability was clearly lowered as the growth proceeded. The ascorbic acid contents of the leaves exceeded 200 mg/100 g fresh weight at the early stage of the growth but decreased with the stage. Thus, it is expected that ascorbic acid can be incorporated at a high content within a short period at the early growth stage when the roots may be more physiologically active.

It has been proved that AsA (or DHA) can be efficiently absorbed by lettuce and bunching onion by the soaking method at a low pH of the ascorbic acid solution and, in the case of lettuce, at the early stage of plant growth. In general, ascorbic acid concentrations in crops vary widely depending on the storage conditions (Zee *et al.*, 1991). Thus, we are going to study further changes in the absorbed ascorbic acid after treatment depending on storage conditions.

Ascorbic acid is produced mainly by plants. Thus, it is common to enrich the endogenous ascorbic acid content of plants by breeding or improving cultivation techniques. In this study, we attempted to introduce exogeneous ascorbic acid into vegetables for several reasons. There has been no cultivation technique established by which endogenous ascorbic acid can be as significantly increased as this study without deteriorating the commercial value of vegetables. Since AsA can be synthesized from glucose on a commercial scale at a relatively low cost, it is expected that the production of ascorbic acid-rich leaf vegetables by this method is applicable in practice. Therefore, we believe that it is socially significant to supply leafy vegetables rich in ascorbic acid because of its contribution to the maintenance of consumers' health.

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〈和文抄録〉

処理液の pH および生育ステージの違いがレタスおよび
葉ネギ葉部の外生アスコルビン酸含量に及ぼす影響

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アスコルビン酸を豊富に含む葉菜類の生産を目的として、レタス(品種:レッドファイヤー)およびネギ(品種:博多黒ねぎ)の根を pH の異なる L-アスコルビン酸の単一溶液 (1500 mg L^{-1}) に 12 時間浸漬し、葉部のアスコルビン酸含量の増加程度を検討した。この処理は、温度 27°C 、湿度 $45\sim 55\%$ 、PPFD $380 \mu\text{mol m}^{-2} \text{s}^{-1}$ に設定した人工気象室で行った。処理液の pH が低下するほど葉部アスコルビン酸含量は増加した。最も pH の低い処理区 (pH 3.1) の葉部アスコルビン酸含量は、レタスで初期値の $26 \pm 8 \text{ mg}$ (新鮮重 100 g あたり、以下同じ) から $191 \pm 21 \text{ mg}$ に、ネギでは $34 \pm 8 \text{ mg}$ から $183 \pm 17 \text{ mg}$ に、それぞれ増加した。レタスの場合、処理時の生育ステージが遅くなるほど根の吸水能が低下し、同時に葉部アスコルビン酸含量の増加率も低下した。以上から、レタスおよびネギは処理液の pH が低いとアスコルビン酸をよく吸収することが認められた。また、レタスでは処理時の生育ステージが早いほど、アスコルビン酸を葉部に高濃度に集積することが示された。