Changing the Concentration of the Culture Solution before Harvesting Affected the Component Quality of Takana (Brassica juncea var. integrifolia)

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INTRODUCTION

Takana (Brassica juncea var. integrifolia), which is widely cultivated in warm regions of western Japan, is mainly pickled in salt. Takana, called Mamba in Kagawa Prefecture, is commonly used as an ingredient in local cuisine. Takana contains lye and has a strong bitter taste. Therefore, it is necessary to remove lye from Takana by boiling and soaking in water before cooking.

Nitrate nitrogen, which is accumulated large amounts in leafy vegetables such as Takana (Yorifuji et al., 2005), is the main source of lye (Noda and Makuta, 2015). Nitrates are reduced to nitrites in the body and can cause methemoglobinemia in infants (Okabe, 1977). In addition, nitrites may combine with secondary amines to form suspected carcinogenic nitrosamines (Okabe, 1977). Boiling and soaking in water has been used to reduce harmful substances, such as nitrate nitrogen in Takana, but causes a reduction in useful components, such as ascorbic acid. Therefore, if Takana with low nitrate nitrogen content could be produced, useful components could be preserved because boiling and soaking would not be required.

The nitrate nitrogen content of vegetables has been studied in many leafy vegetables. For example, studies using lettuce (Lactuca sativa L. var. youmaican), Mizuna (Brassica rapa L. var. perviridis), Cos lettuce (Lactuca sativa L. cv. Parris Island) and spinach (Spinacia oleracea L.) have found that nitrates accumulated in plants is affected by nitrogen application (Takebe et al., 1995; Shinhara et al., 2007; Kondo et al., 2008; Konstantopoulos et al., 2010; Fu et al., 2017). The nitrate content of hydroponic spinach and Mizuna was decreased by changing the culture solution before harvesting (Yoshida et al., 1998; Tsukagoshi et al., 1999; Kawaguchi et al., 2006; Kondo et al., 2008; Konstantopoulos et al., 2010; Fu et al., 2017). Based on these studies, we think it is possible to produce Takana with the low nitrate nitrogen content by controlling fertilizer components.

In this study, the culture solution was changed before harvesting hydroponic Takana to establish a technique for reducing nitrate nitrogen content of Takana, and the effect on nitrate nitrogen content was investigated. The effects of the culture solution concentration on the ascorbic acid content was analyzed, and the growth conditions suitable for the cultivation of high-quality Takana were examined.

MATERIALS AND METHODS

Plant material and growth conditions
Hydroponic Takana (Brassica juncea var. integrifolia ‘Miki Takana’) was cultivated in a natural daylength unheated greenhouse at the Faculty of Agriculture, Kagawa University (34°16′32″N, 134°07′30″E). The temperature during the growing period was measured by a temperature data logger (Ondotori Jr. TR-51, T&D Corporation, Japan) The average temperature during the experiment ranged from 14 to 30°C. On September 28, 2018, Takana were seeded in urethane foam (23.5×23.5×25.5 mm). The urethane foam was filled in holes made in poly-
styrene foam (925×580×30 mm, 137 holes (20×20 mm), drilled at 3.5 cm intervals) and floated in a container (1024×664×200 mm) containing 80 dm³ of a culture solution. The culture solution was adjusted to half the concentration of OAT House solution A (Total nitrogen 130, Ammonia nitrogen 11.5, Nitrate nitrogen 116.5, P₂O₅ 60, K₂O 202.5, CaO 115, MgO 30, MnO 0.75, B₂O₃ 0.75, Fe 1.35, Cu 0.015, Zn 0.045 and Mo 0.015 ppm, EC of 1.4 dS m⁻¹) by mixing 60 g of OAT House 1 (OAT Agrio Co., Ltd., Japan) and 40 g of OAT House 2 (OAT Agrio Co., Ltd., Japan) into 80 dm³ of water. Takana were raised for 20 days after seeding. Raised seedlings were randomly selected for each treatment (48 plants per treatment) and transplanted to a polystyrene foam (925×580×30 mm) container (24 plants per a container). In the polystyrene foam, 6×4 holes (20×20 mm) were drilled at 14 cm intervals. After transplanting, Takana were cultivated using the culture solution adjusted to quarter or half the concentration of OAT House Solution A (EC of 0.7 or 1.4 dS m⁻¹). From this onwards, water was added to the culture solution once a week without changing the culture solution, and electrical conductivity (EC) and potential Hydrogen (pH) of culture solution were measured using a portable electrical conductivity meter (CM-31P type, DKK-TOA Co., Ltd., Japan) and a portable pH meter (HM-30P type, DKK-TOA Co., Ltd., Japan; Fig. 1).

Changing the culture solution treatments

The culture solution was changed before harvest, 22 days after transplanting. In the 1.4-1.4 treatment, the culture solution was readjusted to half the concentration of OAT House solution A (EC of 1.4 dS m⁻¹). In the 0.7-NC and 1.4-NC treatment, the culture solution was not changed. In the 0.7-NF and 1.4-NF treatment, the culture solution was changed to water containing no fertilizer component.

Growth measurement

The number of leaves, plant height, and chlorophyll content (SPAD value) were measured every week after transplantation on October 18. The chlorophyll content was measured using a chlorophyll meter (SPAD-502, Konica Minolta Japan, Inc., Japan).

Harvested leaf size, fresh weight and color

The largest mature leaf leaves of Takana were harvested one by one from 6 plants per container (12 plants per treatment area) on day 1, 3, 5, 7 and 10 after changing the culture solution and the leaf length, leaf width, fresh

![Fig. 1](image-url)
weight, and chlorophyll content were measured. The color of the harvested leaves was measured using a chlorophyll meter.

*Nitrate nitrogen and ascorbic acid determination*

The nitrate nitrogen content and ascorbic acid content of the harvested leaves were measured using a reflective photometer (RQflex 10, Merck, Germany) (Takebe and Yoneyama, 1995).

**Statistical analysis**

All measurements were analyzed by Tukey-Kramer method for significance at $P \leq 0.05$.

**RESULTS AND DISCUSSION**

The number of leaves and plant height in the 1.4-1.4, 1.4-NC and 1.4-NF treatments were no significant difference from October 19 to November 8 (Table 1). The chlorophyll content was no significant difference between treatments on October 19, October 25 and November 8 (Table 1). The 0.7-NC and 0.7-NF treatments showed that the number of leaves decreased significantly from November 1, and the plant height decreased significantly from October 25 (Table 1). In harvested leaves of Takana, the score of number of leaves, plant height, leaf length, leaf width and fresh weight in the 0.7-NF and 0.7-NC treatments were significantly lower than in other treatments from day 1 to day 10 (Table 2). These results indicate that using culture solution an adjusted EC of 0.7 dS m$^{-1}$ from the beginning of growth was inappropriate for agricultural production. The 1.4-NF treatment showed significantly lower leaf length, leaf width and fresh weight compared to the 1.4-1.4 treatment on day 10 (Table 2). There are two ways to harvest Takana: harvesting the leaves one by one or harvesting the whole plant. In this experiment, yield of Takana harvested the leaves one by one may have decreased on the day 10 after changing the culture solution to no fertilizer. Further studies are needed on the yield of Takana harvested the whole plant. The chlorophyll content was no significant difference between treatments from day 1 to day 10 (Table 2). This result indicated that changing the culture solution before harvesting did not affect chlorophyll content of Takana.

In the 0.7-NC and 0.7-NF treatments, the nitrate nitrogen content of leaves was 10 mg/100g FW or less and the ascorbic acid content was 80 mg / 100g or more from the first day of harvest, and this trend was maintained thereafter (Figs. 2, 3). From this, it was likely that the component quality of Takana improved with less fertilizer component from the beginning of growth. In the 1.4-1.4 treatment, the nitrate nitrogen content was high compared to other treatments from day 1 to day 10 (Fig. 2), and the ascorbic acid content was low compared to other treatments on day 7 and 10 (Fig. 3). Accordingly, the addition of fertilizer components before harvesting reduced the component quality of Takana. Nitrate nitrogen decreased in the 1.4-NF and 1.4-NC treatments from day 3 (Fig. 2). However, it decreased more quickly in the 1.4-NF treatment. Yorifuki et al. (2005) reported that the average value of nitrate nitrogen in Takana on the market was ranged from 39.32 to 150.29 mg/100g FW (nitrate nitrogen content [mg/100g FW] = nitrate concentration [mg/kg FW] x 0.0226). In the 1.4-NF treatment, the average value of the nitrate nitrogen content was 33.39±4.06 mg/100g FW on day 1, which was lower than the minimum value of Takana on the market, and the average value was 18.91±2.17 mg/100g FW on day 5, which was about half the minimum value of Takana on the market. Hydroponic spinach has been reported to have a significant decrease in nitrate ion concentration after day 4-6 from the end of supply of fertilizer components before harvesting (Kataoka et al., 1998; Tsukagoshi et al., 1999; Kawaguchi et al., 2006). A similar report was also made in hydroponics Mizuna (Kirimura et al., 2015). In this experiment of Takana, the nitrate nitrogen content was rapidly reduced by changing the culture solution to non-fertilizer before harvesting. In the 1.4-NF treatment, the ascorbic acid content showed a constant value from day 1 to day 5 harvest and increased rapidly from day 7 (Fig. 3). The ascorbic acid content increased

| Treatment | Oct. 19 | Oct. 25 | Nov. 1 | Nov. 8 |
|-----------|---------|---------|--------|--------|
| Number of leaves | 1.4-1.4 | 4.4±0.1 a | 5.9±0.1 a | 7.9±0.1 a | 7.1±0.1 a |
| | 1.4-NC | 4.3±0.0 a | 5.8±0.1 a | 7.7±0.1 a | 6.9±0.1 a |
| | 1.4-NF | 4.3±0.0 a | 5.8±0.1 a | 7.7±0.1 a | 6.9±0.1 a |
| | 0.7-NC | 4.3±0.0 a | 5.7±0.1 a | 7.2±0.1 b | 6.4±0.1 b |
| | 0.7-NF | 4.4±0.0 a | 5.9±0.1 a | 7.2±0.1 b | 6.4±0.1 b |
| Plant height (cm) | 1.4-1.4 | 15.8±0.2 a | 20.7±0.2 a | 27.8±0.3 a | 34.9±0.5 a |
| | 1.4-NC | 15.7±0.2 a | 20.4±0.2 a | 27.8±0.3 a | 35.4±0.5 a |
| | 1.4-NF | 16.2±0.2 a | 20.5±0.2 a | 27.7±0.3 a | 35.2±0.5 a |
| | 0.7-NC | 16.1±0.2 a | 19.4±0.2 b | 24.2±0.3 b | 28.8±0.6 b |
| | 0.7-NF | 16.1±0.2 b | 19.7±0.2 b | 24.6±0.2 b | 29.5±0.4 b |
| Chlorophyll content (SPAD value) | 1.4-1.4 | 32.2±0.3 a | 34.2±0.4 a | 37.0±0.4 ab | 36.4±0.5 a |
| | 1.4-NC | 32.6±0.3 a | 34.6±0.4 a | 38.0±0.5 a | 37.3±0.6 a |
| | 1.4-NF | 31.8±0.3 a | 34.7±0.3 a | 37.5±0.6 a | 38.0±0.6 a |
| | 0.7-NC | 31.5±0.3 a | 34.2±0.4 a | 35.1±0.5 b | 37.1±0.6 a |
| | 0.7-NF | 31.7±0.3 a | 33.5±0.3 a | 35.3±0.5 b | 37.3±0.5 a |

Values indicate mean±SE ($n = 48$). Different letters indicate a significant difference at $P \leq 0.05$ between treatments on each measurement day by Tukey-Kramer method.
from day 8 to day 10 after changing to groundwater without the addition of nutrients in the hydroponic spinach (Yoshida et al., 1998). It was assumed that the increase of ascorbic acid in hydroponic Takana required more than 7 days after nutrient interruption.

Ascorbic acid was negatively correlated with nitrate nitrogen (Fig. 4). Moreover, ascorbic acid increased rapidly when nitrate nitrogen was less than 10 mg/100g FW (Fig. 4). Takebe et al., (1995) reported that the nitrate nitrogen content increases and ascorbic acid content decreases in high nitrogen application to spinach and komatsuna. Accordingly, in our opinion the ascorbic acid content and nitrate nitrogen content may be negatively correlated in other plants.

The relationship between nitrate nitrogen and ascorbic acid has not been elucidated in plants. The main biosynthetic pathway for ascorbic acid is thought to be the D-mannose-L-galactose pathway in plant leaves (Ishikawa, 2011). D-fructose 6-phosphate is converted to L-galactono-1,4-lactone in the cytoplasm, and L-galactono-1,4-lactone is converted into ascorbic acid by dehydrogenase in the mitochondria. Nitrate assimilation requires a carbon skeleton derived from photosynthesis. The flow of carbon shifts from starch synthesis to the synthesis direction of pyruvic acid and oxaloacetic acid as nitrate assimilation proceeds simultaneously with carbon dioxide assimilation (Oji, 1989). Ascorbic acid biosynthesis could compete with nitrate assimilation for photosynthetic products. Interest-

| Treatment   | day 1 | day 2 | day 3 | day 4 | day 5 | day 6 |
|-------------|-------|-------|-------|-------|-------|-------|
| Leaf length (cm) | 1.4-1  | 14-NC | 1.4-NF | 0.7-NC | 0.7-NF | 1.4-NF |
| Leaf width (cm) | 15.6±0.4 | 16.0±0.5 | 16.8±0.4 | 16.1±0.2 | 17.4±0.3 | 17.4±0.3 |
| Fresh weight (g) | 25.8±1.1 | 27.1±1.5 | 30.10±1.34 | 30.97±1.5 | 36.55±1.46 | 36.55±1.46 |
| Chlorophyll content | 40.6±1.4 | 40.1±1.2 | 37.9±1.0 | 36.2±1.5 | 39.4±1.5 | 38.0±1.2 |

Values indicate mean±SE (n = 3). Different letters indicate a significant difference at P ≤ 0.05 between treatments on each measurement day by Tukey-Kramer method.
ingly, ascorbic acid, which reacts with sodium nitrite, was converted to dehydroascorbic acid in a flask (Mori and Mitani, 1979). The nitrite generated in the process of nitrate assimilation could react with ascorbic acid in plants. In addition, nitrite is converted to active nitrogen, such as nitric oxide, in plants (Sakamoto et al., 2004). Active nitrogen species are powerful oxidants and nitro agents. Ascorbic acid may be involved in the metabolism of active nitrogen. The synthesized ascorbic acid is localized in the cytoplasm, chloroplast, vacuole, and apoplast. In particular, the chloroplast contains 30-40% ascorbic acid (Fukusaki and Kobayashi, 1998). The portion of nitrate nitrogen absorbed by plants, that is not assimilated, is stored temporarily in vacuoles (Oji, 1989). Therefore, it is likely that nitrate nitrogen and ascorbic acid may compete for storage. Hence, it can be concluded that ascorbic acid and nitrate nitrogen have some relationship such as biosynthetic pathway, metabolic pathway and storage in the plant body, and the increase or decrease of nitrate nitrogen content may affect the ascorbic acid content. It is important to clarify the relationship between nitrate nitrogen and ascorbic acid in plants to cultivate high quality leafy vegetables.

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