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Internal Blue Discoloration in Daikon, Japanese Radish (*Raphanus sativus* L.) Roots

Katsunori Teranishi[1]* and Nagata Masayasu[2]

[1]*Graduate School of Bioresources, Mie University, Japan*

[2]*Food Research Institute, National Agriculture and Food Research Organization, Japan*

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***Corresponding author:** Katsunori Teranishi, Graduate School of Bioresources, Mie University, 1577 Kurimamachiya, Tsu, Mie, Japan, Tel: +81592319615; Email: teranisi@bio.mie-u.ac.jp

Abstract

Daikon, Japanese radish (*Raphanus sativus* L.) is a major vegetable in Japan. Internal blue discoloration is observed in Daikon roots on a few days after harvest and poses a significant problem to farmers in recent years. To circumvent the blue discoloration, improved cultivation methods, new storage methods after harvest, and/or fundamental development of new Daikon cultivars, which do not lead to the blue discoloration, are required. This mini review concentrates our relevant investigations that contribute to suppression of the blue discoloration. Here we present chemical mechanism underlying the blue discoloration, rapid and simple method for predicting the onset of the blue discoloration at harvest, and useful storage methods for suppression of the blue discoloration.

Keywords: Daikon; Radish; Blue compound; 4-hydroxyglucobrassicin; Chemical mechanism

Mini Review

Daikon, Japanese radish (*Raphanus sativus* L.) is an important agricultural crop in Japan and is most abundantly cultivated for vegetable food (Figure 1). In Japan, Daikon is cultivated on about 33 thousand hectares with about 1.5 million tons annually. In order to improve the appearance, harvesting efficiency, disease resistance, and taste of Daikon roots, Daikon breeding is in progress.

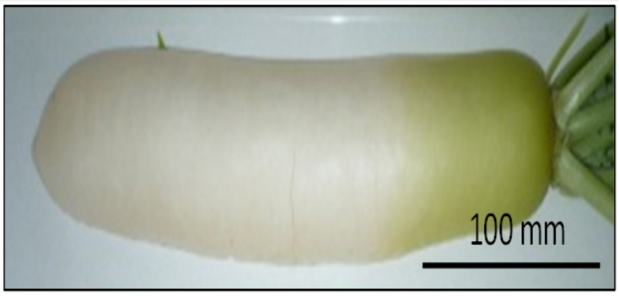

Figure 1: Outward of fresh Daikon root after harvested.

Over the past decade in Japan, the physiological phenomenon of blue component generation has been observed in the Daikon roots during the distribution after harvest (Figure 2) [1]. This physiological phenomenon does not occur at the time of harvest and is often observed by consumers during cooking, leading to complaints to shopkeeper or farmers. Although the internal blue discoloration has not been reported to be harmful, the commodity value of affected Daikon is decreased due to the strange blue color. Thus, the blue discoloration is a serious problem for shopkeepers and farmers.

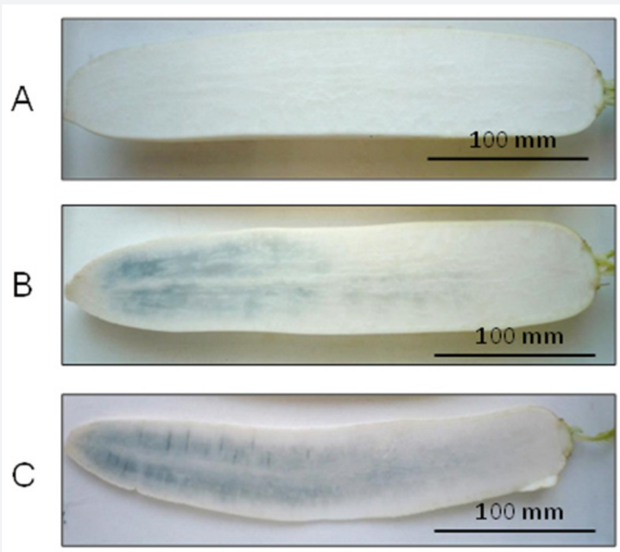

Figure 2: The internal blue discoloration of Daikon roots, which have been known to suffer from blue discoloration in Japan. (A) inside of freshly harvested normal root, (B) internal blue discoloration of root stored at 20 °C for 4 days, and (C) artificial blue discoloration of freshly harvested root sections bysoaking in 0.29 M aqueous hydrogen peroxide **at** 20 °C for 10 min.

Many researches on another physiological phenomenon, internal brown discoloration of Daikon roots have been reported [2-5]. However, little research on the internal blue discoloration of Daikon roots has been reported, and chemical structures of blue components and the mechanism underlying the onset of blue discoloration were unknown. One reason of little research on the blue discoloration is an incorrect prejudice that the blue components are anthocyan compounds. It has been reported that the incidence of the blue discoloration is dependent on the cultivar of Daikon and the condition of the cultivation [6], and the blue discoloration is induced by managed storage at 20 °C for three to five days [1,7]. In order to stably supply Daikon roots to consumer, restrain of the internal blue discoloration is needed.

To circumvent the internal blue discoloration, fundamental development of new cultivars generating no blue discoloration and/or improvement of cultivation methods are needed. Understanding the chemical mechanism of the blue discoloration contributes to these subjects. We discovered that 4-hydroxyglucobrassicin (Figure 3) is only precursor to the blue components in Daikon roots and immediately offers blue components by oxidation reaction with reactive oxygen species [8]. 4-Hydroxyglucobrassicin is generally contained in the seeds, sprouts, and roots of the Brassica plants [9,10]. This is the first discovery that 4-hydroxyglucobrassicin generates the blue compounds in plants.

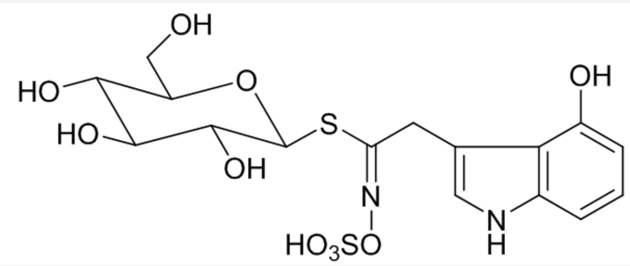

Figure 3: Chemical structure of 4-hydroxyglucobrassicin.

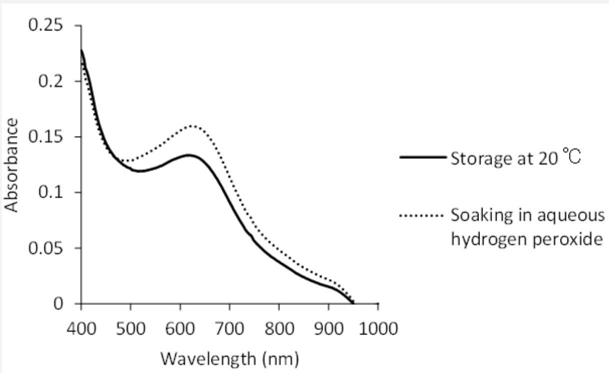

Figure 4: Visible absorption spectra of the blue components. The extracts were prepared from Daikon root stored at 20 °C for 4 days and from Daikon root soaked in 0.29 M aqueous hydrogen peroxide solution. Adapted from Ref. [8].

Our efforts in determining the chemical structures of blue components produced in Daikon roots were unsuccessful due to the ubiquitous types of blue components and the limited amount. 4-Hydroxyglucobrassicin has been known to be unstable to oxidation and easily converted to blue compound(s) [11]. The oxidation mechanism was investigated using 4-hydroxyindole as a model, indicating that oxidation proceeds via the formation of indol-(4,7)-p-quinone [12,13]. The Vis absorption spectrum of oxidation products presented in the literature is similar to the blue components we obtained (Figure 4). Thus, it appears that compounds, which possess an indol-(4,7)-p-quinone skeleton, should be produced as blue components.

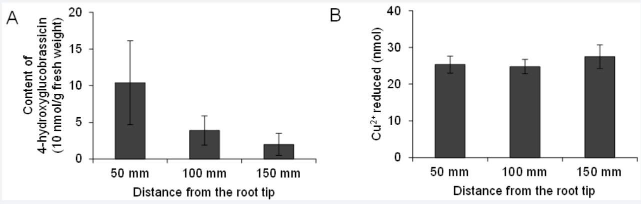

Figure 5: Contents of 4-hydroxyglucobrassicin (A) and total antioxidant capacity (B) in xylem sections obtained at 50, 100, and 150 mm from the root tip of freshly harvested Daikon roots, (n = 6).

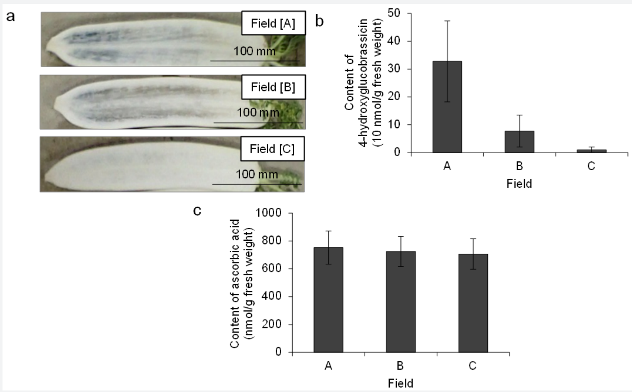

Figure 6: The internal blue discoloration of freshly harvested same cultivar of Daikon cultivated at fields A, B, and C after storage at 20 °C for 4 days (a), contents of 4-hydroxyglucobrassicin (b), and total antioxidant capacity (c) in xylem sections obtained at 50 mm from the root tip of freshly harvested Daikon roots (n = 6). Adapted from Ref. [14].

Subsequently, we indicated that the onset of internal blue discoloration at 20 °C depends on 4-hydroxyglucobrassicin content, oxidative stress involving peroxidase, and antioxidant capacity in Daikon roots [14]. 4-Hydroxyglucobrassicin content in the lower sections of Daikon roots is greater than in the upper sections (Figure 5A) and total antioxidant capacities at all parts are similar (Figure 5B). 4-Hydroxyglucobrassicin content in Daikon roots is directly related to the degree of blue discolorations after storage at 20 °C in Figure 2B. In addition, Figure 6a shows significant differences in the degree of blue discoloration for same cultivar of Daikon cultivated at three different fields. 4-Hydroxyglucobrassicin contents in xylem sections at 50 mm from the root tip of these Daikon roots correspond with the degrees of blue discoloration observed in roots stored at 20 °C (Figure 6b). Antioxidant capacities do not differ significantly among these fields (Figure 6c). Therefore, in these cases, the

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blue discoloration is dependent on the 4-hydroxyglucobrassicin content. The Daikon roots with low 4-hydroxyglucobrassicin content and/or a larger reductive components and/or low peroxidase activity would be desirable to suppress the onset of internal blue discoloration.

A method for predicting the onset of internal blue discoloration has been developed. The soaking of freshly harvested Daikon root sections in 0.29M aqueous hydrogen peroxide solution at room temperature generates immediate blue discoloration consistent with that occurring during storage at 20 °C: consistent location (Figure 2C) and consistent visible absorption spectrum (Figure 4) [8,14]. The correlation was confirmed for 18 cultivars of Daikon using visual assessment on the blue discoloration, affording highly positive correlation [15]. These results indicate that blue discoloration assay with aqueous hydrogen peroxide solution at the time of harvest is a useful way to predict the incidence of internal blue discoloration in Daikon root. This method can be employed easily by farmers due to the low concentrations of hydrogen peroxide, the low costs, and the simple and rapid operation of the method that requires no technical knowledge. Moreover, we developed a seed testing method for the evaluating the risk of internal blue discoloration in Daikon roots [16]. Daikon seeds are soaked in water and the seeds coats are removed. Subsequently, the naked seeds are soaked in 3% aqueous hydrogen peroxide solution at room temperature for 2h and blue components are produced in the seeds. The visual assessment on the blue discoloration in the seeds can predict the onset of internal blue discoloration in roots.

Even when the 4-hydroxyglucobrassicin content in Daikon roots is high, internal blue discoloration can be circumvented by suppression of oxidative stress. Ikeshita et al. showed that the blue discoloration in Daikon roots, which have been known to suffer from the discoloration in Japan, can be suppressed by storage at 5 °C after harvest [7]. We confirmed that the 4-hydroxyglucobrassicin content for 8 days during storage at 5 °C was almost same with that before the storage (unpublished data). Therefore, it appears that the storage at 5 °C can suppress the oxidative stress.

Recently, we showed that Daikon roots in polyethylene bags with 0.03 and 0.05 mm thickness and without bag generated internal blue discoloration on days 7th, 8th, and 5th, respectively, at 20 °C in the dark [17]. On day 5th from the start of storage, concentrations of molecular oxygen in these bags were 7 and 5 %, respectively, and both concentrations of carbon dioxide were 8 %. Thus, condition with low concentration of molecular oxygen can suppress the blue discoloration. It appears that low concentration of molecular oxygen decreases oxidative stress in roots, whereas effect of oxygen concentration on 4-hydroxyglucobrassicin content has not yet known.

In Japan, Daikon roots after harvest are sold out at supermarkets in a week. Then, when the prediction assay for the onset of interanal blue discoloration using hydrogen peroxide by sampling inspection at the time of harvest shows high risk of the blue discoloration onset, storage at 5 °C or in polyethylene bags with 0.05 mm thickness at even 20 °C for these Daikon lots can circumvent the blue discoloration in a week.

At present, we are aiming to develop novel cultivars with no internal blue discoloration and cultivation methods able to suppress internal blue discoloration based on results so far. We are hoping to successfully develop a blue discoloration-free Daikon and/or cultivation method in the near future. Moreover, greater efforts in other research fields are necessary to fully resolve the blue discoloration problem.

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