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Salted Radish Root Biology during Food Processing

*Hiroki Matsuoka, Kei Kumakura, Taito Kobayashi,
Wataru Kobayashi and Asaka Takahashi*

Abstract

White radish root (daikon) is an important vegetable in Japanese food culture and has spawned the development of various cooking and processing methods. takuan-zuke is the major processed food derived from daikon. Takuan-zuke is prepared by the dehydration of the root using a salt-press or a weighted stone, or by sun-drying, followed by salt-aging using salt or salted rice bran. The color of takuan-zuke changes to yellow during salt-aging. We determined the effects of dehydration and salt-aging on the metabolism of daikon using takuan-zuke. In the yellowing reaction, the generation of daikon isothiocyanate was significant, requiring a temperature of $\geq 10^{\circ}\text{C}$ and $\text{pH} \geq 5$. The color change of the sun-dried takuan-zuke was the most significant. Moreover, we investigated the nutritional characteristics of takuan-zuke. In the sun-dried daikon, metabolism progressed for 3 weeks during drying, with increase in the concentrations of γ -aminobutyric acid (GABA) and proline as well as drying stress metabolites. In the salt-pressed daikon, GABA concentrations temporarily increased due to osmotic stress but then decreased on metabolic inhibition by salt permeation. In addition, no change in the concentration of proline was observed under salt-press conditions. The results showed a marked difference between the stress response of the living and processed root.

Keywords: salted radish root, takuan-zuke, *Tsukemono*, isothiocyanates, metabolome analysis, GABA

1. Introduction

Daikon (Japanese white radish, *Raphanus sativus* L. var. *longipinnatus* Bailey) is an important vegetable in Japanese food culture (*Washoku*). The literal translation of daikon to English is big (or giant) root. In Japan, the weight of one root is 1.5–2 kg. Daikon is an indispensable ingredient of *Washoku* and has been used in salads, simmered dishes (*Nimono*), pickles (*Tsukemono*), and in the form of grated radish root (*Daikon oroshi*). In Japan, pickled daikon (salted radish root), which has been salt-aged for several months with salt or salted rice bran, is called takuan-zuke. It is presumed that the original form of takuan-zuke was made over 300 years ago. Currently, takuan-zuke is made by dehydrating raw daikon by sun-drying or salt-pressing followed by aging with salt or salty bran. The amount of salt and the length of salt-aging are dependent on the shipping time. With the modernization

Isothiocyanate	Relative Amount
$\text{CH}_3-(\text{CH}_2)_4-\text{N}=\text{C}=\text{S}$	+
$(\text{CH}_3)_2-\text{CH}-(\text{CH}_2)_2-\text{N}=\text{C}=\text{S}$	++
$\text{CH}_3-(\text{CH}_2)_5-\text{N}=\text{C}=\text{S}$	+
$\text{CH}_3\text{S}-(\text{CH}_2)_3-\text{N}=\text{C}=\text{S}$	++
$\text{C}_6\text{H}_5-\text{CH}_2-\text{N}=\text{C}=\text{S}$	+
$\text{CH}_3\text{S}-\text{CH}=\text{CH}-(\text{CH}_2)_2-\text{N}=\text{C}=\text{S}$	+++++
$\text{CH}_3\text{S}-(\text{CH}_2)_4-\text{N}=\text{C}=\text{S}$	++
$\text{CH}_3\text{S}-(\text{CH}_2)_5-\text{N}=\text{C}=\text{S}$	+++

Table 1. Volatile isothiocyanates in Japanese radish roots [1]. Relative data was obtained by gas chromatograph with a flame ionization detector [1]. Symbols: +, <1%; ++, <1–5%; +++, 5–10%; +++++, >70%.

of pickles, the term “Genboku” refers to aged takuan-zuke, while low-salted radish roots stored at low temperatures in seasoning liquid are currently known as takuan-zuke. In Japan, takuan-zuke is a representative pickle, eaten as a side dish to steamed rice (*Gohan*), while in Europe and the United States, pickles are used as ingredients for cooking.

The flavor of raw daikon and its processed products is derived from a characteristic, pungent chemical component [1]. As shown in **Table 1**, this component, 4-methylthio-3-butenyl isothiocyanate (MTBITC), is formed by the enzymatic conversion of glucoraphasatin (GRH), which is a glucosinolate (GSL), by myrosinase (**Figure 1**). Isothiocyanates (ITCs) act as protective agents against pests in plants. In processed radish roots, such as takuan-zuke, MTBITC contributes by imparting its characteristic flavor and color [2–4]. Nakamura et al. reported the presence of 37–420 $\mu\text{mol}/100\text{ g}$ of MTBITC and 280–1270 $\mu\text{mol}/100\text{ g}$ of GRH in raw radishes, based on analysis results from a total of 83 samples from 7 varieties [5].

Owing to their antimicrobial properties, naturally occurring GSLs and ITCs have been studied for a long time, with 132 types identified by 2011 [6–9]. Recently, the role of ITC in human redox regulation and the activation of detoxification enzymes which act against carcinogens have been studied [10, 11]. It has also been reported that the phototropism of radish hypocotyls promotes myrosinase gene expression, leading to MTBITC production in the illuminated side of the plant [12, 13]. Increased MTBITC has been shown to induce expression of a heat shock protein that increases the heat resistance of the plant [14]. In shredded cabbage, allyl isothiocyanate (AITC) has been shown to inhibit browning, ethylene production, and respiration [15]. Furthermore, downregulation of phenylalanine ammonia-lyase gene expression by AITC treatment has been observed [16].

However, ITCs are unstable in aqueous solution, and their retainment in processed foods with long shelf lives is difficult. Specifically, the degradation of MTBITC is faster than other ITCs and is completely gone within a few hours in processed *Daikon oroshi* (grated radish) [17, 18].

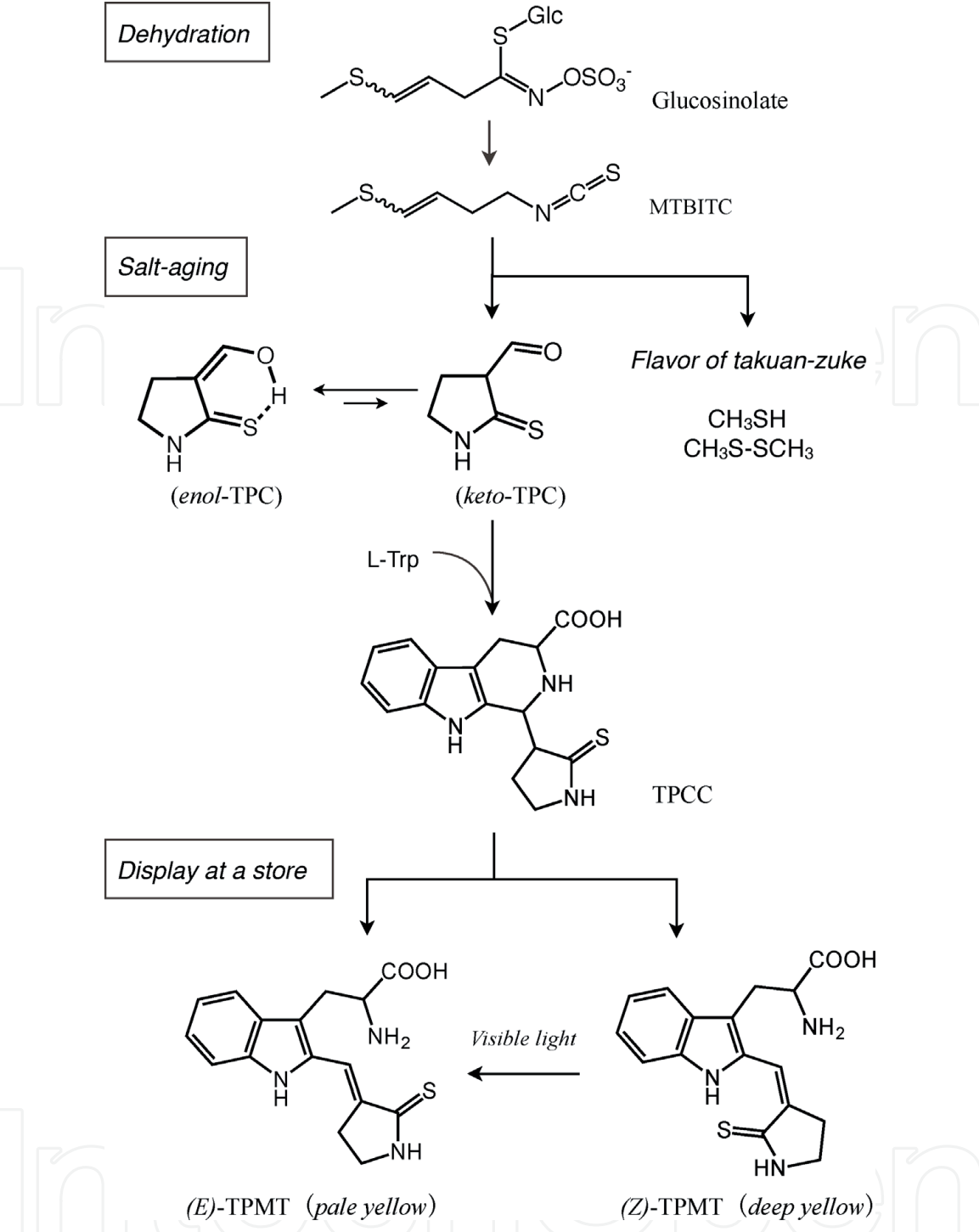


Figure 1.
Pathway of yellowing reaction of radish isothiocyanate.

We present a study of the multistep mechanism of white daikon to yellow takuan-zuke during the process of pickling. This process has shown to induce secondary metabolism in the radish leading to accumulation of hypotensive factor, γ -aminobutyric acid (GABA). We also show that intake of takuan-zuke, containing GABA, is effective in hypertensive model rats. Therefore, our study provides useful information to both consumers and pickle manufacturers.

2. Yellowing mechanism of salted radish root (takuan-zuke)

For takuan-zuke production, dehydration of the raw radish is required as a pre-treatment process. This process destroys the cells and tissues of the plant and

activates isothiocyanate production. The yellow-color change of takuan-zuke is the result of a four-step reaction (**Figure 1**).

Step 1: Physical damage to plant cells and tissues by osmotic shock or stone weight activates the myrosinase reaction to generate MTBITC from GRH.

Step 2: MTBITC is converted to 2-thioxo-3-pyrrolidinecarbaldehyde (TPC)-releasing methanethiol due to high reactivity with water molecules [17, 19].

Step 3: The aldehyde group of TPC and the amino group of tryptophan, which is produced by microorganisms or self-maturation in the fermentation process, are condensed by the Pictet-Spengler reaction and converted to 1-(2-thioxopyrrolidine-3-yl)-1,2,3,4-tetrahydro- β -carboline-3-carboxylic acid (TPCC) [2, 20]. This reaction occurs under acidic pH conditions.

Step 4: The ring structure of TPCC is cleaved to form a yellow pigment, 2-[3-(2-thioxopyrrolidin-3-ylidene)methyl]-tryptophan (TPMT). This reaction can occur under either weakly acidic or neutral pH conditions [21].

Steps 1 and 2 occur during the dehydration process. TPC, which is a major degradation product of 4-methylthio-3-butenyl (MTBI), is an important intermediate. In Daikon oroshi, the MTBI produced is rapidly converted to TPC, with the conversion rate reaching 85% after 2 hours [17]. In takuan-zuke, the radish turns yellow, without browning, by passing through this reaction pathway. Therefore, the yellowing reaction is an indicator of the degree of salt-aging.

2.1 Behavior of yellow pigment and related components during the salt-aging process

We prepared sun-dried and salt-pressed takuan-zuke under normal-temperature conditions (0–2 months at 5°C, 2–4 months at 10°C, and 4–8 months at 20°C) and low-temperature conditions (0–8 months at 5°C). The salt-aging conditions of takuan-zuke were as follows: sun-dried normal-temperature (DN) takuan-zuke, sun-dried low-temperature (DL) takuan-zuke, salt-pressed normal-temperature (SN) takuan-zuke, and salt-pressed low-temperature (SL) takuan-zuke. After 8 months, the salinity of the DL and SL samples was 8–9%, and the salinity of DN and SN samples was 15–16%.

Figure 2 shows the yellowing of DN samples. The effect of pH on the TPCC/TPMT yellow-color change reaction of the long-term salt-aging process was analyzed over time (**Figure 3**). The yellow pigment, TPMT, was produced at $9 \pm 3 \mu\text{mol/kg}$ after 4 months of salt-aging and reached $63 \mu\text{mol/kg}$ after 8 months in the DN sample. Although the other samples did turn yellow, compared to the DN sample, they had smaller amounts of TPMT.

The pH of the DN sample was maintained at ≥ 5 , while the other samples became acidic during the salt-aging treatment. It was suggested that the pH of the sample during salt-aging contributes to the yellowing reaction. However,

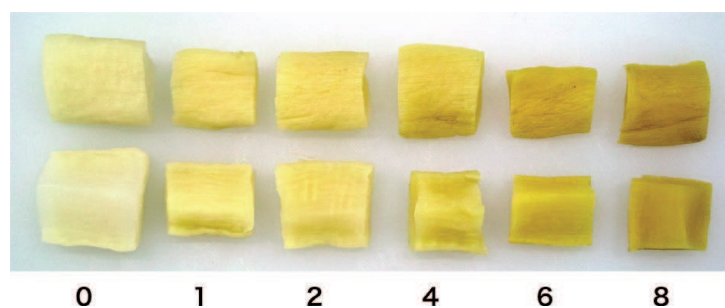


Figure 2. Yellowing of sun-dried normal-temperature takuan-zuke (DN). Zero number indicated sun-dried daikon, other numbers indicate salt-aging time (months).

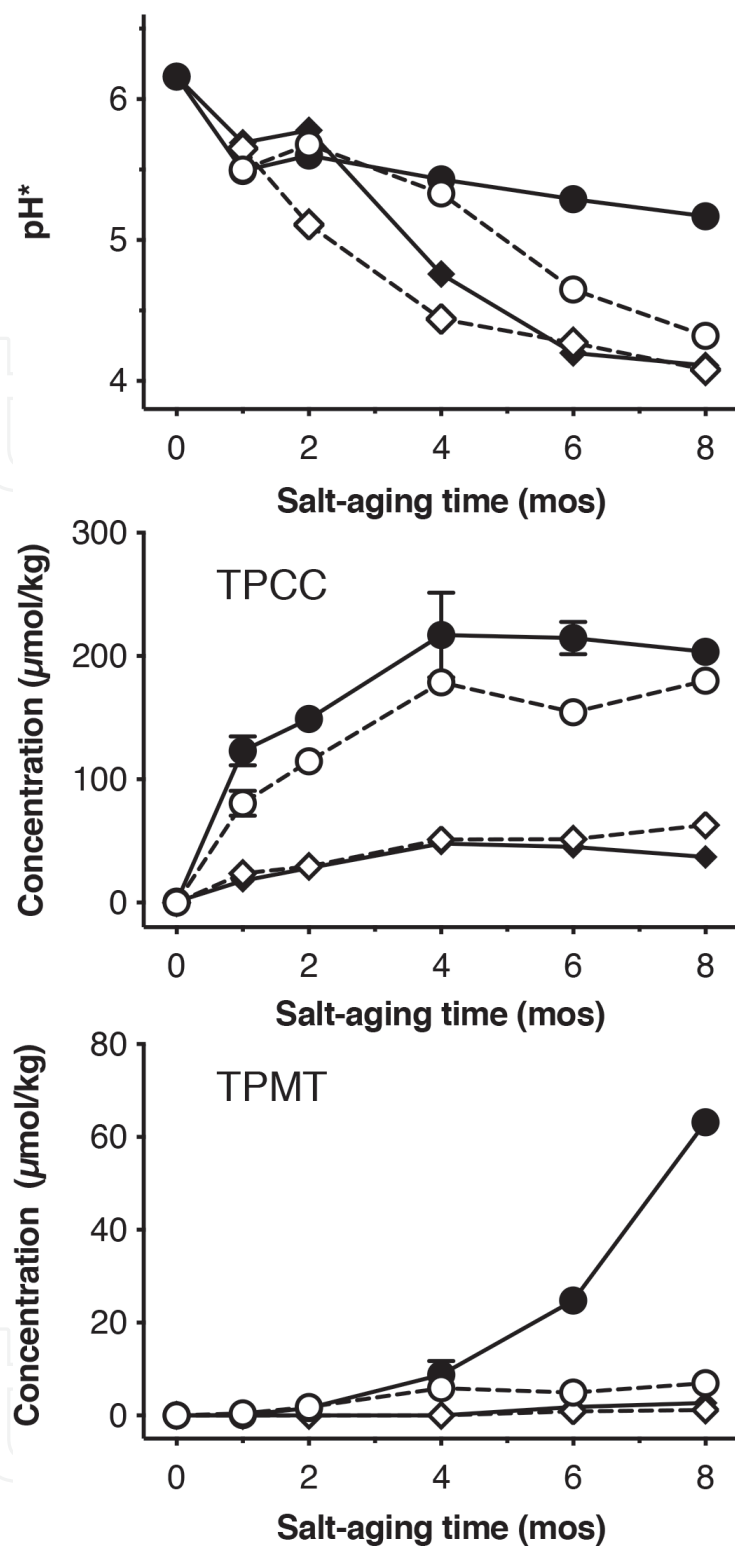


Figure 3. Change in pH value and concentration of yellow pigment and related components in takuan-zuke. pH value of the brined liquid during the salt-aging process. ◆, salt-pressed normal-temperature takuan-zuke (SN); ◇, salt-pressed low-temperature takuan-zuke (SL); ●, sun-dried normal-temperature takuan-zuke (DN); ○, sun-dried low-temperature takuan-zuke (DL).

it is unclear what role the reaction has on the radish itself. It is reported that TPC has biological functions in humans, including antibacterial activity against food poisoning and cariogenic bacteria and antimutagenic activity against carcinogenic heterocyclic amines [22, 23]. Recently, TPCC and TPMT, which increase with salt-aging, have also been revealed, in our research to have antioxidant activities [24], thereby enabling elucidation of the health benefits of takuan-zuke.

3. Metabolomic analysis of salted radish root (takuan-zuke)

Recently, metabolomic analyses involving nuclear magnetic resonance and mass spectrometry have been introduced in the field of food science [25, 26]. Metabolomic analysis enables determination of features by performing comprehensive instrumental and multivariate analyses on a metabolite at a specific moment in time. In the fields of agronomics and food science, it is used for the determination of characteristic secondary metabolites through analysis of variations in different varieties of samples during cultivation, storage, and processing. A mass spectrometer is nonessential, as it is possible to perform multivariate analysis on results obtained from a conventional detector. A description of the application of the latter method to the metabolomic analysis of takuan-zuke can be found in our previously published study [27].

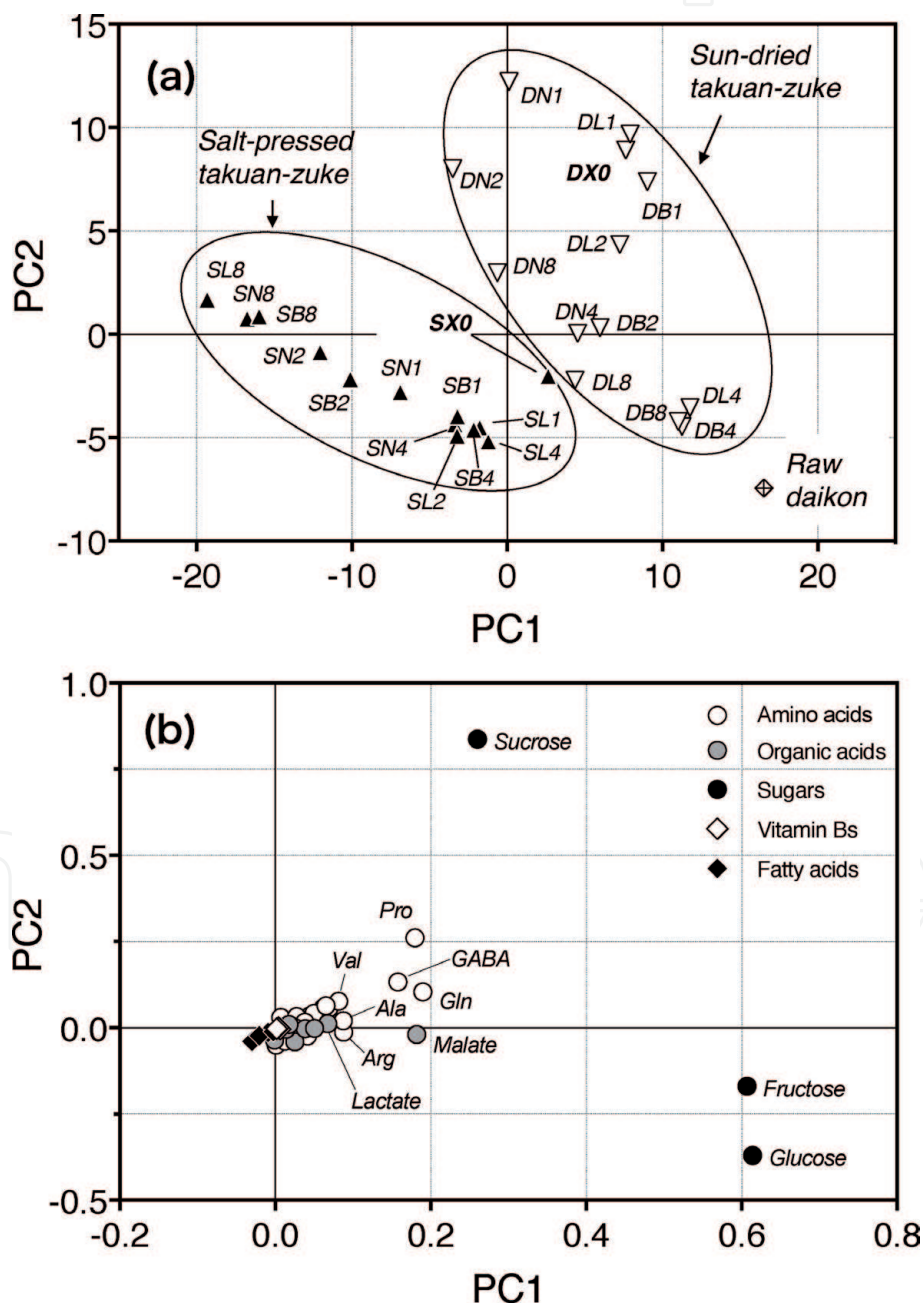


Figure 4. Multivariate analysis of all takuan-zuke groups. (a) PCA score plots generated using all samples. (b) PCA loading plots from all samples. SN, salt-pressed normal-temperature takuan-zuke; SL, salt-pressed low-temperature takuan-zuke; SB, salt-pressed low-temperature takuan-zuke with rice bran; DN, sun-dried normal-temperature takuan-zuke; DL, sun-dried low-temperature takuan-zuke; DB, dried low-temperature takuan-zuke with rice bran. Numbers indicate salt-aging time (months).

Past research of daikon has focused on its characteristic, pungent flavors. Therefore, we focused on hydrophilic components including amino acids, organic acids, and sugars that were different from those of the pungent flavor. We examined the differences between salt-pressed and sun-dried takuan-zuke, conventional high-salt normal-temperature storage and more recent low-salt low-temperature storage, and the influence of rice bran during salt-aging using the metabolomic analysis method.

We analyzed the components over time in the salt-pressed and sun-dried takuan-zuke for 8 months under the conditions of normal temperature (high-salt), low temperature (low-salt), and low temperature (low-salt, rice bran). In takuan-zuke prepared in 2010, amino acids (glutamine and GABA), organic acid (malic acid), sugars (glucose and fructose), and free fatty acids (α -linolenic, palmitic, and linoleic acid) were detected as major components. Principal component analysis (PCA) shows that the differences in the components of each takuan-zuke depend on the method of dehydration applied (**Figure 4a**). The results of the PCA loading plot show components such as sucrose, proline, and GABA were detected in the first quadrant of **Figure 4b** which corresponds to sun-dried takuan-zuke. In addition, fructose and glucose were detected in the fourth quadrant, which corresponds to raw daikon. However, no characterization of salt-pressed takuan-zuke was found.

Plants under osmotic stressors such as salt and dryness promote the synthesis of proline, a low-toxicity, and compatible solute for water retention [28, 29]. In the

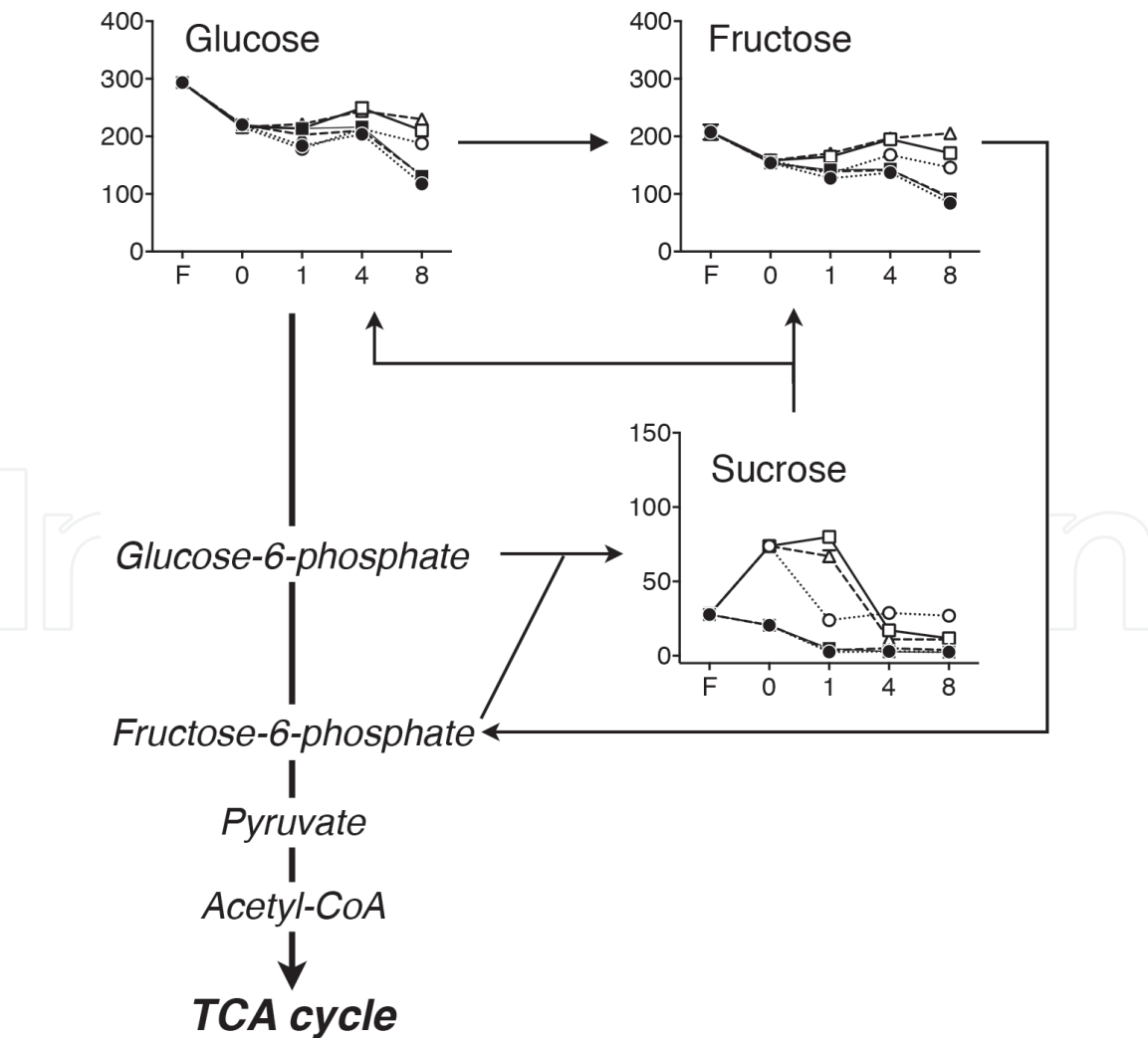


Figure 5. Time-dependent changes in metabolite concentrations. The x axis denotes processing time (months), and the y axis denotes concentration (mg/g of dry weight, mean \pm SD). Zero time denotes the start of salt-aging for dehydration. Symbols denote the following: ○, DN; □, DL; △, DB; ●, SN; ■, SL; ▲, SB.

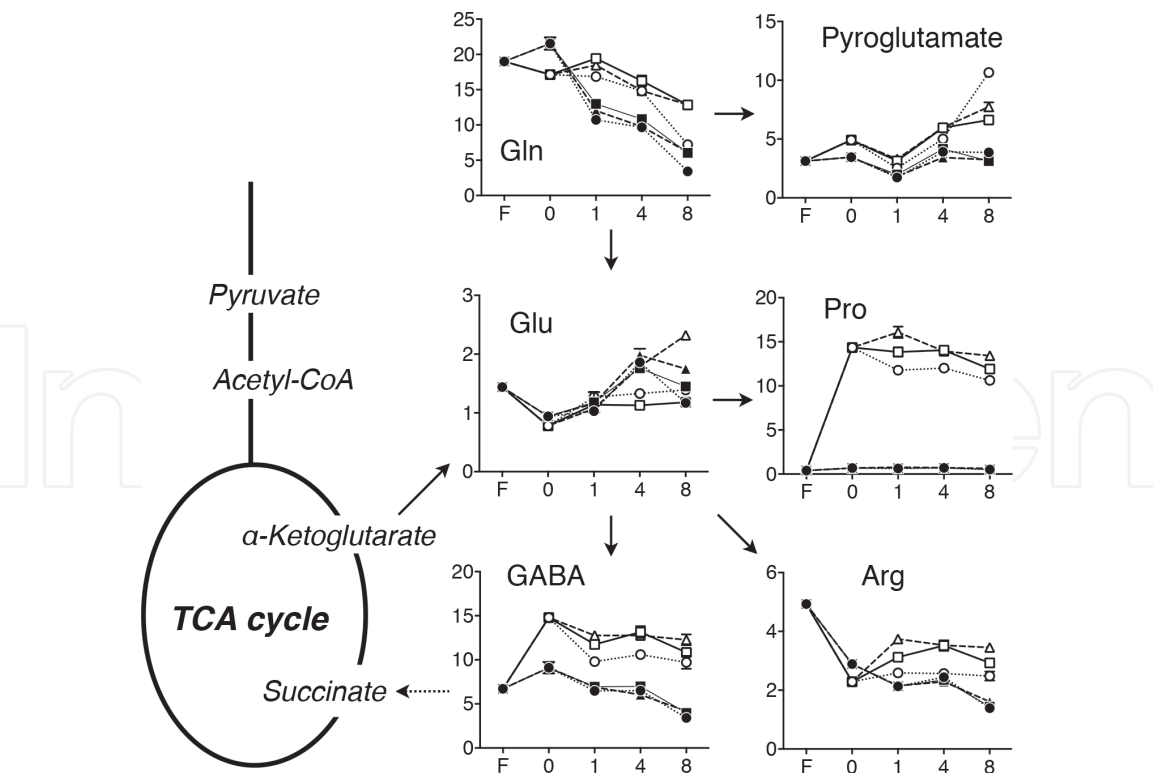


Figure 6. Time-dependent changes in concentrations of metabolites related to Glu and GABA. The x axis denotes processing time (months), and the y axis denotes concentration (mg/g of dry weight, mean ± SD). Zero time denotes the start of salt-aging for dehydration. Symbols denote the following: ○, DN; □, DL; △, DB; ●, SN; ■, SL; ▲, SB.

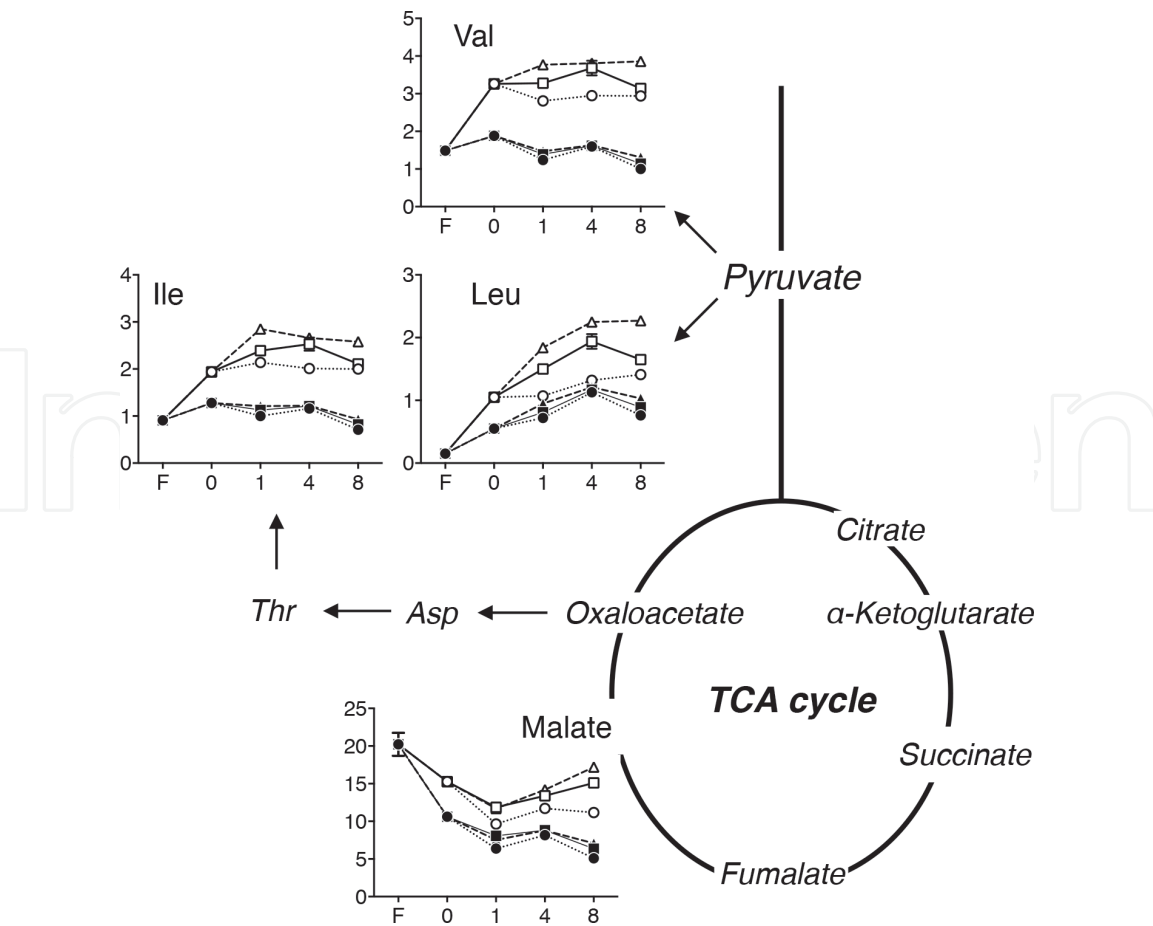


Figure 7. Time-dependent changes in concentration of metabolites related to BCAA. The x axis denotes processing time (months), and the y axis denotes concentration (mg/g of dry weight, mean ± SD). Zero time denotes the start of salt-aging for dehydration. Symbols denote the following: ○, DN; □, DL; △, DB; ●, SN; ■, SL; ▲, SB.

sun-dried takuan-zuke samples, concentrations of sucrose and proline increased significantly with processing time. In the salt-pressed radish samples, it was suggested that metabolic rates decreased due to the mechanical pressure and osmotic dehydration treatments imposed upon the raw daikon. This dehydration process is called *shio-goroshi* among Japanese manufactures.

Figures 5–7 show analyses of carbohydrate, Glu-GABA, and BCAA composition in the form of metabolic maps. Under both dehydrating conditions, branched-chain amino acids (Val, Leu, Ile), GABA, and other minor components with known functionalities tended to increase with processing time. Furthermore, free polyunsaturated fatty acids and pyroglutamic acid increased with salt-aging time. In salt-aging samples treated with rice bran (SBs and DBs), niacin, glutamic acid, and acetic acid, derived from the rice bran, were found to be dependent on salt-aging time rather than the method of dehydration when under the same temperature conditions (data not shown).

The above reactions are significant for sun-dried takuan-zuke, relating to its yellowing reaction tendency. The concentration of total metabolites in the sun-dried samples is higher than those of salt-pressed takuan-zuke. This shows that in the sun-dried takuan-zuke, it is not the simple effect of drying but the induced secondary metabolic reaction from dehydration that affects the metabolism of the functional components.

4. GABA accumulation during daikon dehydration

Plants accumulate GABA in their cells when they are subjected to physicochemical stressors. This is because the proton-consuming glutamate decarboxylase (GAD) reaction is activated to prevent acidification in cells due to stress, and the pH is simultaneously neutralized with GABA production [30, 31]. This pathway is called “the GABA shunt” and involves synthesis of glutamate from α -ketoglutaric acid, an intermediate of the tricarboxylic acid cycle (TCA), to the synthesis of GABA by the GAD reaction. Following the removal of the stressor, succinic acid is further synthesized by GABA transaminase (GABA-T) and succinic semialdehyde dehydrogenase (SSADH) and enters the TCA cycle. Bown reported that the metabolism of glutamate to succinate via the GABA shunt is energetically less favorable than it is via the TCA cycle [32].

The dehydration treatments imposed by salt and weight are strong stressors on vegetables. As a result, they are convenient for activating GABA synthesis with simultaneous inhibition of GABA metabolism. Since our metabolomic analyses of takuan-zuke (Section 2) demonstrated an increase in GABA production, we further studied Glu-GABA during dehydration [33].

4.1 Effects of dehydration processes on GABA concentration and GAD activity

The GABA content of raw daikon harvested in 2013 was 0.28 ± 0.01 mg/g, which increased to 4.9 ± 0.0 mg/g (DW) with salt-pressing treatment and 9.1 ± 0.1 mg/g with sun-drying treatment; the substrate glutamate decreased (**Figure 8**). In general, the GABA content of Japanese radish differs from harvest year to harvest year due to various factors such as weather, temperature, and soil moisture. Analysis of raw radish from 2012 to 2015 revealed a negative correlation between GABA and the glutamate content ($y = -0.429x + 3.14$, $r^2 = 0.884$). It also seemed that the GABA content fluctuated with postharvest storage conditions. Therefore, all daikon samples were frozen within 8 hours of their harvest.

For the salt-pressed daikon samples, GABA production reached a plateau 2 days after salting. In contrast, in the sun-dried daikon samples, GABA synthesis

continued for 3 weeks. The pH values of the sun-dried daikon samples remained between 6.0 and 6.5, while those of the salt-pressed daikon samples decreased over time.

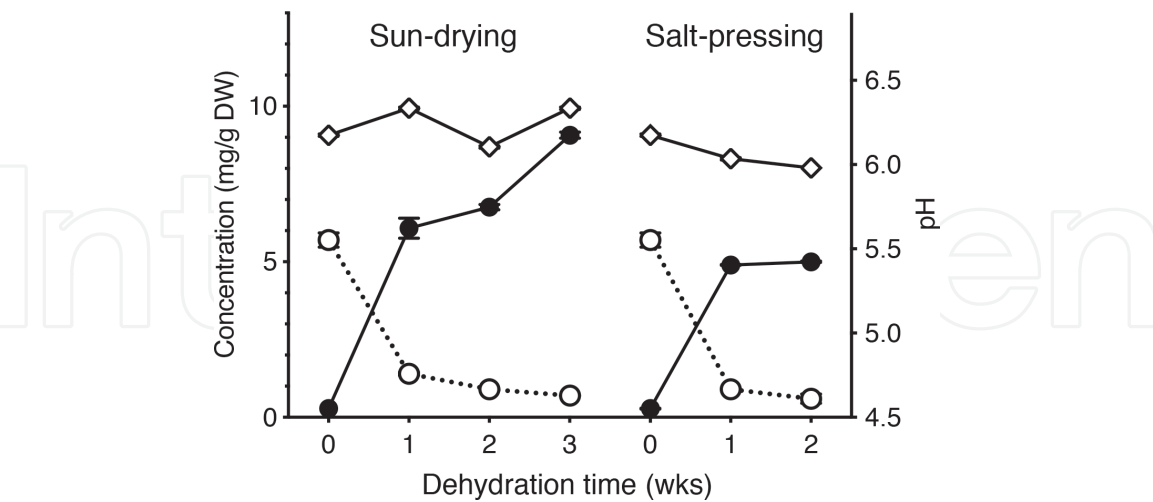


Figure 8. Time-dependent changes of GABA, Glu, and pH during dehydration process. Symbols denote the following: ◇, pH; ○, Glu; ●, GABA.

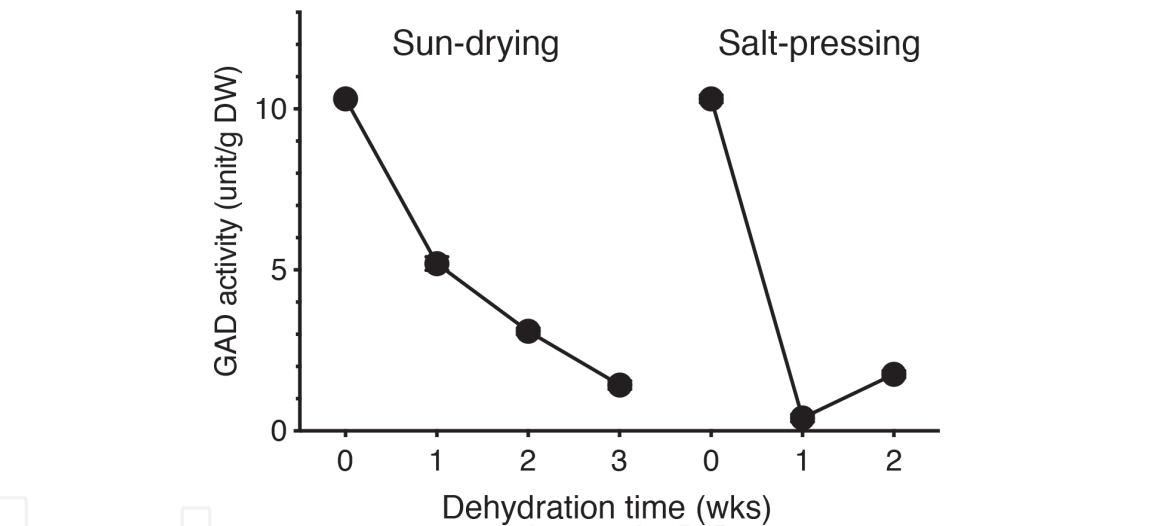


Figure 9. Time-dependent changes of GABA, Glu, and pH during dehydration process.

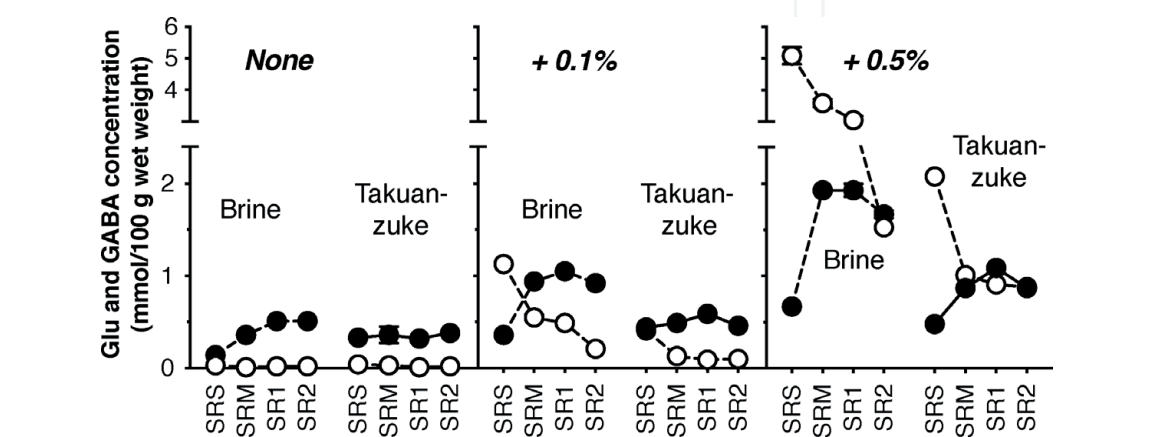


Figure 10. Effect of monosodium glutamate on GABA concentration in the salt-pressed takuan-zuke and the brine during dehydrating and salting processes. The symbols denote the following: ○, Glu; ●, GABA.

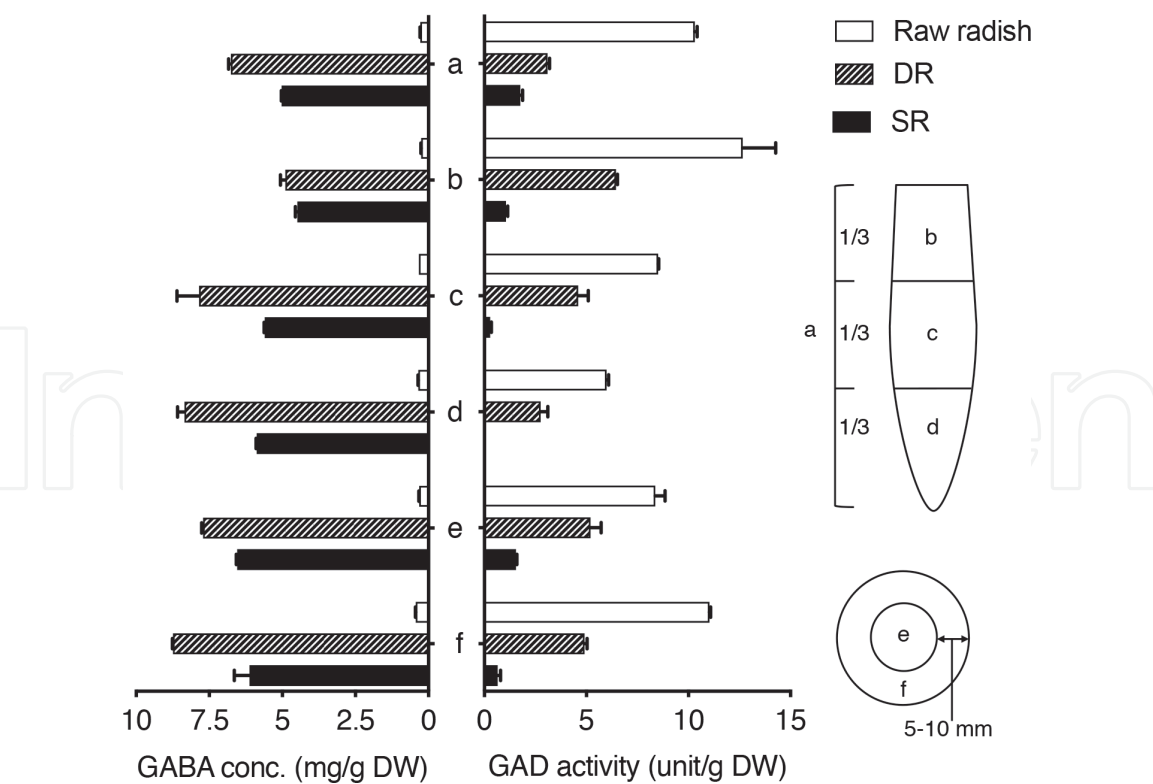


Figure 11.
Distribution of GABA and GAD in dehydrated radish roots. DR, sun-dried radish after 2 weeks; SR, salt-pressed radish after 2 weeks.

Regarding GAD activity, which is involved in GABA synthesis and pH control, the fresh radish had the highest activity, and activity decreased with dehydration treatment time. The enzyme activity decreased gradually with the sun-drying treatment, while it decreased rapidly with the salt-pressing treatment (**Figure 9**). From these results, it was suggested that the glutamate biosynthesis pathway of glutamine is maintained during sun-drying and the GABA synthesis reaction is maintained. It was also revealed that the addition of high amounts of salt inactivated GAD activity, reducing its pH control function.

We prepared GABA-enriched takuan-zuke with the addition of monosodium glutamate (MSG) at the start of salting. As a result, not only GABA concentration in the salted radish increased but also that in the brine (**Figure 10**). The penetration rate of MSG into the radish root is slower than that of NaCl. Thus, GABA, which was converted from MSG in the brine, penetrated into the radish root depending on the concentration gradient. Ueno et al. isolated high-GABA-producing lactic acid bacteria from Senmaizuke, a traditional Kyoto pickle [34]. In takuan-zuke, it is expected that a high-GABA-producing lactic acid bacterium is activated by MSG addition.

4.2 Distribution of GABA concentration and GAD activity in dehydrated radish roots

The distribution of GAD in radish samples was relatively high in the upper root portions, including the base and outer vascular cambium (**Figure 11**). There was no correlation found between residual GAD activity and GABA production. It has been reported that myrosinase, an enzyme involved in MTBITC synthesis, is localized to the epidermis and cambium, and its activity ratio is reported to be 19-fold [35]. The lack of GAD localization showed that the system responding to environmental stressors is functional entirely at the base and root.

5. Conclusion

Takuan-zuke, which is a fermented food, changes the microflora such as lactic acid bacteria and yeast during salt-aging. However, it was not possible to find a dynamic chemical change contributing to microbial fermentation, because the chemical changes derived from the endogenous radish component are large. Kato reported that the presence of long-term-salt-aging-activated *Debaryomyces hansenii*, which is a halo-tolerant yeast; consumption of lactic acid; and generation of ammonia from amino acid during fermentation suppress the decrease in pH [36]. This is presumed to be the reason why the pH of sun-dried normal-temperature takuan-zuke (DN samples) was maintained. The pH condition was optimal for the yellowing reaction.

Recently, taste research studies have revealed that GABA, which in the past had been considered tasteless, affects other tastes. It has also been shown that GABA has an enhancement effect on salty taste [37, 38]. We reported that the intake of takuan-zuke, containing GABA antihypertensive factor, improves renal function and suppresses blood pressure elevation in hypertension model rats [39]. The report also revealed that antihypertensive effects are high in salt-pressed takuan-zuke, which has low GABA content. These results suggest the presence of another antihypertensive factor, different from GABA. In particular, GABA production by simple pickle processing seems to be the key to developing future pickles in terms of both taste function and health benefits.

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Conflict of interest

The authors declare no conflict of interest.

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Author details

Hiroki Matsuoka^{1*}, Kei Kumakura¹, Taito Kobayashi¹, Wataru Kobayashi¹
and Asaka Takahashi²

¹ Department of Health and Nutrition, Takasaki University of Health and Welfare,
Takasaki, Gunma, Japan

² Faculty of Nutritional Sciences, Tohto University, Fukaya, Saitama, Japan

*Address all correspondence to: matsuoka@takasaki-u.ac.jp

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