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Nutritional content and health benefits of sun-dried and salt-aged radish (*takuan-zuke*)

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ABSTRACT

We investigated the nutritional characteristics of salted radish roots (*takuan-zuke*) prepared using different methods: *takuan-zuke* based on sun-drying (*hoshi*) or salt-pressing (*shio-oshi*) dehydration, different salt-aging temperatures, and salting with rice bran. We examined differences in nutritional substances in salted radish using chromatographic analysis, bioassay methods, and multivariate analysis. We previously reported that the amount of γ-aminobutyrate in *takuan-zuke* was increased by both dehydration treatments. In the present study, we observed that sucrose and proline were increased by sun-drying treatment, while little change occurred with salt-pressing treatment. Branched-chain amino acids were increased by both treatments. Interestingly, free fatty acids increased with salt-aging duration, irrespective of the dehydration method. Addition of rice bran to long salt-aging treatment increased the levels of niacin, glutamate, and acetate. Metabolite concentrations were higher in *hoshi takuan-zuke* than *shio-oshi takuan-zuke*. Our comprehensive analysis reveals effects of specific manufacturing conditions on beneficial components of *takuan-zuke*.

1. Introduction

Takuan-zuke, or pickled radish root, is one of the most popular and traditional *tsukemono* in Japan. *Takuan-zuke* is classified into two types depending on the dehydration method used: *hoshi takuan-zuke* is sun-dried, while *shio-oshi takuan-zuke* is dehydrated with salt and pressed. Dehydrated radish root is pickled in salty rice bran. The salt-aging process that gives rise to *takuan-zuke* involves fermentation by microorganisms (Kato, Kitamura, & Ohshima, 1991; Kato, Kitamura, Yamamoto, & Ohshima, 1991; Kato & Nakase, 1986; Kato, Seki, Kaneuchi, & Nakase, 1989). The amount of salt and bran, temperature, and period of salt aging differ depending on the shipping time of the merchandise. High-salted radish undergoes a second processing by pickling in seasoning liquid after desalting. On the other hand, low-salted radish undergoes a second processing without desalting. Salted radish is used for various *tsukemono* (Japanese pickles) such as *asa-zuke*, *fukuzin-zuke*, and *sakura-zuke*. Therefore, clarification of the nutritional value of *takuan-zuke* is important for a review of Japanese traditional food and to promote increased consumption of *tsukemono*.

Many studies have reported that fermented products such as soy sauce, one of the Japanese traditional foods, have antioxidant activity (Esaki, Kawakishi, Morimitsu, & Osawa, 1999) and angiotensin-converting enzyme inhibitory activity (Okamoto et al., 1995). Free fatty acids (FFA) that are produced during the processing of miso are reported to have antimutagenic activity (Watanabe, Owari, Hori, & Takahashi, 2004). Recent investigations have demonstrated that the intake of Japanese traditional miso inhibits salt-induced hypertension in Dahl salt-sensitive rats (Du, Yoshinaga, Sonoda, Kawakubo, & Uehara, 2014; Watanabe, Kashimoto, Kajimura, & Kamiya, 2006).

We previously reported that FFAs in salted radish show antimutagenic activity (Matsuoka et al., 2013). Yellowing-related compounds that are produced from radish isothiocyanate during long-term salting have been shown to possess antioxidant activity (Takahashi et al., 2009). However, few studies have described the

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dynamics of general components, such as functional amino acids, during the unique process of pickling vegetables (Kato et al., 2015).

Metabolomic analysis has become widely used in many research areas. In food science, such studies include investigating the relationship between changes in flavor and metabolites during the preservation of green soy beans (*Edamame*) (Sugimoto et al., 2010) and evaluating the quality of *Matsutake* (*Tricholoma matsutake* Sing) using liquid chromatography-mass spectrometry (Cho, Kim, & Choi, 2007). However, the relationship between metabolites and the nutritional properties of *tsukemono* has not been confirmed.

The present study aimed to compare the nutritional composition of *takuan-zuke* prepared under different treatment conditions using traditional manufacturing methods *via* targeted metabolomic analysis.

2. Materials and methods

2.1. Preparation of salted radish roots (takuan-zuke)

Preparation of *takuan-zuke* was performed using a previously reported method (Takahashi et al., 2015). We used radish cultivar *Hoshi-riso* radish (263 kg) (Takii & Co., Kyoto, Japan), which was cultivated at Juumonji-machi, Takasaki-city, Gunma Prefecture (Japan) between August and November, 2009. The cultivated acreage (740 m^2), which was acidic, was improved by adding 80 kg magnesium lime and 80 kg chemical fertilizer (14% nitrogen, 14% phosphate, and 14% potash). Pesticide (10% pyridalyl, Sumitomo Chemical Co., Ltd.) was sprayed only once.

Takuan-zuke samples used in the present study were self-manufactured under six different sets of conditions involving dehydrating methods, temperature, and presence or absence of rice bran (Fig. 1). The radish root (daikon) was dehydrated *via* sun drying (*hoshi*) or salt pressing (*shio-oshi*). Salt pressing was performed using 8% salt (by weight) and a stone weighing double that of the fresh daikon (137 kg) for 3 weeks at 5 °C. Sun-drying of fresh daikon (126 kg) was performed in a greenhouse for 3 weeks.

Hoshi normal temperature *takuan-zuke* (DN) and *shio-oshi* normal temperature *takuan-zuke* (SN) were pickled in salt (18% and 5% by weight of the dehydrated radish; 15.0 kg and 30.9 kg, respectively). The salting temperature was programmed as follows: 5 °C for 2 months, then 10 °C for 2 months, and 20 °C for 4 months.

Hoshi low-temperature *takuan-zuke* (DL) and *shio-oshi* low-temperature *takuan-zuke* (SL) were pickled for 8 months in salt at 8% or 2% by weight of the dehydrated daikon (13.0 kg and 28.2 kg, respectively). The salting temperature was maintained at 5 °C.

Hoshi low-temperature *takuan-zuke* with bran (DB) was pickled in 7.1% salt and 3.3% bran by weight of the *hoshi* daikon (11.7 kg) for 8 months at 5 °C. *Shio-oshi* low-temperature *takuan-zuke* with bran (SB) was pickled in 2% salt and 1% bran by weight of the *shio-oshi* daikon (23.8 kg) for 8 months at 5 °C. To keep *takuan-zuke* submerged in the brine, a stone weight (1.5-fold the weight of the dehydrated daikon) was placed on top of the barrel. One week after the start of salting, 1.1 kg of 7.4% saline (by weight) and 2 kg of 7.1% saline (by weight) was added to the SB and DB, respectively.

The present study was performed using a single replicate for each treatment group. The headspace of the pickling barrel was replaced with nitrogen gas. Samples were taken through a sample reduction process every 1–2 months. Samples were vacuum packaged and stored at −30 °C. Table 1 shows the average weights, moisture content, and salt content of the radishes. Moisture content was measured using the oven-drying method at 105 °C, while

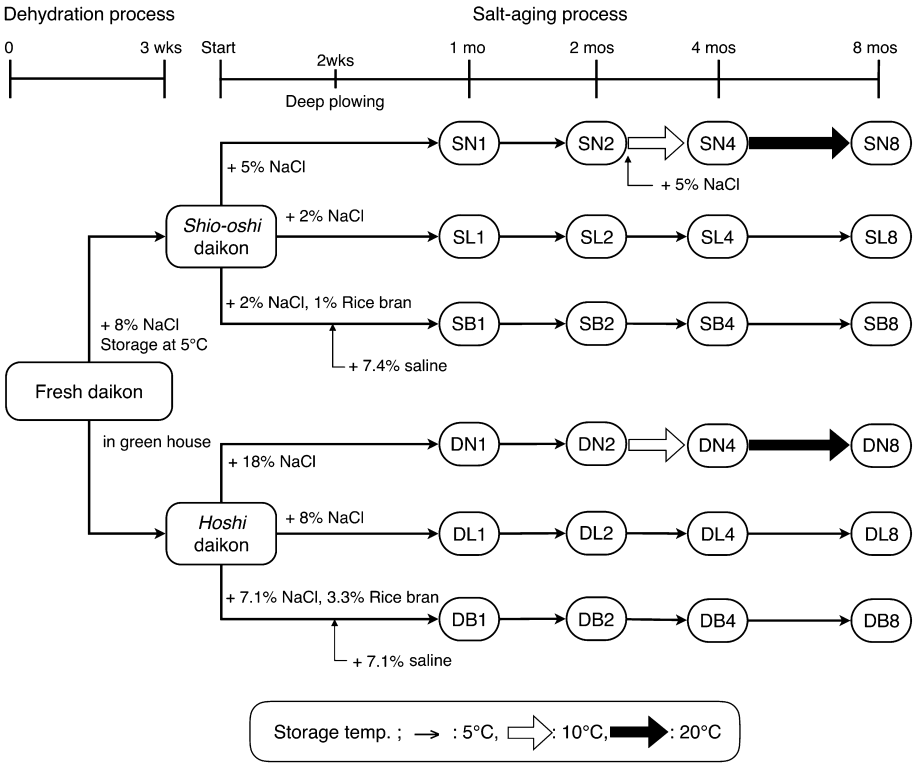

Fig. 1. Time frame for processing of *takuan-zuke*. The harvested sample is labeled "Fresh daikon." Fresh daikon salted in 8% NaCl for 3 weeks at 5 °C is labeled "*Shio-oshi* daikon." Further salted samples under each condition are labeled SN, SL, and SB. Fresh daikon dried in a greenhouse for 3 weeks is labeled "*Hoshi* daikon." *Hoshi* daikon further salted under each condition is labeled DN, DL, and DB. The number next to the label indicates the salt-aging time.

Table 1
Weight, water contents and salt contents in fresh daikon and *takuan-zuke*.

	Hoshi takuan-zuke				*Shio-oshi takuan-zuke*			
		DN	DL	DB		SN	SL	SB
Weight (kg/body)	Fresh	1.07	–	–	Fresh	1.06	–	–
	Hoshi	0.35	–	–	*Shio-oshi*	0.76	–	–
	1 mo	0.26	0.30	0.33	1 mo	0.64	0.67	0.74
Water (%)	Fresh	95.0 ± 0.0	–	–	Fresh	95.0 ± 0.0	–	–
	Hoshi	83.7 ± 0.2	–	–	*Shio-oshi*	85.3 ± 0.2	–	–
	1 mo	67.4 ± 0.2	76.8 ± 0.3	77.2 ± 1.0	1 mo	80.6 ± 0.1	82.7 ± 0.1	82.8 ± 0.2
	2 mos	69.8 ± 0.2	76.0 ± 1.0	77.7 ± 0.4	2 mos	80.1 ± 0.1	83.1 ± 0.3	82.0 ± 0.6
	4 mos	70.2 ± 0.2	76.5 ± 0.9	79.6 ± 1.1	4 mos	79.7 ± 1.1	82.5 ± 1.2	82.3 ± 1.8
	8 mos	70.2 ± 0.2	79.8 ± 0.4	80.6 ± 0.0	8 mos	76.3 ± 0.8	82.0 ± 0.1	81.3 ± 0.1
NaCl (%)	Fresh	–	–	–	Fresh	–	–	–
	Hoshi	–	–	–	*Shio-oshi*	5.5 ± 0.1	5.5 ± 0.1	5.5 ± 0.1
	1 mo	11.7 ± 0.0	6.1 ± 0.0	5.0 ± 0.0	1 mo	9.2 ± 0.0	7.1 ± 0.1	6.8 ± 0.1
	2 mos	10.7 ± 0.2	5.9 ± 0.0	5.5 ± 0.1	2 mos	8.6 ± 0.0	6.4 ± 0.1	7.0 ± 0.0
	4 mos	12.5 ± 0.1	6.2 ± 0.0	5.5 ± 0.0	4 mos	9.9 ± 0.0	7.4 ± 0.0	7.4 ± 0.0
	8 mos	11.2 ± 0.1	6.2 ± 0.0	5.6 ± 0.0	8 mos	11.7 ± 0.1	7.0 ± 0.0	6.5 ± 0.0

Weight indicated the value of the total weight divided by the number of radishes.
Water and NaCl concentrations were presented as the mean ± standard deviation in duplicate analysis.

salt content was measured using the coulometric titration method (SAT-210, DKK-TOA Co., Japan).

2.2. Extraction of amino acids, organic acids, and sugars

Half-thawed samples of salted radish were homogenized in a mixer. To 5 grams of homogenized sample 80% ethanol (20 mL) and internal standard solution (5 mL) (2 μmol/mL *S*-carboxymethyl-L-Cys, 2 μmol/mL sarcosine, 5 mg/mL of maltose, 0.5 mg/mL of levulinate) were added. The mixture was homogenized for 2 min and incubated for 20 min at 80 °C. After cooling, the mixture was centrifuged at 15,000 rpm for 10 min. The residue was re-extracted twice with 80% ethanol. Collected supernatants were evaporated with ethanol, 1 M H_2SO_4 (625 μL) was added, and it was then diluted up to 25 mL with water. A portion of this solution was used for amino acid analysis. For organic acid analysis, this solution was passed through Ag-type strong cation exchange resins; for sugar analysis, the solution used in organic acid analysis was further passed through Cl-type strong anion exchange resins. Resulting solutions were stored at −30 °C until analysis.

2.3. Amino acid analysis

High performance liquid chromatography (HPLC) identification of amino acids was performed using the pre-column automatic derivatization method using *o*-phthalaldehyde and 9-fluorenylmethyl chloroformate according to the Agilent application method (Woodward, Henderson, & Wielgos, 2007). *S*-Carboxymethyl-L-Cys and sarcosine were used as the internal standards.

2.4. Organic acid analysis

Organic acids were identified by ion exclusion HPLC and a post-column method with bromothymol blue solution (BTB). HPLC conditions were as follows: Agilent 1100-1200 system; column, ICSep-ION 300 (φ7.8 × 300 mm, 7 μm; Transgenomic, USA); column temperature, 53 °C; eluent, 3.5 mM H_2SO_4; post-column reagent, 0.1 mM BTB in 30 mM Na_2HPO_4; flow rates, 0.4 mL/min for the eluent and 0.4 mL/min for the post-column reagent. Detection was conducted at a wavelength of 450 nm. Levulinate was used as an internal standard.

2.5. Sugar analysis

Sugar content was determined using normal phase HPLC with a post-column pulsed amperometric detector. HPLC conditions were as follows: instrument, Agilent 1100-1200 system with pulsed-amperometric detector (Antec Leyden, Netherland); column, Unizon UK-Amino (φ2.0 × 250 mm, 3.5 μm, Imtakt, Kyoto); column temperature, 60 °C; eluent, 80% acetonitrile; post-column reagent, 0.3 M LiOH; flow rate, 0.25 mL/min for the eluent and 0.5 mL/min for the post-column reagent. Maltose was used as an internal standard.

2.6. Thiamin, riboflavin, and niacin analysis

Thiamin, riboflavin, and niacin analysis was performed as previously reported (Okano et al., 2010). Thiamin was analyzed using post-column derivatization and fluorescence detection with column-switching HPLC (Motoe, 1994; Yoshida, Hishiyama, & Igarashi, 2008). HPLC conditions were as follows: separation column, Inertsil ODS-3 (φ3 × 150 mm, 3.5 μm, GL science); pre-column, CAPCELL PAK MF SCX S-5 (φ 4 × 10 mm, 5 μm, Shiseido); column temperature, 40 °C for both columns; eluent, H_2O for precolumn and 0.01 M NaH_2PO_4-0.15 M $NaClO_4$ (pH 2.2)/methanol (9:1 v/v) for separation column; post-column reagent, 0.025% $K_3[Fe(CN)_6]$ in 5% NaOH; flow rate, 0.64 mL/min for both eluent and post-column reagent. Fluorescence intensity was monitored at 440 nm with an excitation wavelength of 445 nm.

Riboflavin was analyzed using HPLC with a fluorescence detector. HPLC conditions were as follows: column, Inertsil ODS-3 (φ3 × 150 mm, 3.5 μm, GL science); column temperature, 40 °C; eluent, sodium acetate buffer (pH4.5)/methanol (65:35 v/v); flow rate, 0.64 mL/min. Fluorescence intensity was monitored at 530 nm with an excitation wavelength of 445 nm.

Niacin analysis was performed using microbiological assay with *Lactobacillus plantarum.*

2.7. Free fatty acid analysis

FFA analysis was performed in accordance with our previous report (Matsuoka et al., 2013). Five grams of homogenized half-thawed salted radish was added to a 25-mL solution of chloroform/methanol (1:2 v/v containing 0.2% (w/v) pyrogallol). The mixture was incubated for 20 min at 80 °C. After cooling, it was homogenized for 2 min and extracted for 16 h at 4 °C. Next, the

mixture was centrifuged at 15,000 rpm for 10 min. The residue was re-extracted twice using an extraction solvent. The collected supernatants were partitioned by a separating funnel and the obtained crude lipid phase. The organic layer was evaporated and diluted with 10 mL chloroform, including 0.02% butylated hydroxytoluene. This solution was used for fatty acid analysis. The sample solution was treated in accordance with *Kaluzny's* method (Kaluzny, Duncan, Merritt, & Epps, 1985). That is, the crude lipid extraction was applied to solid-phase extraction by aminopropylsilica gel. Following methyl esterification, free fatty acid methyl esters were analyzed with Agilent 7890A gas chromatography using a flame ionization detector equipped with an HP-88 column (100 m × φ250 μm, 0.25 μm; Agilent). Methyl nonadecanoate was used as the internal standard.

2.8. Data analysis

Multiple *t*-tests were performed with a false discovery rate approach (Q = 1%) using the Graphpad Prism software ver. 7 (Graphpad Software, Inc., USA). The SIMCA software ver. 13.0.3 (Umetrics, Sweden) was used for principal component analysis (PCA). PCA was used to compare the metabolite contents of different products. Orthogonal partial least square (OPLS) regression analysis was performed using an S-plot™ filter, which is an OPLS model, with the y-variable set as salt-aging time.

3. Results

3.1. Nutritional contents of fresh radish and takuan-zuke

Comparisons of weight, water content, and salt content between fresh daikon and *takuan-zuke* are shown in Table 1. To investigate the metabolic differences between fresh daikon and *takuan-zuke*, 43 metabolites were used to construct multivariate statistical models. PCA score plots showed a clear distinction between *shio-oshi takuan-zuke* and *hoshi takuan-zuke*, with high statistical values of R2 (0.955) and Q2 (0.803), as shown in Fig. 2A. The contributions of principal component 1 (PC1) and PC2 were 66.7% and 21.6%, respectively. Salted radishes were divided into two groups according to the dehydration process they were subjected to (Fig. 2A). The scatter plot of fresh daikon differed from those of *hoshi* and *shio-oshi* groups. The plots of *hoshi* groups DN, DL, and DB were mainly in the positive direction as in t[1] and t[2] (t[1], −3.51 to 11.3; t[2], −4.46 to 12.2) whereas those of *shio-oshi* groups SN, SL, and SB were mainly in the negative direction, as in t[1] and t[2] (t[1], −19.3 to 2.65; t[2], −5.19 to 1.68). Distinctive components were analyzed using PCA loading plots. These findings showed that sugar, proline, γ-aminobutyrate (GABA), glutamine, and malate content differed between groups (Fig. 2B). Concentrations of sucrose, proline, GABA, and glutamine were distinctive in *hoshi takuan-zuke*, while concentrations of malate, fructose, and glucose were distinctive in fresh daikon. No distinctive components were observed in *shio-oshi* salted radishes or bran salted radishes.

3.2. Nutritional contents of sun-dried and salt-pressed takuan-zuke

OPLS regressive analysis was performed and S-plot filters were applied to reveal the time-dependent changes in components of *hoshi takuan-zuke*, *shio-oshi takuan-zuke*, and the bran-salted radish (DB and SB). As shown in Fig. 2C, t[1] shifted from negative to positive over time in all groups during the salt-aging process. S-plot filter results for the *hoshi* groups show that sucrose, glutamine, and tryptophan correlate negatively with time (Fig. 2D), while pyroglutamate, formate, and FFAs correlate positively with time. S-plot filter data for the *shio-oshi* groups show that glucose, fructose, glutamine, GABA, alanine, arginine, and tryptophan decreased over time while acetate and FFAs increased over time (Fig. 2E). In the salty bran groups (DB and SB), glutamate, acetate, niacin, and FFA content correlated positively with salt-aging time, while glutamine content correlated negatively with salt-aging time (Fig. 2F).

3.3. Temporal changes in the nutritional content of each product

Multivariate analysis revealed temporal changes in the content of specific components. Metabolic pathways related to amino acids, organic acids, and sugars are summarized in Fig. 3. For sugar metabolism, dehydration by sun-drying markedly elevated the concentration of sucrose at harvest (2.6-fold, $p = 0.0047$). *Shio-oshi* salt aging (SN, SL, and SB) and sun-drying followed by salt aging (DN, DL, and DB) resulted in reduced sucrose concentrations with increasing duration of salt aging. Fructose and glucose levels were highest at harvest, and both dehydration treatments decreased these levels. Time-dependent changes in the levels of fructose and glucose in the *hoshi* groups were small.

Analysis of the GABA pathway revealed that both dehydration treatments resulted in increased GABA levels at harvest (*hoshi* processing: 2.2-fold, $p = 0.0009$; *shio-oshi* processing: 1.6-fold, $p = 0.044$). In contrast, proline levels of fresh daikon and *shio-oshi* radishes were low while *hoshi* processing remarkably increased the proline level (36.5-fold; $p = 0.00002$) with no observed reduction during the salt-aging process. Glutamate, a substrate for GABA and proline synthesis, was reduced by the dehydration but increased with salt-aging time in the DB radish group. Although glutamine increased slightly with *shio-oshi* dehydration, the salt-aging process decreased the concentration of this component in all types of processing. The reduction in glutamine in the *hoshi* radish group was more gradual than that in the *shio-oshi* radish group. Pyroglutamate increased from 3.1 ± 0.1 mg/g dry weight (DW) in fresh daikon by 5.0, 2.1, and 2.5-fold after 8 months of DN, DL, and DB, respectively. Arginine content was reduced by dehydration; however, this change was small in all processing groups.

Malate is converted biosynthetically to isoleucine and threonine. The level of malate was decreased by dehydration and salt aging. In contrast, levels of threonine and isoleucine were elevated, and this increase was greater with *hoshi* processing than *shio-oshi* processing. These compounds did not change during the long salt-aging process.

Analysis of metabolites in the pyruvate pathway showed that leucine (which was at low levels in fresh radish) increased as a consequence of all dehydration processes. Valine and isoleucine levels increased during the sun-drying process and were maintained during salting afterwards. Alanine and lactate levels were elevated by the sun-drying process and decreased during salting afterwards.

Analysis of the shikimate pathway showed that the conversion of phosphoenolpyruvate to aromatic amino acids increased during dehydration *via* sun drying and salt-pressing. Changes in tyrosine and phenylalanine content during the salt-aging process were small. In contrast, tryptophan levels decreased during the salt-aging process, and this reduction was remarkable after 4 and 8 months.

Temporal changes in FFA content are shown in Fig. 4. Total FFA content of fresh daikon was 0.38 ± 0.5 mg/g DW. With subsequent salt-pressed dehydration, the FFA content increased to 1.6 ± 0.2 mg/g DW. In contrast, sun drying resulted in little change. FFAs also increased with longer salt-aging time after dehydration (DN8: 3.5 ± 0.1 mg/g DW, DL8: 5.3 ± 0.4 mg/g DW, DB8: 6.6 ± 0.2 mg/g DW, SN8: 4.2 ± 0.4 mg/g DW, SL8: 6.1 ± 0.4 mg/g DW, SB8: 6.0 ± 0.1 mg/g DW). The ratio of FFAs to TFAs increased during the salt-aging process. For FFAs, palmitate, stearate, oleate,

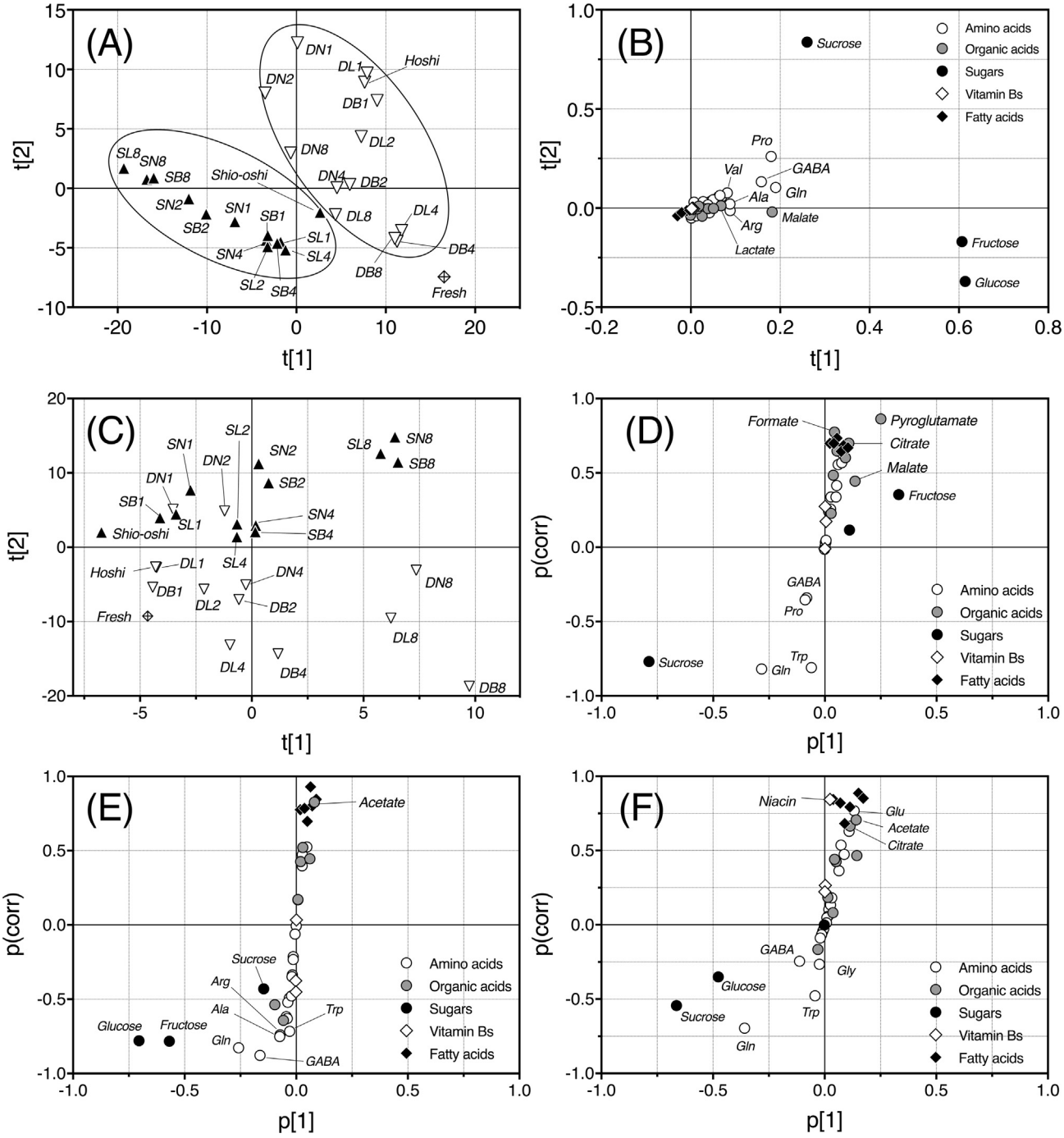

Fig. 2. Multivariate analysis of all *takuan-zuke* groups. (A) PCA score plots generated using all samples. (B) PCA loading plots from all samples (C) OPLS score plots from all samples. (D) S-plot of *hoshi takuan-zuke* (DN, DL, and DB) (E) S-plot of *shio-oshi takuan-zuke* (SN, SL, and SB). (F) S-plot of *hoshi* and *shio-oshi takuan-zuke* with rice bran (DB and SB).

and (*Z*)-vaccenate, the highest concentrations were observed in SB8. In contrast, concentrations of polyunsaturated fatty acids such as linoleate and α-linolenate were highest in DB8.

Temporal changes in niacin, thiamin, and riboflavin concentrations are shown in Fig. 5. Distinct elevation in niacin concentrations was observed in the salty-bran radish groups with 58.7 ± 11.7 μg/g DW in fresh daikon, 108 ± 7.8 μg/g DW in DB8, and 74.2 ± 6.1 μg/g DW in SB8. Both dehydration treatment methods decreased thiamin and riboflavin concentrations, while the concentration of riboflavin in the DB group increased slightly.

4. Discussion

The present study used targeted metabolomic analysis to investigate the secondary metabolites and content dynamics during the dehydration and salt aging processes of *takuan-zuke* production. The metabolite concentrations in *hoshi takuan-zuke* were higher than those of *shio-oshi takuan-zuke*, because tissues of the *hoshi* daikon shrank more than those of the *shio-oshi* daikon, and metabolites were transferred to the brining liquid (*agari-mizu*) during salt-pressing.

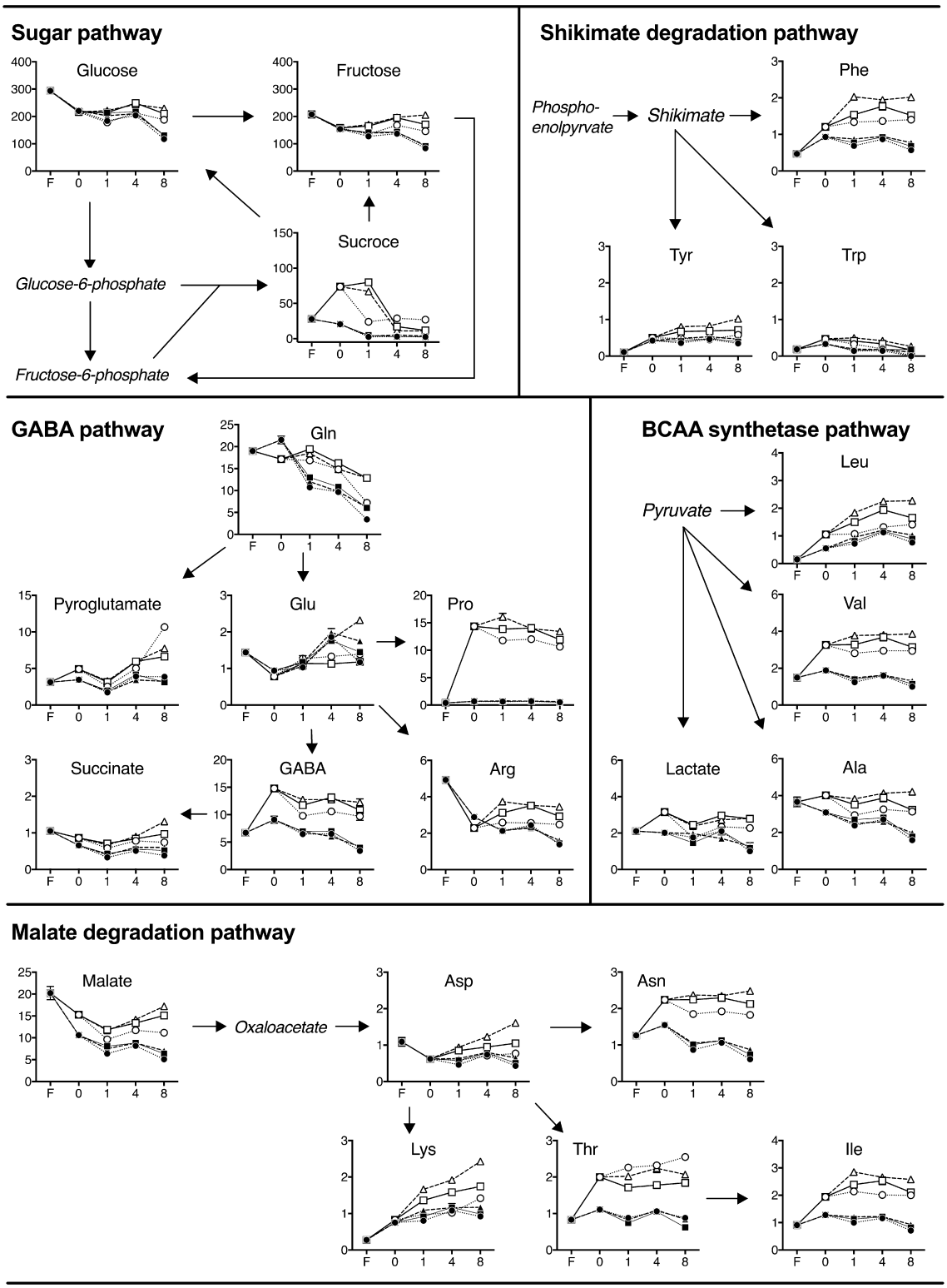

Fig. 3. Time dependent changes in metabolite concentrations. The x-axis denotes processing time (months), and the y-axis denotes concentration (mg/g of dry weight, mean ± SD). Zero time denotes the start of salt aging for dehydration. Symbols denote the following: ○, DN; □, DL; △, DB; ●, SN; ■, SL; ▲, SB.

Hoshi processing is thought to promote secondary metabolism, while *shio-oshi* processing (called "*shio-goroshi*" among Japanese manufacturers) suppresses secondary metabolism. Typically, water stress in higher plants results in the accumulation of γ-aminobutyrate (GABA) and proline (Liu, Zhao, & Yu, 2011). We previously reported that dehydration induces GABA production in the radish root (Kato et al., 2015). Although the proline content of radish root was elevated through *hoshi* processing, no change was observed with *shio-oshi* processing. In higher plants, glutamate is a precursor for proline and GABA synthesis. Plants protect their cells by pooling proline for osmotic adjustment during induced stress (Liu et al., 2011). Hara et al. reported that proline biosynthesis was induced by drying and salting stress during the growing phase of radish sprouts (Hara, Sugano, & Kuboi, 2003). Because

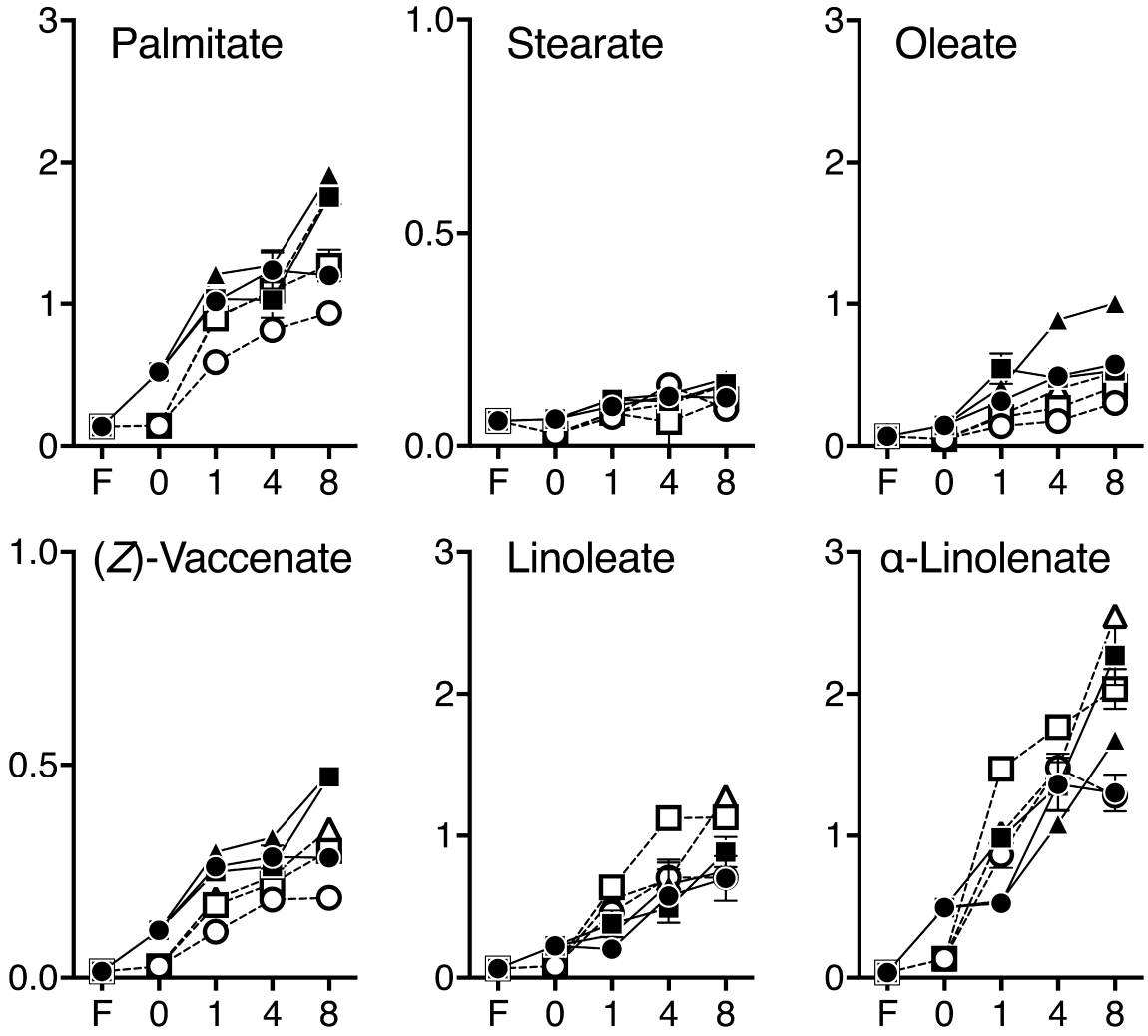

Fig. 4. Changes in free fatty acid concentration during the salt-aging process in *takuan-zuke*. The x-axis denotes processing time (months), and the y-axis denotes concentration (mg/g of dry weight, mean ± SD). Zero time denotes the start of salt aging for dehydration. Symbols denote the following: ○, DN; □, DL; △, DB; ●, SN; ■, SL; ▲, SB.

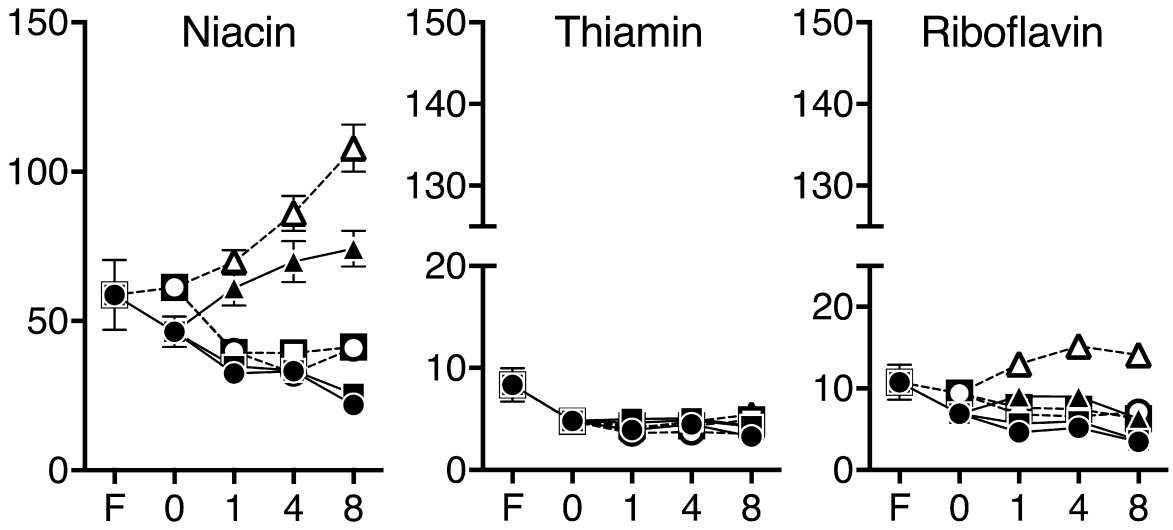

Fig. 5. Changes in niacin, thiamine, and riboflavin concentrations during salt-aging processing of *takuan-zuke*. The x-axis denotes processing time (months) and the y-axis denotes concentration (μg/g of dry weight, mean ± SD). Zero time denotes the start of salt aging for dehydration. Symbols denote the following: ○, DN; □, DL; △, DB; ●, SN; ■, SL; ▲, SB.

salting induced intense osmotic stress, the metabolic system related to proline in *shio-oshi* processing was blocked. These observations suggest that GABA synthesis *via* glutamate metabolism is the dominant pathway and that the enzymes involved in proline biosynthesis have low salt tolerance. Thus, GABA is a unique marker of dehydration stress, unlike other metabolites such as proline and sucrose.

GABA is known to lower blood pressure by decreasing noradrenalin secretion through the inhibition of firing from the peripheral sympathetic nerves (Hayakawa, Kimura, & Kamata, 2002; Hayakawa, Kimura, & Yamori, 2005). Branched-chain amino acid (BCAA) is reported to decrease muscle wastage (Buse & Reid, 1975; Fulks, Li, & Goldberg, 1975). Thus, it is expected that dehydration of *takuan-zuke* will increase its health-promoting properties.

Of the organic acids investigated, malate was highest in fresh daikon and decreased after salting. Pyroglutamate increased twofold in DL8 and DB8 and threefold in DN8 compared with fresh daikon and *shio-oshi* radishes. Since pyroglutamate is derived from unstable glutamine, its synthesis strongly depends on the dehydration method chosen and the temperature used during salt aging. Yoshinari et al. reported that pyroglutamate has activities that counteract type II diabetes, antineoplastic activity, and antimetastatic activity (Yoshinari & Igarashi, 2011). Therefore, long-term salting of *hoshi takuan-zuke* increases its health benefits.

Sucrose accumulated during sun drying and decreased during salting afterwards. Concentrations of glucose and fructose were highest in fresh daikon and were reduced by the dehydration process. It is well known that the sucrose synthetic enzyme becomes active in radish after harvest (Rouhier & Usuda, 2001). This observation corroborates sun-drying triggered sucrose storage. The dehydration process is reported to activate enzymatic reactions of carbohydrates and the amino-carbonyl reaction (Mochimaru, Tomita, Ohtani, Yoshino, & Minamide, 2007). Free sugars are major components of radish that are susceptible to dehydration and salting and affect the sensory function of *takuan-zuke*.

Fatty acid concentrations were observed to increase in all test categories upon extension of the salting period (Fig. 4). The ratio of FFAs to total fatty acids increased with salting time. The FFAs/total fatty acid ratio was 8% for sun-dried radishes and 24% for those subjected to *shio-oshi* processing. Hashimoto et al. and Matsuoka et al. estimated the remaining activity of ester degradative enzyme such as lipase (Hashimoto & Tatokoro, 1991; Matsuoka et al., 2013). Our results indicate that a slow enzymatic reaction was in progress without lipase deactivation, even after *shio-oshi* dehydration and long term salting. For each fatty acid, stearate, oleate, and (*Z*)-vaccenate levels were highest in SB8. In contrast, linoleate and α-linolenate, the polyunsaturated fatty acids, were highest in DB8. (*Z*)-Vaccenate occurs widely in *Cruciferous* vegetables (Kaymak, 2015) and possesses high antimutagenic activity in salted radish (Matsuoka et al., 2013). N-3 type polyunsaturated fatty acid is known to lower systolic blood pressure (Sekine, Sasanuki, Aoyama, & Takeuchi, 2007). Linolate is known to have antimutagenic activity resulting from metabolic deactivation of heterocyclic amines (Hayatsu, Arimoto, Togawa, & Makita, 1981). Palmitate contained in yogurt possesses inhibitory activity against *N*-methyl-*N*′-nitrosoguanidine (MNNG), a direct mutagen. Therefore, the salt aging related increase in *takuan-zuke* FFAs is beneficial.

Niacin content increased with salting time *via* the addition of bran at the start of salting (Fig. 5); this increase was proportional to the amount of bran added. After one month of salting, *agarimizu* contained 9.3–51.4 mg/100 g (wet weight) of niacin, and the abundance ratio was 30–80 times more than that of the fresh daikon. These results indicate that niacin in the bran is transferred to the salted radish during the maturation period. Thus, it is expected that other auxiliary materials such as the peels of persimmon and red pepper provide components in addition to those in the salted radish itself.

In addition to *takuan-zuke*, radish is processed into *asa-zuke*, *fukuzin-zuke*, and *sakura-zuke*. The availability of these varieties depends on the timing of production, which aims to provide a stable supply of radish irrespective of the season. Because the present study dealt with only one variety of radishes, further studies are required to clarity the influence of other factors such as radish type, pickling season, soil type, and climate.

5. Conclusion

We identified dehydration process dependent changes in the chemistry of *takuan-zuke*, with long salt aging and bran salt aging dependent alterations in its components. In particular, *hoshi takuan-zuke* prepared at normal temperature in high salt concentration, has greater nutritional value and health benefits than those prepared using different processing methods. Dehydration and salt aging of radish resulted in increased levels of GABA, polyunsaturated fatty acids, and branched chain amino acids. We therefore concluded that the process of pickling not only serves to dehydrate vegetables and concentrate their functional components but also increases concentrations of secondary metabolites that confer health benefits.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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References

Buse, M. G., & Reid, S. S. (1975). Leucine. A possible regulator of protein turnover in muscle. *Journal of Clinical Investigation, 56*(5), 1250–1261.

Cho, I. H., Kim, Y. S., & Choi, H. K. (2007). Metabolomic discrimination of different grades of pine-mushroom (*Tricholoma matsutake* Sing.) using ^{1}H NMR spectrometry and multivariate data analysis. *Journal of Pharmaceutical and Biomedical Analysis, 43*(3), 900–904.

Du, D. D., Yoshinaga, M., Sonoda, M., Kawakubo, K., & Uehara, Y. (2014). Blood pressure reduction by Japanese traditional Miso is associated with increased diuresis and natriuresis through dopamine system in Dahl salt-sensitive rats. *Clinical and Experimental Hypertension, 365*(5), 359–366.

Esaki, H., Kawakishi, S., Morimitsu, Y., & Osawa, T. (1999). New potent antioxidative *o*-dihydroxyisoflavones in fermented Japanese soybean products. *Bioscience, Biotechnology, and Biochemistry, 63*(9), 1637–1639.

Fulks, R. M., Li, J. B., & Goldberg, A. L. (1975). Effects of insulin, glucose, and amino acids on protein turnover in rat diaphragm. *Journal of Biological Chemistry, 250*(1), 290–298.

Hara, M., Sugano, Y., & Kuboi, T. (2003). Drought-regulated expression of prolyl-tRNA synthetase genes in radish (*Raphanus sativus*) seedlings. *Plant Science, 165*(1), 129–137.

Hashimoto, T., & Tatokoro, Y. (1991). Analysis of hydroxycinnamic acids in salted daikon (*Raphanus sativus* L.) roots. *Nippon Shokuhin Kogyo Gakkaishi, 38*(6), 494–498 (in Japanese).

Hayakawa, K., Kimura, M., & Kamata, K. (2002). Mechanism underlying γ-aminobutyric acid-induced antihypertensive effect in spontaneously hypertensive rats. *European Journal of Pharmacology, 438*(1–2), 107–113.

Hayakawa, K., Kimura, M., & Yamori, Y. (2005). Role of the renal nerves in γ-aminobutyric acid-induced antihypertensive effect in spontaneously hypertensive rats. *European Journal of Pharmacology, 524*, 120–125.

Hayatsu, H., Arimoto, S., Togawa, K., & Makita, M. (1981). Inhibitory effect of the ether extract of human feces on activities of mutagens: Inhibition by oleic and linoleic acids. *Mutation Research, 81*(3), 287–293.

Kaluzny, M. A., Duncan, L. A., Merritt, M. V., & Epps, E. D. (1985). Rapid separation of lipid classes in high yield and purity using bonded phase columns. *Journal of Lipid Research, 26*(1), 135–140.

Kato, R., Hayashi, S., Kobayashi, T., Takahashi, H., Kimura, N., Takahashi, A., et al. (2015). Behavior analysis of γ-aminobutyrate and glutamate decarboxylase activity in salted radish roots (*takuan-zuke*). *Nippon Shokuhin Kagaku Kogaku Kaishi, 62*(10), 492–500 (in Japanese).

Kato, S., Kitamura, E., & Ohshima, S. (1991). Factors influencing the growth of *Debaryomyces hansenii* and *Saccharomyces servazii* isolated from salted "Daikon" (Japanese radish). *Nippon Shokuhin Kogyo Gakkaishi, 38*(4), 357–359.

Kato, S., Kitamura, E., Yamamoto, S., & Ohshima, S. (1991). Effects of sodium chloride, ethanol, glucose content and pH on the growth of lactic acid bacteria isolated from salted "Daikon" (*Raphanus sativus*). *Nippon Shokuhin Kagaku Kogaku Kaishi, 38*(10), 962–966.

Kato, S., & Nakase, T. (1986). Yeast flora in soaked fluid of salted "Daikon" under a nitrogen packing system. *Nippon Shokuhin Kogyo Gakkaishi, 33*(9), 659–666 (in Japanese).

Kato, S., Seki, M., Kaneuchi, C., & Nakase, T. (1989). Flora of lactic acid bacteria in a soaked fluid of salted "Daikon" under a nitrogen gas-packing system. *Nippon Shokuhin Kogyo Gakkaishi, 36*(5), 357–363 (in Japanese).

Kaymak, H. C. (2015). Profile of (n-9) and (n-7) isomers of monounsaturated fatty acids of radish (*Raphanus sativus* L.) seeds. *Journal of the American Oil Chemists' Society, 92*, 345–351.

Liu, C., Zhao, L., & Yu, G. (2011). The dominant glutamic acid metabolic flux to produce γ-amino butyric acid over proline in *Nicotiana tabacum* leaves under water stress relates to its significant role in antioxidant activity. *Journal of Integrative Plant Biology, 53*(8), 608–618.

Matsuoka, H., Sunaga, K., Miyashita, S., Sunahara, Y., Hukasawa, S., Shimoda, M., et al. (2013). Identification and quantification of antimutagenic compounds in salted radish. *Nippon Shokuhin Kagaku Kogaku Kaishi, 60*(3), 117–124 (in Japanese).

Mochimaru, Y., Tomita, K., Ohtani, K., Yoshino, Y., & Minamide, T. (2007). Effect of season, dry processing and reconstitution on the sugars and minerals in radish. *Journal of cookery science of Japan, 40*(6), 456–461.

Motoe, K. (1994). Improved determination methods of thiamin and 2-(1-hydroxyethyl)thiamin in foods by high performance liquid chromatography. *Vitamins, 68*(7), 379–384 (in Japanese).

Okamoto, A., Hanagata, H., Matsumoto, E., Kawamura, Y., Koizumi, Y., & Yanagida, F. (1995). Angiotensin I converting enzyme inhibitory activities of various fermented foods. *Bioscience, Biotechnology, and Biochemistry, 59*(6), 1147–1149.

Okano, T., Kanaya, K., Kawamura, N., Saito, K., Sakurai, K., Sano, M., et al. (2010). Vitamin. In T. PS. o. Japan (Ed.), *Method of analysis health science* (pp. 227–258). Tokyo: Kenehara Shuppan.

Rouhier, H., & Usuda, H. (2001). Spatial and temporal distribution of sucrose synthase in the radish hypocotyl in relation to thickening growth. *Plant and Cell Physiology, 42*(6), 583–593.

Sekine, S., Sasanuki, S., Aoyama, T., & Takeuchi, H. (2007). Lowering systolic blood pressure and increases in vasodilator levels in SHR with oral α-linolenic acid administration. *Journal of Oleo Science, 56*(7), 341–345.

Sugimoto, M., Goto, H., Otomo, K., Ito, M., Onuma, H., Suzuki, A., et al. (2010). Metabolomic profiles and sensory attributes of edamame under various storage duration and temperature conditions. *Journal of Agricultural and Food Chemistry, 58*, 8418–8425.

Takahashi, A., Matsuoka, H., Watanabe, E., Shinagawa, A., Ozawa, Y., Yamada, Y., et al. (2009). Quantitative analysis of yellow pigment in *takuan-zuke* and their ABTS radical cation scavenging activity. *Food Science and Technology Research, 15*(3), 337–342.

Takahashi, A., Yamada, T., Uchiyama, Y., Hayashi, S., Kumakura, K., Takahashi, H., et al. (2015). Generation of the antioxidant yellow pigment derived from 4-methylthio-3-butenyl isothiocyanate in salted radish roots (*takuan-zuke*). *Bioscience, Biotechnology, and Biochemistry, 79*(9), 1512–1517.

Watanabe, H., Kashimoto, N., Kajimura, J., & Kamiya, K. (2006). A miso (Japanese soybean paste) diet conferred greater protection against hypertension than a sodium chloride diet in Dahl salt-sensitive rats. *Hypertensive Research, 29*, 731–738.

Watanabe, T., Owari, K., Hori, K., & Takahashi, K. (2004). Selection of koji mold strain for making functional miso as rich antimutagenic activity. *Nippon Shokuhin Kagaku Kogaku Kaishi, 51*(12), 698–702 (in Japanese).

Woodward, C., Henderson, J. W., Jr., & Wielgos, T. (2007). *High-speed amino acid analysis (AAA) on 1.8 μm reversed-phase (RP) columns*. Agilent Technologies Inc.. Application 5989-629EN 1–14.

Yoshida, M., Hishiyama, T., & Igarashi, T. (2008). A novel method for determining total vitamin B1 in processed food enriched with dibenzoyl thiamine. *Nippon Shokuhin Kagaku Kogaku Kaishi, 55*(9), 421–427 (in Japanese).

Yoshinari, O., & Igarashi, K. (2011). Anti-diabetic effect of pyroglutamic acid in type 2 diabetic Goto-Kakizaki rats and KK-Ay mice. *British Journal of Nutrition, 106*(7), 995–1004.