DOI:10.1021/jf101471d

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Metabolomic Profiles and Sensory Attributes of Edamame under Various Storage Duration and Temperature Conditions

Masahiro Sugimoto,*[,†,‡] Hajime Goto,[†,‡,§] Kazuko Otomo,[‡] Masanori Ito,[∥] Hiromi Onuma,[‡] Asako Suzuki,[‡] Maki Sugawara,[‡] Shinobu Abe,[‡] Masaru Tomita,[‡] and Tomoyoshi Soga[‡]

[‡]Institute for Advanced Biosciences, Keio University, Tsuruoka, Yamagata 997-0052, Japan, [§]Shonai Area General Branch Administration, Yamagata Prefectural Government, Mikawa, Yamagata 997-1392, Japan, and [∥]Yamagata Shonai Agricultural Technique Improvement Research Office, Yamagata 998-0112, Japan. [†] These authors contributed equally to this work.

Its high nutritional content and sensory characteristics make edamame a popular vegetable bean. However, because of its short shelf-life, it is important to optimize the storage conditions to maintain its quality during distribution to consumers. We focused on storage conditions to investigate the temporal changes in the metabolic profiles and sensory characteristics of edamame during transportation from the site of harvest to the site of purchase/consumption. We conducted metabolomic analysis and sensory evaluation tests of edamame stored for different lengths and at different temperatures. Charged metabolites were profiled by capillary electrophoresis−mass spectrometry, and free sugars were quantified by liquid chromatography−tandem mass spectrometry. In comparison to the gradual decrease in its sensory characteristics over time, the changes in metabolite profiles manifested four different patterns. In particular, changes in amino acid levels were related to sensory attributes. The downstream metabolites of shikimate as well as phospholipids and γ-aminobutyric acid increased with increasing storage temperatures.

KEYWORDS: Edamame; metabolome; sensory evaluation; storage; CE−MS; LC−MS/MS

INTRODUCTION

Because soybeans [*Glycine max* (L.) Merr.] are nutrition-rich and possess favorable sensory characteristics, particularly, taste, they are an important and popular crop consumed worldwide. There are two general categories of soybean: the grain type and the vegetable type. Grain soybeans are primarily used for oil production and are processed as foodstuffs, including soy sauce, soybean, soy curd, and fermented soybean (*1*). The vegetable-type soybean, usually harvested while immature, is called edamame in Japan. Although some of the harvested edamame is processed for use in snacks or salads, most is boiled in the pods and eaten with or without salt. Edamame plays an important role in the food culture of Japan and other Asian countries.

Edamame is grown and harvested at many locations in Japan, principally in the Tohoku region and transported to the site of purchase/consumption either frozen or at or below room temperature. Freezing is essential for long-term storage if consumption is to occur several months after harvest (*2*). On the other hand, short-term storage, e.g., for several days, does not require freezing to maintain the high quality of the bean and to minimize loss of its nutritional and sensory properties (*3*). Optimization of the storage conditions during transport from the site of harvest to the point of sale/consumption is important.

Vegetable properties, including the vitamin, free sugar, fatty acid, chloroplast, and amino acid content and titratable acidity, fluctuate with storage conditions. Beans contain high levels of ascorbic acids, free sugars, such as glucose and sucrose, and amino acids. Because a decreases in the level of these components affects their sensory attributes negatively (*4*), the correlation between sensory evaluation values and the ascorbic acid content of beans under different storage conditions has been widely studied (*2*, *5*, *6*).

The components of edamame, such as isoflavones (e.g., genistein), which may confer cancer-risk reduction (*7*), and lipids (*8*) and amino acids, important cellular components relating to taste, have been extensively analyzed (*1*). Asparagine, alanine, and glutamate are the principal amino acids in grain and vegetable soybeans (*9*). Comparative profiles of edamame cultivars revealed that the total content of free amino acids and γ-aminobutyric acid (GABA) contributes to their sensory and flavor characteristics (*10*). Of note, decreases in these organoleptic sensory and color characteristics have been observed after frozen storage of soybeans (*11*). Furthermore, decreases in the amino and ascorbic acid content have been reported during cold-temperature storage of edamame (*12*). These studies focused on a few similar metabolites and the relationship among the metabolites and sensory attributes. Elsewhere, we reported that a diverse range of metabolites, including dipeptides and amino acids, are strongly correlated with the sensory properties of a beverage (*13*). However, the

*To whom correspondence should be addressed: Institute for Advanced Biosciences, Keio University, 246-2 Kakuganji, Tsuruoka, Yamagata 997-0052, Japan. Telephone: +81-235-29-0528. Fax: +81-235-29-0574. E-mail: msugi@sfc.keio.ac.jp.

 Published on Web 07/01/2010

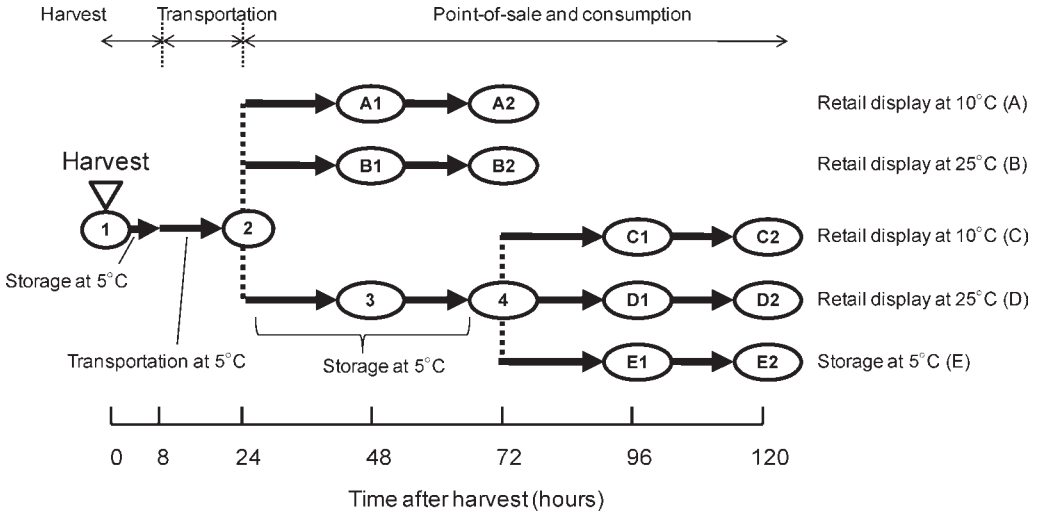

Figure 1. Study design. The originally harvested sample is labeled sample 1. Samples stored for 8 h at 5 °C (corresponding to storage after harvest) and stored for a further 16 h at 5 °C (corresponding to transportation to the point of sale; 24 h after harvest) were labeled sample 2. Further samples were then stored for an additional 1 or 2 days (to mimic retail display) at 10 and 25 °C and labeled A1 and B1 (48 h after harvest) and A2 and B2 (72 h after harvest). Additional samples were stored at 5 °C for 1 or 2 days after initial storage and labeled samples 3 (48 h after harvest) and 4 (72 h after harvest). These samples were then stored at 10, 25, or 5 °C and for a further 1 or 2 days and labeled C1, D1, and E1 (96 h after harvest) and C2, D2, and E2 (120 h after harvest).

relationship between a broad variety of metabolites and sensory properties has not been confirmed in beans. Therefore, a comprehensive evaluation of metabolite profiles was needed to understand the contribution of the overall profile and the individual metabolites to sensory characteristics.

The aim of this study was to explore the relationship between changes in metabolic profiles and the sensory characteristics of edamame stored under different conditions. The conditions applied in this study were intended to represent the typical storage conditions between harvest and consumption, including transportation, cold storage, and retail display, for different terms and at different temperatures. We used capillary electrophoresis–mass spectrometry (CE–MS) (*14*) in a comprehensive analysis of charged metabolites, such as amino and organic acids, and liquid chromatography–tandem mass spectrometry (LC–MS/MS) to quantify free sugars. Our comprehensive metabolomic analysis, combined with sensory evaluation tests, revealed the storage condition-specific effects on the properties of edamame.

MATERIALS AND METHODS

Design of the Field Experiment and Storage Conditions. The Shonai 3 cultivar of edamame harvested in Yamagata prefecture in the Tohoku area of Japan was used in this study. We conducted blanching, metabolite measurements, and sensory evaluation tests at each of the time points detailed below.

In the field, plug seedlings were planted at 30 cm intervals in furrows spaced 90 cm. All edamame pods were manually harvested within several hours after dawn, transported to a shaded area, and immediately moved to an incubator. To ensure consistent growth, only pods with a thickness (the shortest length across three dimensions) of approximately 8 mm were selected.

Harvested beans were either processed immediately (sample 1) or stored in the incubator at 5 °C for 8 h and then at 5 °C for 16 h (sample 2) to mimic the typical time required for transport of the beans from the Tohoku area to Tokyo, one of the largest consuming regions in Japan. Then, the edamame beans were stored at room (25 °C, case A) or cold (10 °C, case B) temperature for 1 or 2 days to represent retail display. As an alternative storage/transport condition, we also stored the beans at 5 °C for 2 days and then mimicked retail display at room temperature (25 °C, case C), 10 °C (case D), or 5 °C (case E) for 2 days. Samples were packed in P-plus for edamame (Sumitomo Bakelite, Tokyo, Japan) and stored in an incubator in the dark for all experiments, including storage, transport, and retail display. The experimental design and sample groups are summarized in **Figure 1**.

Chemicals. Ophthalmate was purchased from Bachem AG (Bubendorf, Switzerland). Acetohydroxamate and azetidine-2-carboxylate were purchased from Chem Service (West Chester, PA). Glucose 1-phosphate and 5-aminovalerate were purchased from Fluka (Buchs, Switzerland). Betaine was purchased from Tokyo Chemical Industry (Tokyo, Japan). The other compounds were purchased from Sigma-Aldrich (St. Louis, MO) and Wako (Osaka, Japan).

Sample Preparation. Six edamame pods were placed in a metal basket and immersed in 10 L of boiling water for 90 s; this volume of water was required to avoid temperature deviations. The samples were then retrieved and dried under an electric fan for 5 min. Edamame beans for metabolomic analysis were homogenized without solvent using a multi-bead chocker at 2000 rpm for 60 s, and three replicates were used for subsequent analyses. The positively charged (cationic) internal samples were methionine sulfone and 3-aminopyrrolidine, and the negatively charged (anionic) internal standards were 2-(*N*-morpholino)ethanesulfonic acid (MES), D-camphor-10-sulfonic acid, and trimesate. We added 2.5 mL of MeOH containing 200 μM of the internal standards to each sample. Then, 2.5 mL of $CHCl_3$ and 1.0 mL of Milli-Q water (Millipore, Bedford, MA) were added, and the samples were centrifuged at 3000 rpm for 20 min. Next, 250 μL of the aqueous solution was centrifugally filtered at 10 000 rpm for 3 min through a 5 kDa cutoff filter (Millipore) to remove large molecules. Finally, 100 μL of the filtrate was centrifugally concentrated and dissolved in 50 μL of Milli-Q water.

CE–MS Equipment and Conditions for Charged Metabolite Analysis. CE–MS and the conditions for cationic and anionic metabolite analysis were as described elsewhere (*13*), with the following modifications. To analyze anion metabolites, an Agilent CE capillary electrophoresis system, an Agilent G6210A LC/MSD TOF system, an Agilent 1200 series isocratic HPLC pump, a G1603A Agilent CE–MS adapter kit, and a G1607A Agilent CE–electrospray ionization (ESI) source–MS sprayer kit (Agilent Technologies, Waldbronn, Germany) were used. All of the other instruments and conditions were as described elsewhere (*13*).

LC–MS/MS Conditions for Free Sugar Analysis. The LC–MS/MS experiments were performed using an Agilent 1100 series vacuum degasser, an Agilent G6410AA triple quadrupole mass spectrometer, and an ESI source. Samples were separated on a TSKgel amide-80 column (2.0 mm inner diameter × 250 mm; Tosoh). The initial mobile phase consisted of 75% acetonitrile and 25% Milli-Q water at a flow rate of 0.2 mL/min; the gradient profile of acetonitrile was 65, 25, and 10% acetonitrile at 15, 25, and 30 min, respectively. The post time period was 15 min. The temperature of the column oven was set at 80 °C; 1.0 μL aliquots of sample were injected into the column. The turbospray mode was selected in the negative-ion mode. The nebulizer gas pressure, gas flow, nitrogen turbo gas temperature, and ion spray voltage were set at 20 psig, 10 L/min, 300 °C, and 4.0 kV, respectively.

The mass spectrometer was set to run a multi-channel reaction monitoring (MRM) experiment. It was operated in unit resolution for both Q1 and Q3 in the MRM mode with a dwell time of 333 ms for the MRM channels. For glucose and fructose, Q1 and Q3 were m/z 179 and 89, and the fragmenter voltage and collision energy were 60 and 0 V, respectively. For sucrose, Q1 and Q3 were m/z 341 and 59, respectively, and the fragmenter voltage and collision energy were 120 and 30 V, respectively. For maltose, Q1 and Q3 were m/z 341 and 161, respectively, and the fragmenter voltage and collision energy were 60 and 0 V, respectively. All data were acquired using MassHunter (Agilent Technologies).

Sensory Evaluation Test. All edamame samples were evaluated by 15 researchers at the Agricultural Technique Improvement Research Office, Agricultural Technique Popularization Division, Shonai Area General Branch Administration, Yamagata Prefectural Government. The following four features were graded from 1 to 5 at a resolution of 0.5 points, and the average scores were used in subsequent analyses. Grade 5 indicated the highest grade, and grade 1 indicated the lowest grade, for sweetness, umami, flavor, and total taste intensity. Grade 3 was assigned when the features were considered to be the same as those of the control sample, the Shonai 1 cultivar, which has similar sensory characteristics. The sensory evaluation tests at each time point, e.g., at 48 h after harvest, were conducted simultaneously and also immediately after blanching and cooling of the edamame samples. The control samples were blanched under the same conditions as the other samples, immediately frozen at −45 °C, and thawed before each sensory evaluation test.

Raw CE−MS data were analyzed with our proprietary software called MasterHands (*13*, *15*). Briefly, first, the peaks were detected from sliced electropherograms (m/z 0.02 width), and accurate m/z values were calculated by Gaussian curve fitting. Second, the migration times of the detected peaks were normalized by a time-warping function, whose numerical parameters were optimized by a simplex method with peak matching across multiple data sets based on dynamic programming techniques (*16*). Third, any redundant features, such as isotopic peaks, fragments, and adduct ions, were removed. Finally, the metabolites contained in the standard compounds were assigned to the remaining features by matching their m/z values and normalized migration times. LC−MS/MS data were analyzed by MassHunter (Agilent Technologies).

Data Analysis. JMP, version 7.0.1 (SAS, Cary, NC), and Mev TM4 software (Dana-Farber Cancer Institute, Boston, MA) were used for principal component analysis (PCA) and heat map analysis, respectively. The Pearson correlation coefficient and two-tailed p values were calculated using GraphPad Prism, version 5.02 (GraphPad Software, Inc., San Diego, CA).

RESULTS AND DISCUSSION

Sensory Evaluation Test and Metabolite Profiles. Figure 2 depicts the overall metabolite profile and sensory properties. CE−MS detected 337 peaks on average (±98, standard deviation). After peaks observed in less than 75% of the samples were removed, 126 peaks remained. Of these, 76 were matched with compounds in our standard libraries based on m/z and normalized migration times. **Figure 2A** is a heat map visualization of these 76 annotated metabolites and 4 free sugars quantified by LC−MS/MS and sensory evaluation scores; the correlation between these metabolites and sensory evaluation scores is also shown.

The metabolites on the heat map (green−black−red color scheme) exhibited four major clusters. Amino acids were included in clusters A and B, while the free sugars were included in cluster D. In clusters B and D, the initial metabolite concentrations in samples 1 and 2, used in all experimental cases, were relatively high and decreased with storage time. Overall, these metabolites also showed a high correlation with the sensory evaluation scores (rainbow color scheme) because the sensory evaluation scores tended to decrease with the storage time (black−yellow color scheme). On the other hand, the metabolites in clusters A and C generally increased with storage time and were negatively correlated with sensory evaluation scores. Notably, cluster A included the metabolites showing higher concentrations after retail display at 25 °C (i.e., cases B and D), while cluster C included the metabolites that showed increased concentrations in cases C, D, and E, demonstrating the effect of 2 days of storage after transportation.

The sensory evaluation scores (black−yellow color scheme) for the four criteria scored in this study were correlated with each other. Even in the case with the weakest correlation, the correlation between flavor and the total score was statistically significant ($r = 0.829$, $p = 0.0009$). The relationship between storage temperature and sensory evaluation scores was different at 72 and 120 h after harvest; at 72 h, the umami, flavor, and total scores for sample B2 (at 25 °C) were slightly better than for sample A2 (at 10 °C) and the sweetness score was the same. In contrast, at 120 h after harvest, except for flavor, the sweetness, umami, and total scores for sample C2 (at 10 °C) were better than for sample D2 (at 25 °C).

Free Amino Acid and Free Sugar Profiles at Harvest. The concentration of free amino acids and free sugars at harvest is depicted in **Figure 2B**. Glutamate, asparagines, and alanine showed the highest concentrations, while cysteine, although detectable, exhibited the lowest concentration. This finding is consistent with other studies, in which the concentration of glutamate, asparagine, and alanine was significantly higher than of other amino acids and in which cysteine was not detectable in edamame without blanching (*1*, *9*). Of the free sugars measured here, the concentration of sucrose was significantly higher than that of other free sugars, including maltose (2.34-fold, $p = 0.028$), fructose (14.8-fold, $p = 0.0004$), and glucose (19.1-fold, $p = 0.003$). These observations are consistent with earlier reports regarding fructose and glucose (*17*, *18*). The concentration of maltose, which is generated from starch by β-amylase during boiling (*19*), was significantly higher than that of fructose (6.34-fold, $p = 0.0003$) and glucose (8.17-fold, $p = 0.0002$).

Effect of 2 Days of Storage after Transportation on Metabolite Profiles. To understand the effect of 2 days of storage after transportation, we compared the metabolite profiles of cases A and B (measured 72 h after harvest) to those of cases C and D (measured 120 h after harvest). Samples obtained at harvest (0 h), after transportation (24 h), and after 2 days of storage at 5 °C (120 h, case E) were also analyzed using PCA. **Figure 3A** shows the score plot for PCA using the concentrations of all detected metabolites. Samples 1 (0 h) and 2 (24 h) were closely located, indicating only slight changes in the metabolite profiles after a short period of storage and transportation at 5 °C after harvest. Except for A2, plots B2, C2, D2, and E2 were distributed at broadly similar distances from each other and showed no remarkable clusters. Loading plots (Figure S1 in the Supporting Information) distributed most of the metabolites at almost equal distance from the origin point, and none of the plots was of exceptional distance from the origin relative to the other plots. PCA was also conducted using the metabolites related to sensory characteristics, namely, the amino acids and free sugars. Score plots (**Figure 3B**) showed a cluster consisting of B2 (72 h) and D2 (120 h), indicating similar profiles for retail display at 25 °C with/without storage for 2 days after transportation. Plots C2 and E2 (120 h) formed another cluster, and the plots for samples 2 and A2 also formed a cluster. Loading plots (**Figure 3C** and Figure S2 in the Supporting Information) showed that only tryptophan and aspartate, reflecting bitter and sour tastes, respectively, were located in the minus region along the first principal component (PC) axis. Free sugars and glutamate, reflecting umami (*20*), were located closely along the second PC axis, while the other metabolites were distributed globally.

Storage Effect on Sugar Profiles. To demonstrate the effects of storage time and temperature on metabolic profiles, the time

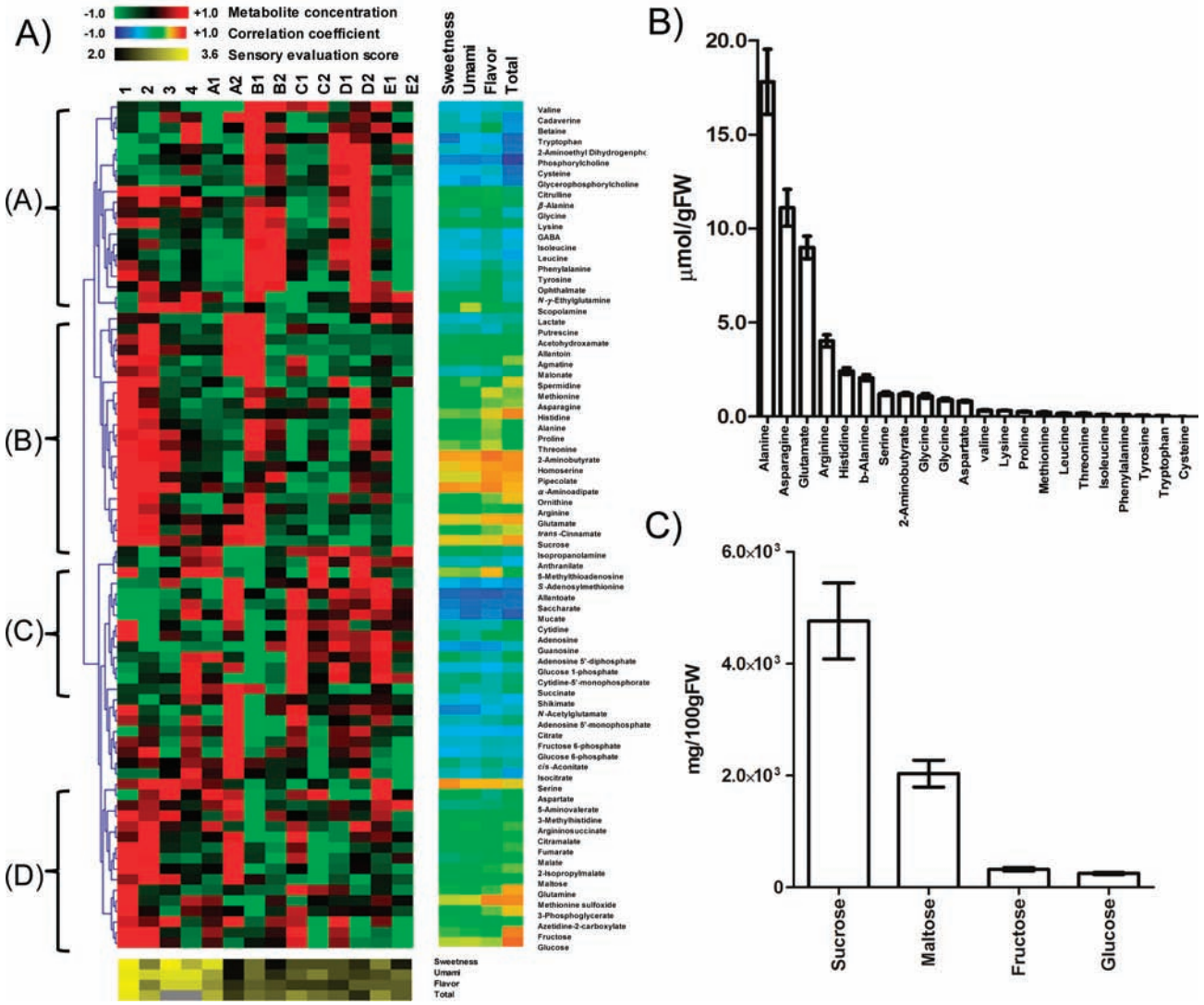

Figure 2. Metabolite profiles and sensory evaluation scores. (A) Heat maps showing the quantified metabolic profiles of the edamame samples (black−green−red color scheme), sensory evaluation scores (black−yellow color scheme), and their correlations (rainbow color scheme). All of the concentrations shown in the heat map were transformed into *z* scores, and Pearson correlation analysis was performed. The gray areas in the heat map indicate missing data. See the text for alphabetical labels. (B) Quantified amino acids and (C) free sugars at harvest. The error bars in panels B and C indicate standard deviations.

course of principal metabolites is shown in **Figure 4**, including free sugars (**Figure 4A**), the serine degradation pathway (**Figure 4B**), the shikimate pathway (**Figure 4C**), branched metabolites from pyruvate (**Figure 4D**), the GABA pathway (**Figure 4E**), and the asparate degradation pathway (**Figure 4F**).

As shown in the free sugar profile (**Figure 4A**), there were small temperature-specific differences in the glucose concentration. Overall, the concentration of maltose, glucose, sucrose, and fructose decreased over time during retail display at 25 and 10 °C, except for a slight increase in the glucose and fructose concentrations at 96 h after harvest. Shono et al. (*5*), who monitored the free sugar content in green peas during 6 days of storage, found that the decrease in the sucrose and glucose concentration was greater at higher (20 °C) than lower (10 °C) temperatures. In contrast, the sucrose concentration at higher storage temperatures (25 °C, cases B and D) was slightly lower than at lower storage temperatures (10 °C, case A and C), except for 48 h after harvest. The sucrose time course at 5 °C (case E) was similar. Furthermore, the effect of the storage temperature on the glucose concentration was different from that reported by Shono et al. (*4*); they reported that changes in the sucrose concentration in edamame beans were enhanced by translocation from the pod and decreased by bean growth. Song et al. (*17*) and Mozzoni et al. (*21*) reported significant decreases in the sucrose concentration with subtle changes in the amino acid profiles during a short blanching time. These factors may account for the disparity in the results.

Storage Effect on Serine Pathway Profiles. A feature consistent at 72 and 120 h involves the metabolites that play a role in the serine degradation pathway (**Figure 4B**). Although there was little difference in the serine concentration, the concentration of glycine and cysteine increased after storage at 25 °C (cases B and D) compared to 10 °C (cases A and C). The time course showed a slight decrease in the serine concentration and a concomitant increase in the cystein and glycine concentrations, particularly, in cases B and D. In contrast, the time course of changes in the 2-aminobutyrate concentration was broadly independent of storage conditions. Thus, considering the initial low concentration of cysteine, the concentration of glycine and serine is expected to increase as a result of synthesis from serine during storage.

Storage Effect on Shikimate Pathway Profiles. The shikimate pathway, which is present in all vascular plants, starts from shikimate as a precursor for various phenolic and aroma compounds, such as lignin and tannins (*22*). The concentration of tyrosine and phenylalanine, two intermediates in the shikimate

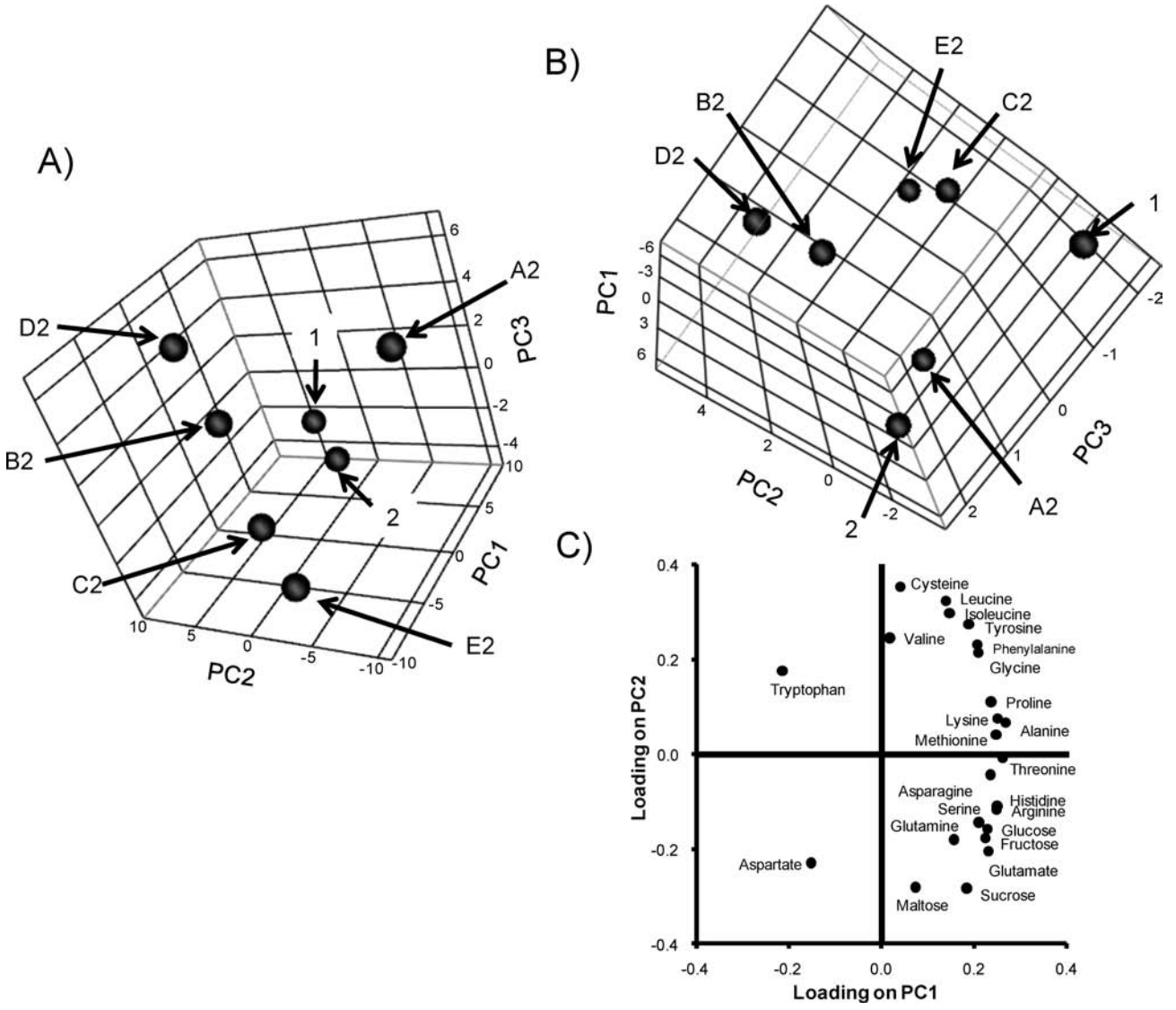

Figure 3. PCA for metabolite profiles in sample 1 (0 h at harvest), 2 (24 h after harvest), A2 and B2 (72 h after harvest), and C2, D2, and E2 (120 h after harvest). (A) PCA score plots generated using all of the detected metabolites. The cumulative proportions of the first three PCs were 36.3, 63.6, and 79.5%, respectively. Loading plots are shown in Figure S1 in the Supporting Information. (B) Score plots for PCA using amino acids and free sugars. The cumulative proportion of the first three PCs were 55.0, 83.5, and 91.1%, respectively. (C) Loading plots for the first and second PCs. The other loading plots are shown in Figure S2 in the Supporting Information.

pathway that are synthesized from shikimate through anthrailate and prephenate, respectively, was increased after retail display at 25 °C (cases B and D). Shikimate decreased at 24 h after harvest and decreased further after retail display at 25 °C (case B) compared to its decrease at 10 °C (case A) (**Figure 4C**). On the other hand, cases C, D, and E exhibited similar time-course profiles. The concentration of tyrosine, tryptophan, and phenylalanine increased during retail display at 25 °C (cases B and D) compared to their concentration at 10 °C (cases A and C). Because phenylalanine and tryptophan correspond to a bitter taste (*23*), the accumulation of these metabolites at higher temperatures is expected to reduce the sensory evaluation scores. In particular, the time course for changes in the tryptophan content, which was notably different from that of the other metabolites in the PCA loading plots (**Figure 2C**), was unique in that it increased over time under all conditions, even after storage at 5 °C. Phenylalanine is a precursor of lignin, which serves as a matrix around the polysaccharide components in cell walls to provide physical strength (*24*), and may be involved in the hardening of edamame (*25*).

Storage Effect on Sweet Amino Acid Profiles. The concentration of leucine and alanine, which are derived from pyruvate, was higher after retail display at 25 °C (cases B and D) than at 10 °C (cases A and C) (**Figure 4D**). On the other hand, the differences in the concentration of valine were subtle among the four cases. Leucine contributes to a bitter taste (*23*), while alanine contributes to sweetness, which is especially important for the overall taste of edamame (*26*). However, there were no or only small differences in the sweetness scores at 72 h (2.1 for cases A and B) and 120 h (2.5 for case C and 2.3 for case D), and there was no correlation between alanine and the sweetness ($r = 0.276$, $p = 0.340$) and the total score ($r = -0.256$, $p = 0.426$). Leucine showed a weak but not significant negative correlation with sweetness ($r = -0.2921$, $p = 0.311$) and the total score ($r = -0.793$, $p = 0.225$). Thus, these metabolites did not greatly influence the sensory characteristics.

Storage Effect on GABA Profiles. Miyashita and Good (*27*) reported that alanine and GABA in the root of *Arabidopsis* accumulated in response to hypoxic stress, while Urano et al. (*28*) observed the accumulation of alanine in a strain of *Arabidopsis* with

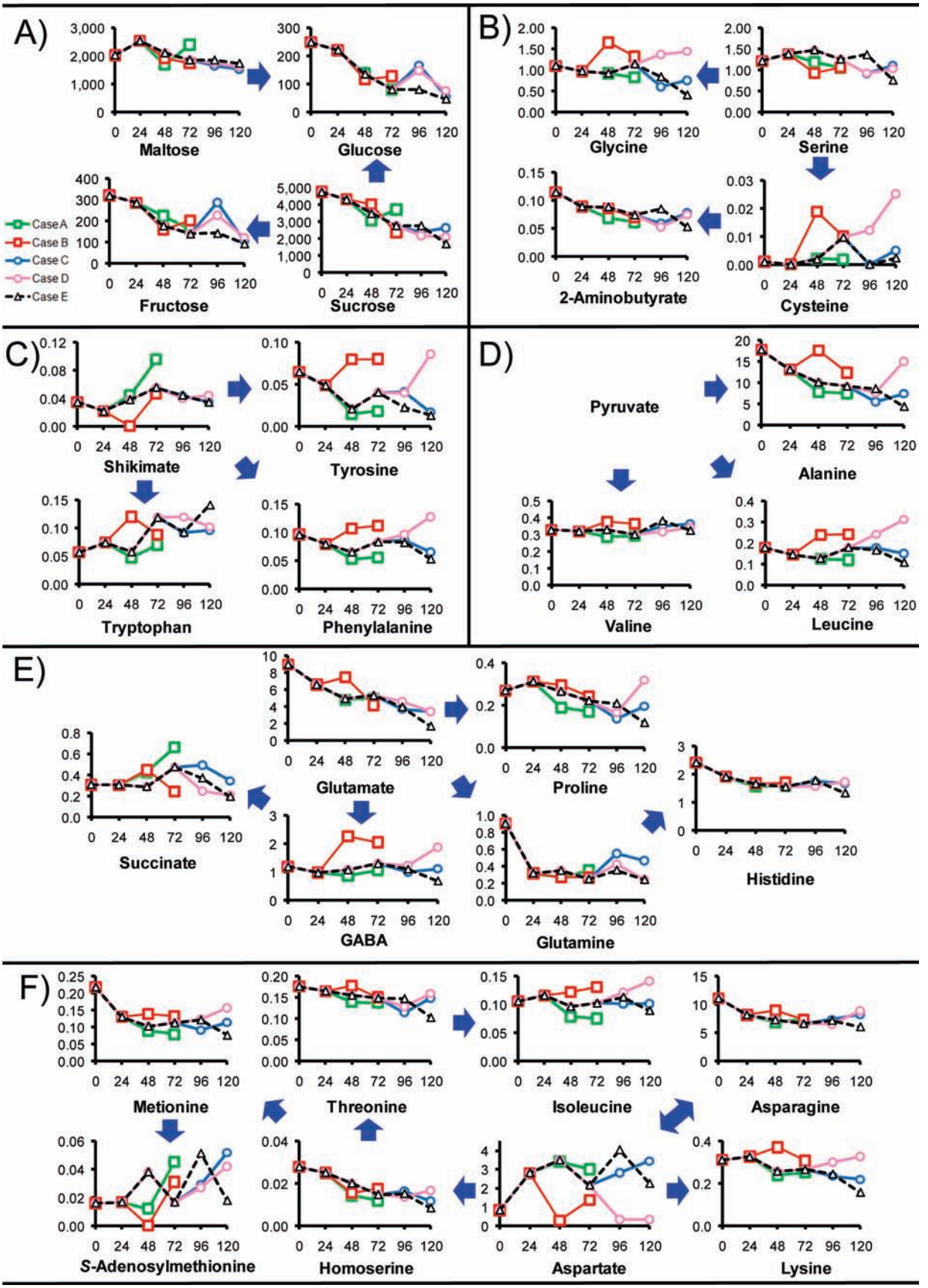

Figure 4. Time course of changes in metabolite concentrations. Green, red, blue, pink, and black lines represent cases A, B, C, D, and E, respectively, as defined in **Figure 1**. (A) Free sugars, (B) serine degradation pathway, (C) shikimate degradation pathway, (D) valine, alanine, and leucine, (E) GABA pathway, and (F) aspartate degradation pathway. The *x* axis indicates storage time (hours), and the *y* axis indicates the concentration. The units on the *y* axis are mg/100 g of FW for panel A and μmol/g of FW for panels B−F.

a mutation in the 9-*cis*-epoxycarotenoid dioxygenase (NCED) gene, which plays a role in the dehydration stress-inducible biosynthesis of abscisic acid, a plant hormone. Thus, the accumulation of alanine may be attributable to hypoxia, dehydration, or similar stressors derived from elevated respiration during storage at high temperatures for several days after harvest (*2*).

Metabolites belonging to the glutamate pathway and the time course of changes in the glutamate, glutamine, and histidine concentration (**Figure 4E**) were similar to those at 5 °C (case E). The concentration of proline and GABA at 25 °C (cases B and D) was higher than at 10 °C (cases A and C), while the concentration of succinate, which is derived from the tricarboxylic acid cycle, was higher at 10 °C. Proline plays a role in tolerance to dehydration (*29*), and the respiration rate increases during storage at high temperatures (*2*); this is consistent with the increased concentration of proline at high temperatures.

GABA accumulates by several fold in response to diverse stimuli, including heat shock, mechanical stimulation, hypoxia, and phytohormone activity (*30*). In our study, the concentration of GABA was higher after retail display at 25 °C (cases B and D) than at 10 °C (cases A and C) and 5 °C (case E) (**Figure 4E**). Although a corresponding decrease in the glutamate concentration by glutamine synthetase (EC 6.3.1.2) (*31*) was expected, the storage temperature scarcely affected its concentration. Succinate, a metabolite of the tricarboxylic acid cycle that is synthesized from GABA by 4-aminobutyrate aminotransferase (EC 2.6.1.19) and succinate semialdehyde dehydrogenase (EC 1.2.1.24), showed a different pattern of changes. In fact, the concentration of succinate was greater at 72 and 120 h after storage at 10 °C (cases A and C) than after storage at 25 °C; this was the inverse of the changes in GABA (**Figure 4E**) and suggests activation of the GABA−succinate metabolic pathway. Further analyses are needed to confirm the underlying mechanism(s), taking into account changes in related metabolites and the activity of the GABA shunt (*32*).

Storage Effect on Asparate Pathway Profiles. Aspartate is a precursor of two major pathways: (1) the synthesis and degradation of asparagine by asparagine synthetase (EC 6.3.5.4) and asparaginase (EC 3.5.1.1), respectively, and (2) the downstream activity of aspartate kinase (EC 2.7.2.4) for the synthesis of the essential amino acids lysine, threonine, methionine, and isoleucine. We found that the concentration of isoleucine was greater at 25 °C (cases B and D) than at 10 °C (cases A and C), while the concentration of adenosylmethionine, which is synthesized from methionine, was higher at 10 °C than 25 °C (**Figure 4F**). However, the concentration of aspartate noticeably decreased after storage at 25 °C (cases B and D). The change in asparagine was expected because this metabolite is an important nitrogen reservoir in plants, it accumulates in response to dark/light transitions and to various stressors, such as mineral deficiencies, salt, or drought, and asparagine as well as glutamine are released as ammonium and nitrogen during sugar starvation (reviewed in ref *33*). However, we observed only minor differences at 48 and 120 h after harvest. Changes in methionine, isoleucine, and lysine were the inverse of changes in aspartate, indicating their role in the storage temperature-specific changes in aspartate degradation.

Study Limitations. We studied one kind of edamame cultivar. Minamide et al. (*12*) found cultivar-specific differences in the total concentration of free sugars and free amino acids, except for ascorbic acid, and Zarkadas et al. (*34*) observed differences in individual amino acids. In addition, Kirimura et al. (*23*) reported marked changes in the content of the three principal amino acids of edamame during the harvest season, including asparagine (sour taste), alanine (sweet taste), and glutamic acid (umami). Thus, further analyses of edamame from various cultivars and at different times in the harvest season should be conducted to confirm the observations in this study.

To reduce the time-dependent variations in the grading of sensory scores, the samples separated for the sensory evaluation test should be immediately stored and evaluated simultaneously. Guo et al. (*2*) reported that frozen samples of green beans that were stored for several days showed no change in weight but did show a large decrease in the total sugar content. We froze control samples and found a sugar loss (**Figure 4A**). If this sugar loss dramatically decreases the sensory scores of the control samples, the scores of the other samples, which were graded relative to the control sample, may be higher. Nonetheless, in our study, the decrease in the sensory scores of samples over time indicates that freezing the control samples had no or only a subtle influence on the scores.

Our study took a top-down approach; we examined overall features and explored the metabolites relevant to specific taste properties based on statistical criteria. This strategy does not yield proof; rather, it provides a correlative relation between metabolites and taste. In contrast, the conventional bottom-up approach is used to determine the concentration threshold that facilitates the sensory perception of individual metabolites and to confirm taste changes under conditions of artificial perturbation of the metabolite level. This approach makes sense only when each molecule is independently attributed to taste and requires extensive experiments if taste changes depend upon multiple metabolites. Thus, both approaches should be combined in future studies to narrow down the metabolite candidates and to confirm their contribution to taste.

In the time course of the metabolite profiles (**Figure 4**), it is of particular interest that the concentration of all four free sugars decreased over time, while downstream metabolites in the shikimate pathway, including tyrosine and phenylalanine, increased independent of the storage temperature. On the other hand, the concentration of GABA, which is affected by various stressors, and phosphorylcholine, a precursor of cell membrane phospholipids, was markedly affected by the storage temperature. Thus, while shorter storage times are recommended to maintain the sensory attributes and reduce the loss of free sugars and changes in components related to the cell membrane, the selected storage temperature is dictated by the goal. For example, a comparison of cases A and B at 72 h after harvest showed that the higher storage temperature (case B) improved the nutritional content, such as GABA and alanine, and yielded better sensory attributes. The opposite was true in comparisons of cases C and D at 120 h after harvest. Studies using other omics analyses are underway for a better understanding of the mechanisms involved in changes in metabolite levels and their relationship to changes in the sensory characteristics of edamame during short periods of storage during transportation and retail display.

ABBREVIATIONS USED

CE−MS, capillary electrophoresis−mass spectrometry; LC−MS/MS, liquid chromatography−tandem mass spectrometry; ESI, electrospray ionization; PCA, principal component analysis; GABA, γ-aminobutyric acid.

ACKNOWLEDGMENT

We thank the professional panelists and Ando of the Agricultural Technique Improvement Research Office, Agricultural Technique Popularization Division, Shonai Area, General Branch Administration, Yamagata Prefectural Government. We also thank Masayo Mori and Atsuko Tomita at the Institute of Advanced Biosciences for fruitful discussions.

Supporting Information Available: PCA loading plot using all metabolites (Figure S1) and PCA loading plots using amino acids and free sugars (Figure S2). This material is available free of charge via the Internet at http://pubs.acs.org.

LITERATURE CITED

(1) Akazawa, T.; Yanagisawa, Y.; Sasahara, T. Concentrations of water-soluble nitrogen and amino acids as criteria for discriminating vegetable-type and grain-type soybean cultivars. *Breed. Sci.* **1997**, *47*, 39–44.

(2) Guo, L.; Ma, Y.; Sun, D.-W.; Wang, P. Effects of controlled freezing-point storage at 0 °C on quality of green bean as compared with cold and room-temperature storages. *J. Food Eng.* **2008**, *86*, 25–29.

(3) Rostagno, M. A.; Palma, M.; Barroso, C. G. Short-term stability of soy isoflavones extracts: Sample conservation aspects. *Food Chem.* **2005**, *93*, 557–564.

(4) Shono, Y.; Yoshimura, M.; Kimura, S.; Yamauchi, N. Sucrose metabolism in stored green peas. *Food Sci. Technol. Int., Tokyo* **1997**, *3*, 41–45.

(5) Sánchez-Mata, M. C.; Cámara, M.; Díez-Marqués, C. Extending shelf-life and nutritive value of green beans (*Phaseolus vulgaris* L.), by controlled atmosphere storage: Micronutrients. *Food Chem.* **2003**, *80*, 317–322.

(6) Berger, M.; Küchler, T.; Maassen, A.; Busch-Stockfisch, M.; Steinhart, H. Correlations of ingredients with sensory attributes in green beans and peas under different storage conditions. *Food Chem.* **2007**, *103*, 875–884.

(7) Simonne, A. H.; Smith, M.; Weaver, D. B.; Vail, T.; Barnes, S.; Wei, C. I. Retention and changes of soy isoflavones and carotenoids in immature soybean seeds (edamame) during processing. *J. Agric. Food Chem.* **2000**, *48*, 6061–6069.

(8) Wang, L.; Wang, T.; Fehr, W. R. Effect of seed development stage on sphingolipid and phospholipid contents in soybean seeds. *J. Agric. Food Chem.* **2006**, *54*, 7812–7816.

(9) Yanagisawa, Y.; Akazawa, T.; Abe, T.; Sasahara, T. Changes in free amino acid and Kjeldahl N concentrations in seeds from vegetable-type and grain-type soybean cultivars during the cropping season. *J. Agric. Food Chem.* **1997**, *45*, 1720–1724.

(10) Abe, T.; Takeya, K. Difference in γ-aminobutyric acid content in vegetable soybean seeds. *J. Jpn. Soc. Food Sci. Technol.* **2005**, *52*, 545–549 (in Japanese).

(11) Urbanski, G. E.; Wei, L. S.; Nelson, A. I. Effect of freeze damage on soybean quality and storage stability. *J. Food Sci.* **1980**, *45*, 208–212.

(12) Minamide, T.; Hata, A. Effect of harvest time and storage temperature on the quality of green soybean seeds (edamame). *Sci. Rep. Kyoto Prefect. Univ.* **1990**, *41*, 23–28 (in Japanese).

(13) Sugimoto, M.; Koseki, T.; Hirayama, A.; Abe, S.; Sano, T.; Tomita, M.; Soga, T. Correlation between sensory evaluation scores of Japanese sake and metabolome profiles. *J. Agric. Food Chem.* **2010**, *58*, 374–383.

(14) Soga, T.; Baran, R.; Suematsu, M.; Ueno, Y.; Ikeda, S.; Sakurakawa, T.; Kakazu, Y.; Ishikawa, T.; Robert, M.; Nishioka, T.; Tomita, M. Differential metabolomics reveals ophthalmic acid as an oxidative stress biomarker indicating hepatic glutathione consumption. *J. Biol. Chem.* **2006**, *281*, 16768–16776.

(15) Sugimoto, M.; Wong, D. T.; Hirayama, A.; Soga, T.; Tomita, M. Capillary electrophoresis mass spectrometry-based saliva metabolomics identified oral, breast and pancreatic cancer-specific profiles. *Metabolomics* **2010**, *6*, 78–95.

(16) Sugimoto, M.; Hirayama, A.; Ishikawa, T.; Robert, M.; Baran, R.; Uehara, K.; Kawai, K.; Soga, T.; Tomita, M. Differential metabolomics software for capillary electrophoresis−mass spectrometry data analysis. *Metabolomics* **2010**, *6*, 27–41.

(17) Song, J. Y.; An, G. H.; Kim, C. J. Color, texture, nutrient contents, and sensory values of vegetable soybeans [*Glycine max* (L.) Merrill] as affected by blanching. *Food Chem.* **2003**, *83*, 69–74.

(18) Masuda, R.; Harada, K.; Saito, M. Enhancement of sweet components in vegetable soybean seeds: Starch degradation during cooking enhance flavor of immature seeds. *Proceedings of the 2nd International Vegetable Soybean Conference*; Lumpkin, T. A., Shanmugasundaram, S., Eds.; Washington State University: Pullman, WA, **2001**; pp 105−108.

(19) Masuda, R. Elucidation of maltose formation reaction involved in the improvement of the eating quality of green soybean. *Agric. Hortic.* **2004**, *79*, 1085–1093.

(20) Kurihara, K.; Kashiwayanagi, M. Physiological studies on umami taste. *J. Nutr.* **2000**, *130*, 931S–934S.

(21) Mozzoni, L. A.; Chen, P.; Morawicki, R. O.; Hettiarachchy, N. S.; Brye, K. R.; Mauromoustakos, A. Quality attributes of vegetable soybean as a function of boiling time and condition. *Int. J. Food Sci. Technol.* **2009**, *44*, 2089–2099.

(22) Jung, H. G.; Fahey, G. C., Jr. Nutritional implications of phenolic monomers and lignin: A review. *J. Anim. Sci.* **1983**, *57*, 206–219.

(23) Kirimura, J.; Shimizu, A.; Kimizuka, A.; Ninomiya, T.; Katsuya, N. The contribution of peptides and amino acids to the taste of foods. *J. Agric. Food Chem.* **1969**, *17*, 689–695.

(24) Whetten, R.; Sederoff, R. Lignin biosynthesis. *Plant Cell* **1995**, *7*, 1001–1013.

(25) Shiga, T. M.; Lajolo, F. M.; Filisetti, T. M. C. C. Changes in the cell wall polysaccharides during storage and hardening of beans. *Food Chem.* **2004**, *84*, 53–64.

(26) Masuda, R. Quality requirement and improvement of vegetable soybean. In *Vegetable Soybean Research Needs for Production and Quality Improvement*; Shanmugasundaram, S., Ed.; Asian Vegetable Research and Development Centre: Taiwan, 1991; pp 92−102.

(27) Miyashita, Y.; Good, A. G. Contribution of the GABA shunt to hypoxia-induced alanine accumulation in roots of *Arabidopsis thaliana*. *Plant Cell Physiol.* **2008**, *49*, 92–102.

(28) Urano, K.; Maruyama, K.; Ogata, Y.; Morishita, Y.; Takeda, M.; Sakurai, N.; Suzuki, H.; Saito, K.; Shibata, D.; Kobayashi, M.; Yamaguchi-Shinozaki, K.; Shinozaki, K. Characterization of the ABA-regulated global responses to dehydration in *Arabidopsis* by metabolomics. *Plant J.* **2009**, *57*, 1065–1078.

(29) Nanjo, T.; Kobayashi, M.; Yoshiba, Y.; Sanada, Y.; Wada, K.; Tsukaya, H.; Kakubari, Y.; Yamaguchi-Shinozaki, K.; Shinozaki, K. Biological functions of proline in morphogenesis and osmotolerance revealed in antisense transgenic *Arabidopsis thaliana*. *Plant J.* **1999**, *18*, 185–193.

(30) Shelp, B. J.; Bown, A. W.; McLean, M. D. Metabolism and functions of γ-aminobutyric acid. *Trends Plant Sci.* **1999**, *4*, 446–452.

(31) Bown, A. W.; Shelp, B. J. The metabolism and functions of γ-aminobutyric acid. *Plant Physiol.* **1997**, *115*, 1–5.

(32) Fait, A.; Fromm, H.; Walter, D.; Galili, G.; Fernie, A. R. Highway or byway: The metabolic role of the GABA shunt in plants. *Trends Plant Sci.* **2008**, *13*, 14–19.

(33) Azevedo, R. A.; Lancien, M.; Lea, P. J. The aspartic acid metabolic pathway, an exciting and essential pathway in plants. *Amino Acids* **2006**, *30*, 143–162.

(34) Zarkadas, C. G.; Gagnon, C.; Gleddie, S.; Khanizadeh, S.; Cober, E. R.; Guillemette, R. J. D. Assessment of the protein quality of fourteen soybean [*Glycine max* (L.) Merr.] cultivars using amino acid analysis and two-dimensional electrophoresis. *Food Res. Int.* **2007**, *40*, 129–146.

Received for review April 19, 2010. Revised manuscript received June 14, 2010. Accepted June 19, 2010. This work was supported by research funds from the Yamagata Prefectural Government and the city of Tsuruka.