Journal of Bioscience and Bioengineering
VOL. 118 No. 1, 56–63, 2014

www.elsevier.com/locate/jbiosc

Analysis of the correlation between dipeptides and taste differences among soy sauces by using metabolomics-based component profiling

Shinya Yamamoto,[1] Kazuki Shiga,[1,2] Yukako Kodama,[2] Miho Imamura,[2] Riichiro Uchida,[2] Akio Obata,[2] Takeshi Bamba,[1] and Eiichiro Fukusaki[1,*]

Department of Biotechnology, Graduate School of Engineering, Osaka University, 2-1 Yamadaoka, Suita, Osaka 565-0871, Japan[1] *and Research and Development Division, Kikkoman Corporation, 399 Noda, Noda City, Chiba 278-0037, Japan*[2]

Received 16 September 2013; accepted 22 December 2013
Available online 1 February 2014

Characterizing the relationships between the components and taste differences among soy sauces can help evaluate and improve the quality of soy sauces. Although previous studies have reported that certain taste-active dipeptides, the relationships between taste differences and dipeptides of soy sauces are unknown. Therefore, our objective in this study was to investigate the correlations between the dipeptides and the taste differences among soy sauces. To analyze the dipeptides, we constructed an analytical method using liquid chromatography/tandem mass spectrometry (LC/MS/MS) in multiple reaction monitoring mode. Based on this method, we detected 237 dipeptides, the largest number ever detected in soy sauce research. Next, orthogonal projections to latent structures regressions were performed. The data matrix of components, including dipeptides and other low-molecular-weight hydrophilic components obtained from gas chromatography/mass spectrometry (GC/MS), served as explanatory variables (366 in total), whereas a sensory data matrix obtained using quantitative descriptive analysis served as the response variable. The accuracy of models for the sweetness and saltiness differences constructed using the LC/MS/MS and GC/MS data matrix were higher than did models constructed using only the GC/MS data matrix. As a result of investigation of the correlation between the dipeptides and taste differences among soy sauces by using variable importance in the projection (VIP) score, many dipeptides showed the high correlation with taste differences. Specifically, Ile–Gln, Pro–Lys, Ile–Glu, Thr–Phe, and Leu–Gln showed the high VIP score on sweet differences. This study is the first report that reveals the correlations between the dipeptides and taste differences among soy sauces.

[**Key words:** Soy sauce; Liquid chromatography/tandem mass spectrometry; Dipeptides; Sensory analysis; Multivariate analysis; Orthogonal projections to latent structures]

Numerous kinds of soy sauces are available worldwide, and the taste of each soy sauce depends on the starting materials and production method used for preparing the soy sauce. For example, typical Japanese soy sauce (*koikuchi shoyu*) is prepared from nearly equal amounts of boiled soybeans and roasted wheat, with common salt also being added (final about 17% w/v NaCl); after fermenting the materials by using mold, lactobacillus, and yeast, the well-aged mash is pressed and pasteurized (1). Conversely, in traditional Chinese soy sauce, less raw wheat is used than in *koikuchi shoyu*, and the strains of microorganisms used, and the fermentation periods are distinct from those used for preparing *koikuchi shoyu* (2). The disparities in the intensity and quality of the tastes of various kinds of soy sauces are considered to reflect the presence of different components in the soy sauces (3). Therefore, revealing the relationship between the components and taste differences of a soy sauce may help evaluate and improve the quality of the sauce. However, only a few studies have been conducted to uncover the differences in soy sauce components.

Amino acids, sugars, organic acids and minerals are considered as main taste components of soy sauce. For example, total nitrogen and concentration of salt are considered as useful evaluation index for the quality of soy sauce. However, describing the taste of soy sauce based on only a few main taste components is difficult primarily because many of these components interact with each other (4). For instance, although an umami taste of soy sauce is considered to depend mainly on the concentration of L-glutamic acid, which is derived from the enzymatic decomposition of proteins, Phe and Tyr have also been reported as umami-related components (5,6). In addition, Imamura et al. reported that the lowest additive concentration of monosodium glutamate (MSG) and glucose to sense the taste differences in *koikuchi shoyu* were 1.9% and 1.8% (w/v), whereas the lowest concentration to sense the taste in aqueous solution of MSG and glucose were 0.026% and 0.57% (w/v) (7). This fact suggests that the high contributor of taste not necessarily be play a large role in the taste differences among soy sauces, and other taste compounds have a significant effect on the taste differences. In addition, these previous studies indicate that the existing index for quality of soy sauces such as total nitrogen and

---

* Corresponding author. Tel./fax: +81 6 6879 7424.
E-mail address: fukusaki@bio.eng.osaka-u.ac.jp (E. Fukusaki).

This study represents a portion of the dissertation submitted by Shinya Yamamoto to Osaka University in partial fulfillment of the requirement for his PhD.

1389-1723/$ – see front matter © 2013, The Society for Biotechnology, Japan. All rights reserved.
http://dx.doi.org/10.1016/j.jbiosc.2013.12.019

concentration of salt are not perfect. Therefore, a wide variety of components should be analyzed to study the taste differences among the soy sauces.

During the process of producing traditional Japanese soy sauce, 70%–90% of the total proteins of soybean and wheat, the main ingredients of soy sauce, are degraded into soluble free amino acids and peptides (2). Low-molecular-weight peptides, which are commonly composed of 2–3 amino acids, constitute 10%–20% of the total nitrogenous compounds in *koikuchi shoyu* and are the second-most abundant component in soy sauce after free amino acids (8).

Recently, low-molecular-weight peptides have been suggested to act as taste-active compounds in soy sauce. For example, a soy sauce fraction containing low-molecular-weight peptides (<500 Da) exhibited the strongest umami taste among all fractions (9,10). Moreover, pyroglutamyl peptides, which are produced by the condensation reaction of N-terminal Glu and Gln that are enzymatic decomposition products of wheat gluten, are known to enhance taste (11). Katsura et al. suggested that a fraction containing pyroglutamyl peptides enhanced the umami taste of Japanese noodle soup (udon-dashi) (12). Therefore, the contribution of low-molecular-weight peptides (especially those <500 Da) to the umami taste of *koikuchi* and *tamari shoyu* (produced using soybeans alone or soybeans and a little wheat) has been evaluated using a taste dilution analysis (TDA) (13). TDA has been used as a tool to trace taste-active compounds in previous studies (6,13). The taste dilution factor—the maximal dilution at which a taste can be detected—of the peptide fraction was reported to be low, and low-molecular-weight peptides were evaluated to be negligible contributors to the umami taste of soy sauce (13). However, Kaneko et al. showed that pyroglutamyl dipeptides help enhance umami taste even at subthreshold concentrations, although these dipeptides are also known to produce sweet taste on their own (14). This study indicated the possibility that dipeptides influence the tastes of soy sauces even at low concentrations.

As noted above, low-molecular-weight peptides, especially the dipeptides in soy sauce, have been suggested to function as taste-active compounds (11,12,14), but only a few dipeptides have been reported to be related to taste. The contribution of dipeptides to the tastes of soy sauces has been evaluated by TDA, but the relation between dipeptides and taste differences among various soy sauces have never been studied.

Recently, metabolomics-based component profiling has been applied to food research. Metabolome analysis, or metabolomics, can estimate the metabolites related to specific biological properties by analyzing the correlation between differences of metabolite profiles and biological properties such as lifespan of yeast (15). By modifying the correlation between metabolite profiles and the properties of organisms to that between components and quality differences of food, the relationship between food components and quality differences can be revealed (16–18). Specifically, multivariate analysis methods such as orthogonal projections to latent structures (OPLS) have helped to identify components that correlate strongly with food quality differences (16). Furthermore, metabolomics-based component profiling can be applied to large numbers of components concurrently. Using gas chromatography/mass spectrometry (GC/MS)-based component profiling, we previously developed highly accurate models for predicting taste differences and further revealed that compounds such as amino acids and sugars are highly correlated with taste differences; however, dipeptides were not included in the low-molecular-weight profiles identified in that study (18).

In this study, our aim was to investigate the correlation between the dipeptides and taste differences among soy sauces by using metabolomics-based component profiling techniques. A detailed analysis of this correlation can provide useful information regarding the relationship between specific dipeptides and taste differences among various soy sauces.

First, we developed an experimental method for analyzing the dipeptides, and the dipeptide data matrix (comprising qualitative and quantitative information on each soy sauce) was acquired by using liquid chromatography/tandem mass spectrometry (LC/MS/MS). Moreover, using GC/MS, we obtained the data matrix for other low-molecular-weight compounds. Next, OPLS was performed to analyze the correlations between the data matrices of components and sensory analysis, with the components' data matrix serving as explanatory variables and the sensory analysis data matrix serving as response variables. After evaluating the predictive ability of the model, the correlations between the dipeptides and taste differences were investigated by using the variable importance in projection (VIP) scores (19).

MATERIALS AND METHODS

Materials Ribitol, ultrapure water, methanol (LC/MS grade), and analytical grade chloroform, pyridine, and formic acid were purchased from Wako Pure Chemical Industries (Osaka, Japan). The derivatization reagents methoxyamine hydrochloride and *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA) were purchased from Sigma (MO, USA) and GL Science, Inc. (Tokyo, Japan), respectively. LC/MS grade acetonitrile was purchased from Kanto Chemical Co. (Tokyo, Japan).

Dipeptide standard materials ($n = 337$) were purchased from AnaSpec (CA, USA), and 15 types of standard pyroglutamyl dipeptides (pGlu-X) (X = amino acid) standards were synthesized using the conventional liquid-phase method in which L-pyroglutamic acid pentachlorophenyl ester, triethylamine, and amino acids are used (20). Table S1 shows the standard compounds used in this study. The dipeptide standards were dissolved in a solvent consisting of methanol, ultrapure water, and formic acid (1:1:0.002, v/v/v). Four standard mixtures were prepared: no. 1 contained Gln-X, Leu-X, Pro-X, Thr-X, Arg-X, His-X, and Gly-X; no. 2 contained Glu-X, Met-X, Ile-X, Phe-X, Ser-X, and Asn-X; no. 3 contained Asp-X, Lys-X, Val-X, Ala-X, Trp-X, and Tyr-X; and No. 4 contained pGlu-X.

In this study, we used 19 soy sauce samples that were also used in a previous study, except for the hydrolyzed vegetable protein; of the 19 sauces, 10, 6, and 3 of the soy sauces were made in Japan, China, and USA, respectively, and of the 10 Japanese soy sauces, 9 were *koikuchi shoyu* and 1 was *shiro shoyu* (made by using a very high ratio of wheat to soybeans) (18).

Sensory analysis In this study, we focused on the 5 basic tastes, sweetness, saltiness, umami, sourness, and bitterness (21), and used the previously reported data matrix of these tastes obtained using quantitative descriptive analysis (QDA) (18). Thirteen panelists were selected who had >1 year experience in sensory analysis of soy sauce samples. We poured 20 mL of each sample into cups bearing 3-digit random numbers and presented them to the panelists in random order. After the panelist had each tasted 0.2 mL of the samples, they were instructed to evaluate the intensities of the 5 basic tastes; a linear scale was used for quantitative evaluation.

GC/MS conditions Sample preparation, peak detection, alignment, and compound identification were based on a previous study (18). Ribitol was used as an internal standard. The first derivatization of the extracts was conducted at 30 °C for 90 min using methoxyamine hydrochloride in pyridine. A second derivatization agent, MSTFA, was then added and the mixture was incubated at 37 °C for 30 min. Gas chromatographic analysis was performed using a GC/MS-QP-2010 system consisting of a GC-2010 gas chromatograph connected to a QP-2010 electron impact mass spectrometer and an AOC-20S autosampler (Shimadzu, Kyoto, Japan). We used a CP-SIL 8 CB low bleed 30 m × 0.25 mm i.d. fused silica capillary column (Agilent Technology, CA, USA). GCMS Solutions Ver.2.7.1 software (Shimadzu) was used for collecting and processing data. The starting helium gas flow rate through the column was 1.12 mL/min under the constant linear velocity. The split ratio, injection volume, and temperature were set at 1:25, 1 μL, and 230 °C, respectively. The column temperature was maintained at 80 °C for 2 min and then raised at a rate of 15 °C/min to 320 °C, where it was maintained for 6 min. The ion source temperature was 200 °C. Ions were generated by electron ionization by using a 70 eV electron beam, and we recorded 20 scans/s over the mass range 85–500 *m/z*. The acceleration voltage was turned on after a solvent delay of 3.5 min.

LC/MS/MS conditions The soy sauce samples were desalted until the concentration of NaCl reached 1%, as determined by using an S1 Micro Analyzer (Asahi Chemical Industry Co. Ltd., Tokyo, Japan). The desalted soy sauces were diluted 100-fold using the solvent mixture of methanol, ultrapure water, and formic acid (1:1:0.002, v/v/v) and filtered with a 0.2 μm polytetrafluoroethylene filter.

Liquid chromatographic analysis was performed using an LCMS-8030 Plus system (Shimadzu). Lab Solutions Ver. 5.53 SP3 (Shimadzu) was used for data collection and processing. Samples were separated on a Discovery HS F5, 2.1 × 150 mm, 3 μm column (Sigma–Aldrich). LC was conducted using a linear gradient of solvent A (ultrapure water containing 0.1% formic acid) and solvent B (acetonitrile containing 0.1% formic acid) for 55 min, with the following linear gradient elution: 0 min, 2% B;