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ORIGINAL ARTICLE

Principal component analysis of the relationship between the D-amino acid concentrations and the taste of the sake

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Abstract We performed sensory evaluations on 141 bottles of sake and analyzed the relationship between the D-amino acid concentrations, and the taste of the sake using principal component analysis, which yielded seven principal components (PC1–7) that explained 100 % of the total variance in the data. PC1, which explains 33.6 % of the total variance, correlates most positively with strong taste and most negatively with balanced tastes. PC2, which explains 54.4 % of the total variance, correlates most positively with a sweet taste and most negatively with bitter and sour tastes. Sakes brewed with "*Kimoto yeast starter*" and "*Yamahaimoto*" had high scores for PC1 and PC2, and had strong taste in comparison with sakes brewed with "*Sokujo-moto*". When present at concentrations below 50 µM, D-Ala did not affect the PC1 score, but all the sakes showed a high PC1 score, when the D-Ala was above 100 µM. Similar observations were found for the D-Asp and D-Glu concentrations with regard to PC1, and the threshold concentrations of D-Asp and D-Glu that affected the taste were 33.8 and 33.3 µM, respectively. Certain bacteria present in sake, especially lactic acid bacteria, produce D-Ala, D-Asp and D-Glu during storage, and these D-amino acids increased the PC1 score and produced a strong taste (*Nojun*). When D- and L-Ala were added to the sakes, the value for the umami taste in the sensory evaluation increased, with the effect of D-Ala being much stronger than that of L-Ala. The addition of 50–5,000 µM DL-Ala did not effect on the aroma of the sakes at all.

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Introduction

D-Amino acids are found in various foods, including wine (Kato et al. 2011), milk (Rubio-Barroso et al. 2006), cocoa (Pätzold and Brückner 2006), and fruit (Gogami et al. 2006). We recently reported for the first time that sake contains the D-amino acid forms of Ala, Asn, Asp, Arg, Glu, Gln, His, Ile, Leu, Lys, Ser, Tyr, Val, Phe and Pro; these D-amino acids were found at concentrations in the range of 0–524.3 µM (D-Ala), 0–68.8 µM (D-Asn), 0–66.9 µM (D-Asp), 0–133.1 µM (D-Arg), 0–132.0 µM (D-Glu), 0–38.8 µM (D-Gln), 0–70.9 µM (D-His), 0–13.6 µM (D-Ile), 0–501.3 µM (D-Leu), 0–31.5 µM (D-Lys), 0–15.7 µM (D-Ser), 0–31.5 µM (D-Tyr), 0–68.4 µM (D-Val), 0–63.9 µM (D-Phe) and 0–6.6 µM (D-Pro) (Gogami et al. 2011a). D-Amino acids are known to have tastes that are different from, and sometimes the opposite of, the tastes of their L-enantiomers and generally have a more sweet taste (Solms et al. 1965). However, because food contains various compounds that confer taste, such as L-amino acids, organic acids, and sugars (Caligiani et al. 2007), it might be difficult to estimate the effect of D-amino acids. To our knowledge, there has been no report that addresses this issue till date. Sake is one of the useful candidate foods to study the effects of D-amino acids on taste because the sensory evaluation method is well established. Furthermore, we have previously measured the concentrations of all the D- and L-amino acids in 141 bottles of sake using high-performance liquid chromatography (HPLC) (Gogami et al. 2011a).

In the present study, we performed sensory evaluations on the same 141 bottles of sake and analyzed the relationship between the D-amino acid concentration and the taste of the sake using principal component analysis.

Materials and methods

Materials

The sakes (141 bottles produced at 51 prefectures in Japan) evaluated in this study were purchased from randomly chosen manufacturers in Japan. The specific classes of sakes purchased were "*Daiginjo*" ($n = 9$), "*Junmai Daiginjo*" ($n = 14$), "*Ginjo*" ($n = 9$), "*Junmai Ginjo*" ($n = 42$), "*Special Honjozo*" ($n = 2$), "*Special Junmai*" ($n = 9$), "*Honjozo*" ($n = 15$), "*Junmai*" ($n = 35$), and "*Futuu*" ($n = 6$). The differences between these specific classes of sake have been described previously (Gogami et al. 2011a). DL-Alanine and L-alanine (food grade) were obtained from Kyowa Hakko Bio Co. Ltd. (Tokyo, Japan).

Measurement of D-amino acid concentrations

We measured the concentrations of D- and L-amino acids in the sakes using (HPLC) with a protocol that combined two

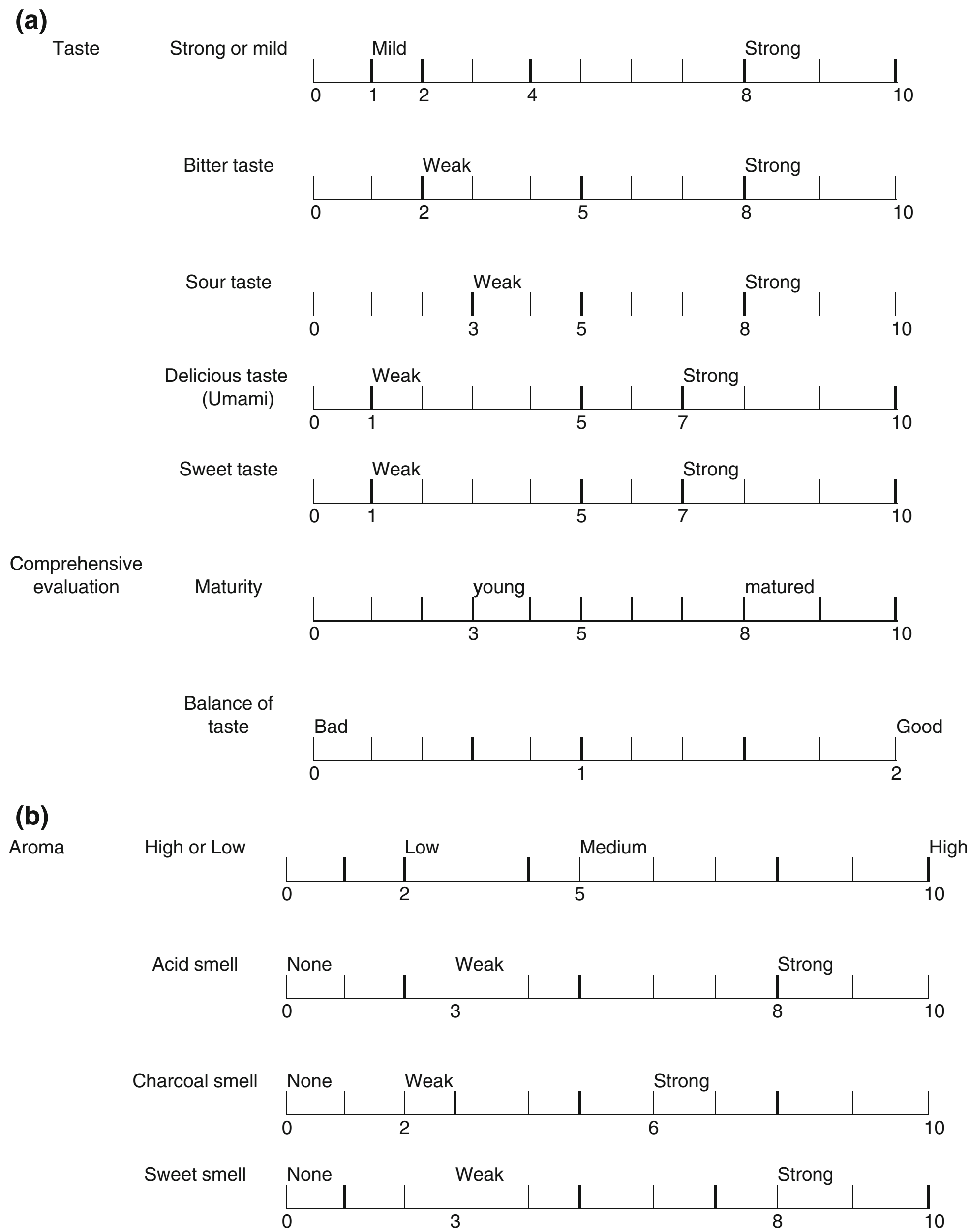

Fig. 1 The sensory evaluation attributes and points: **a** taste and **b** aroma

Table 1 Principal component analysis of sensory evaluation of 141 sakes

	Component						
	1	2	3	4	5	6	7
Strong or mild	0.844*	−0.096	0.039	0.329	−0.186	−0.114	0.348
Bitter taste	0.619	−0.194	−0.299**	−0.212	0.664	0.050	0.046
Sour taste	0.748	−0.408**	0.138	0.273	−0.134	0.301	−0.267
Delicious taste	0.644	0.629	0.039	0.045	0.025	−0.365	−0.230
Sweet taste	0.440	0.612	0.253	−0.498	−0.151	0.297	0.090
Maturity	–0.165	0.000	0.909*	0.167	0.342	−0.022	0.034
Balance of taste	–0.243**	0.690*	−0.257	0.549	0.192	0.245	0.033
Explained variance	33.6	20.8	15.3	11.5	9.6	5.5	3.7
Cumulative variance	33.6	54.4	69.7	81.2	90.8	96.3	100

*Most positively correlated attribute; **most negatively correlated attribute

Table 2 Principal component analysis of sensory evaluation of 141 sakes (after 3 months of storage at room temperature)

	Component						
	1	2	3	4	5	6	7
Strong or mild	0.853*	−0.110	0.388*	0.044	−0.064	−0.035	0.320
Bitter taste	0.586	−0.270**	−0.582**	0.119	0.410	0.246	0.051
Sour taste	0.807	−0.264	0.399	0.041	−0.018	0.175	−0.296
Delicious taste	0.640	0.513	−0.196	0.226	0.089	−0.471	−0.093
Sweet taste	0.306	0.724*	−0.243	0.110	−0.468	0.304	0.011
Maturity	0.190	0.605	0.111	−0.702	0.298	0.066	−0.002
Balance of taste	–0.366**	0.547	0.366	0.505	0.387	0.166	0.021
Explained variance	34.2	23.0	12.7	11.8	9.1	6.3	2.9
Cumulative variance	34.2	57.2	69.9	81.7	90.9	97.1	100

*Most positively correlated attribute; **most negatively correlated attribute

pre-column derivatization methods of amino acid enantiomer detection, OPA-NAC (*o*-phthalaldehyde and *N*-acetyl-L-cysteine) and FLEC/ADAM [(+)-1-(9-fluorenyl) ethyl chloroformate/1-aminoadamantane], and one post-column derivatization method using OPA-NAC (Gogami et al. 2011a).

Sensory evaluation

The sensory evaluation was performed by one of the counselors of the sake sensory evaluation board of the Osaka Regional Taxation Bureau, Osaka, Japan (age 39; sex male). All the sakes were maintained at room temperature (approximately 25 °C) for at least 24 h prior to the analysis. The sensory evaluation of the sakes was conducted at room temperature as follows: (a) sake was poured into about 70 % (v/v) of a tasting cup (about 100 ml) and a cup is slightly shaken to note the aroma rising from the sake; (b) about 5–10 ml of sake was taken into mouth and then turned over the tongue by sucking for noting characteristic taste and taste intensity; (c) the aftertaste or cleanness was noted after the sake was spat out; (d) these procedures were repeated at least three times until the sensory evaluation attributes and points in Fig. 1a, b were determined completely; (e) about 10 sake samples were evaluated per day and next sensory evaluation was done after about one week. Taste was evaluated with regard to five attributes: strong or mild, bitter, sour, delicious and sweet. The total evaluation was performed with regard to two attributes: maturity and balance of taste. The evaluations of strong or mild, bitter, sour, delicious, sweet and maturity were scored using a 10-point scale from a low intensity (1) to a high intensity (10) for each attribute. In the case of the balance of taste, the evaluation was performed using a 3-point scale from a low intensity (0) to a high intensity (2). The sensory evaluation of all the sakes was conducted again after 3 months of storage at room temperature (approximately 25 °C) under the same conditions described above.

Data analysis

The sensory evaluation data were analyzed using IBM SPSS (Statistical Package for Social Science, version 19 for Windows, SPSS Inc., Tokyo, Japan). The relationship between the D-amino acid concentrations and the taste of the sake was modeled using principal component analysis (PCA), which maximized the correlation between the

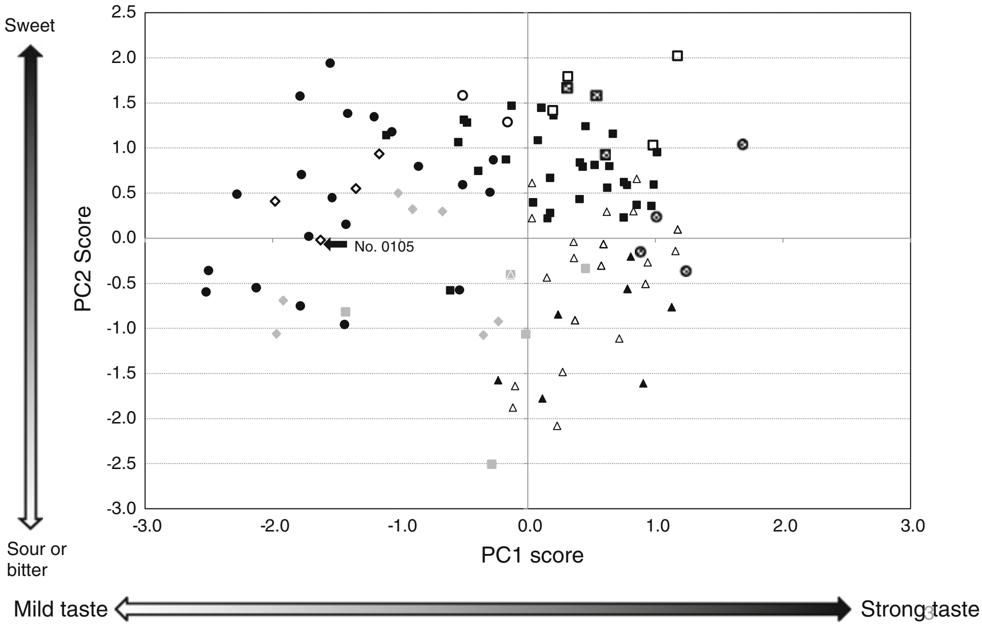

Fig. 2 The first two PC scores plotted against each other in the principal component analysis of the sensory evaluation of 141 sakes. *Filled circle* good balance and mild taste, *open circle* good balance, mild and sweet taste, *filled square* good balance and strong taste, *open square* good balance, strong and sweet taste, *filled gray diamond* mild taste, *open diamond* mild and sweet taste, *open triangle* strong taste, *checked square* strong and sweet taste, *filled gray square* bad balance and mild taste, *filled gray triangle* bad balance, mild and sweet taste, *filled triangle* bad balance and strong taste, *checked square* bad balance, strong and sweet taste

original variables to form new variables that are mutually orthogonal. PCA is a special type of factor analysis that transforms the original set of independent, uncorrelated variables into a new set of an equal number of independent, uncorrelated variables or principal components (PCs) that are linear combinations of the original variables.

Effects of the addition of DL-Ala or L-Ala on the taste and aroma of sake

To determine the effect of D-Ala on the taste of sake, we added food-grade DL-Ala or L-Ala to selected sakes and compared their tastes using sensory evaluation. The final

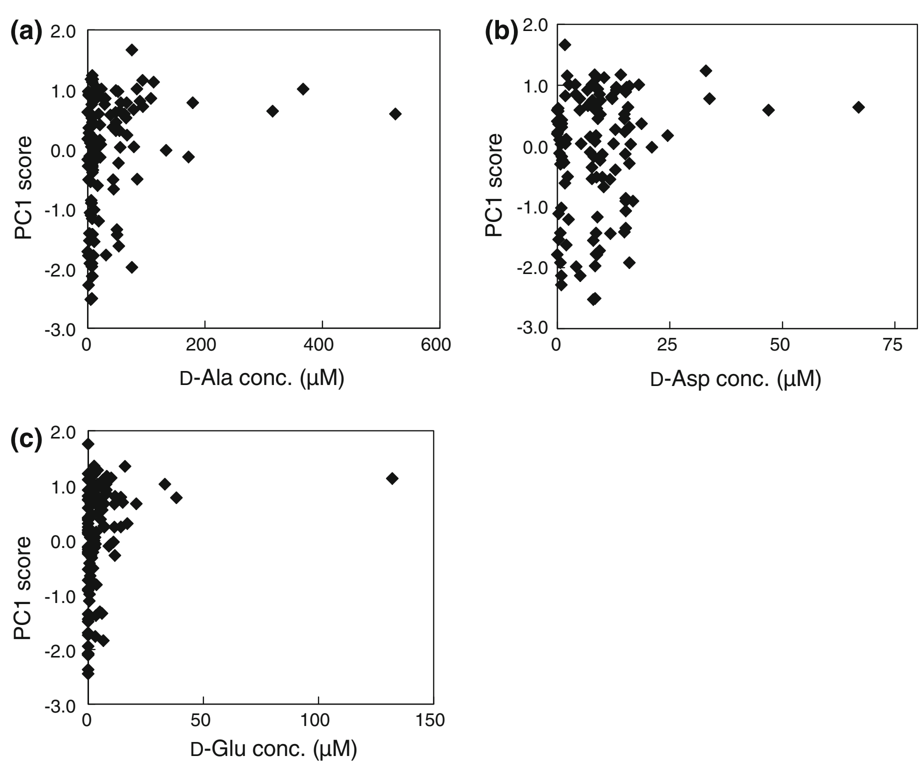

Fig. 3 The effects of the D-Ala, D-Asp, and D-Glu concentrations in 141 sakes on the PC1 score. **a** D-Ala, **b** D-Asp and **c** D-Glu

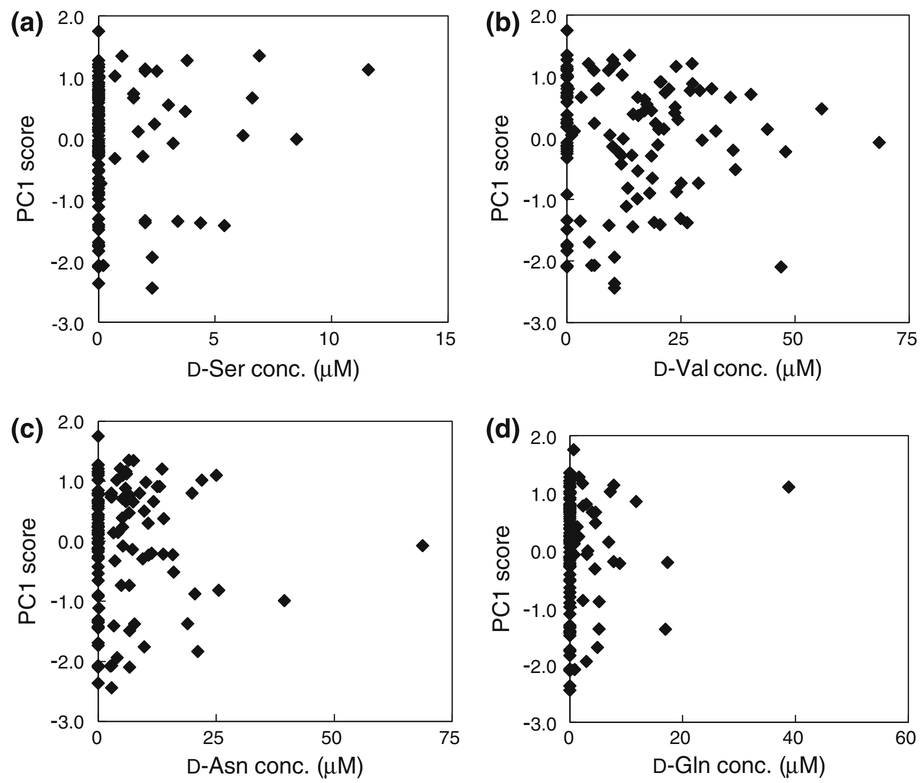

Fig. 4 The effects of the D-Ser, D-Val, D-Asn, and D-Gln concentrations in 141 sakes on the PC1 score. **a** D-Ser, **b** D-Val, **c** D-Asn and **d** D-Gln

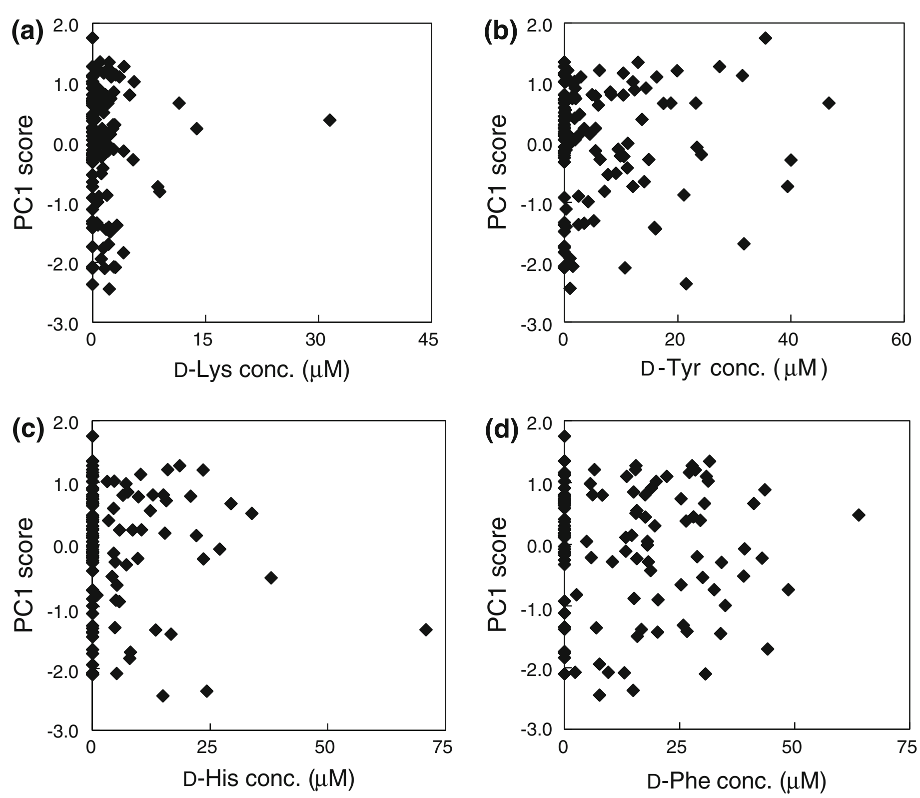

Fig. 5 The effects of the D-Lys, D-Tyr, D-His, and D-Phe concentrations in 141 sakes on the PC1 score. **a** D-Lys, **b** D-Tyr, **c** D-His and **d** D-Phe

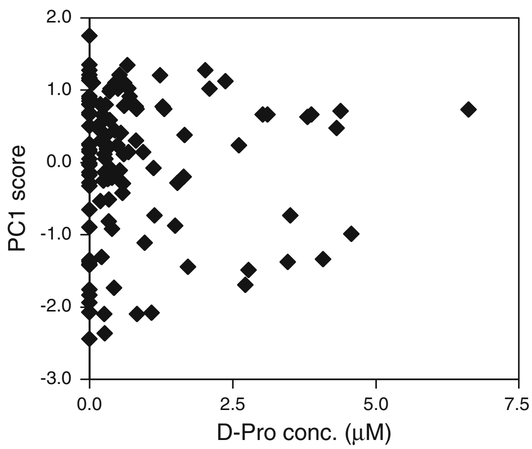

Fig. 6 The effects of the D-Pro concentration in 141 sakes on the PC1 score

concentrations of DL-Ala were 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0 and 10 mM. L-Ala was also added independently to final concentrations of 0.005, 0.01, 0.025, 0.05, 0.1, 0.25, 0.5, 1.0, 2.5 and 5.0. Accordingly, the highest concentration of D-Ala was to be 5 mM (about 10 times higher concentration of D-Ala in the sake we have already measured), to examine the effect of high concentration of D-Ala on the taste and aroma of sake. The sakes used in this experiment [Nos. 4003 (Table 3), 4504 (Table 4), 2402 (Table 5)] were chosen based on the results of our D-amino acid analysis and sensory evaluation analysis; these sakes contained low D-Ala concentrations and had sensory evaluation scores for delicious that were low.

Results and discussion

Principal component analysis

The 141 bottles of sake were arranged in rows of the input matrix. The variables (columns) were standardized to a zero mean and a unit variance, i.e. the column means were subtracted from each matrix entry and the entry was then divided by the standard deviation of the columns. The principal component analysis (PCA) yielded seven principal components that explained 100 % of the total variance in the data. The loading values +0.844 and −0.243 (PC1), +0.690 and −0.409 (PC2), and +0.909 and −0.299 (PC3) are asterisked in Table 1 and express how well the new PCs correlate with the old variables. The first PC, which explains 33.6 % of the total variance, correlates most positively with a strong taste and most negatively with a balance of tastes. The second PC, which explains 54.4 % of the total variance, correlates most positively with a sweet taste and most negatively with a bitter and sour taste. The third PC loaded heavily for maturity and a bitter taste, explaining 69.7 % of the variation. None of the variables

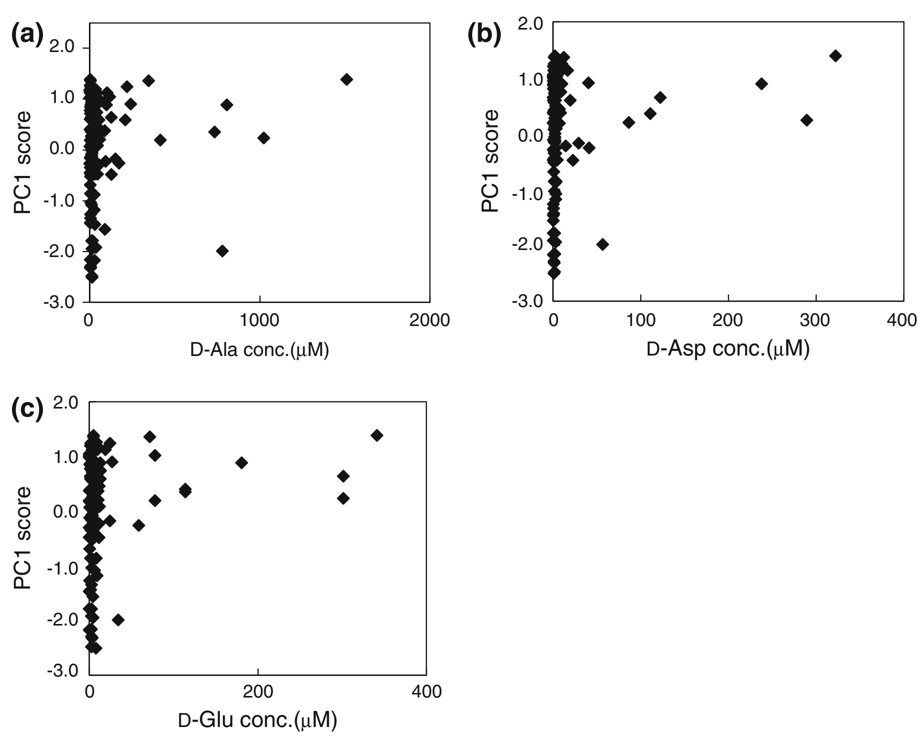

Fig. 7 The effects of the D-Ala, D-Asp, and D-Glu concentrations in sake on the PC1 score after 3 months of storage at room temperature

Table 3 Effects of addition of DL-Ala or L-Ala on taste and aroma of sake No.4003

Ala concentration (μM)	Taste					Comprehensive evaluation		Aroma			
	Strong or mild	Bitter	Sour	Delicious (Umami)	Sweet	Maturation	Balance of taste	High or Low	Acid	Charcoal	Sweet
Blank											
0	4.0	5.0	5.0	1.0	5.0	5.0	1.0	5.0	0	2.0	0
L-Ala											
5	4.0	6.0	5.0	1.0	3.7	5.0	1.0	5.0	0	2.0	0
10	7.3	6.0	5.0	3.0	5.0	5.0	1.0	5.0	0	2.0	0
25	7.7	6.0	6.0	3.0	5.0	5.0	1.0	5.0	0	2.0	0
50	7.7	7.0	7.0	5.7	3.7	5.0	0.7	5.0	0	2.0	0
100	7.3	8.0	7.0	6.3	3.7	5.0	0.7	5.0	0	2.0	0
250	7.3	8.0	7.0	6.3	3.7	5.0	0.7	5.0	0	2.0	0
500	9.3	8.0	8.0	6.3	2.3	5.0	0.7	5.0	0	2.0	0
1000	9.0	8.0	8.0	7.0	2.3	5.0	0.3	5.0	0	2.0	0
2500	9.0	8.0	8.0	7.0	2.3	5.0	0.3	5.0	0	2.0	0
5000	9.0	8.0	8.0	7.0	2.3	5.0	0.3	5.0	0	2.0	0
DL-Ala											
10	5.3	6.0	6.0	4.3	3.7	5.0	1.0	5.0	0	2.0	0
20	7.0	6.0	6.0	6.3	5.0	5.0	1.0	5.0	0	2.0	0
50	7.0	7.0	6.0	6.3	5.0	5.0	0.7	5.0	0	2.0	0
100	9.3	7.0	8.0	6.3	5.0	5.0	0.7	5.0	0	2.0	0
200	9.3	7.0	8.0	6.3	2.3	5.0	0.3	5.0	0	2.0	0
500	9.3	7.0	8.0	6.3	3.7	5.0	0.7	5.0	0	2.0	0
1000	9.0	8.0	8.0	6.3	2.3	5.0	0.3	5.0	0	2.0	0
2000	9.0	8.0	8.0	7.0	3.7	5.0	0.3	5.0	0	2.0	0
5000	9.0	8.0	8.0	7.0	2.3	5.0	0.3	5.0	0	2.0	0
10000	9.0	8.0	8.0	7.0	3.7	5.0	0.0	5.0	0	2.0	0

was decisive for the remaining principal components (PC4–7). If we consider the high correlations (loading values of +0.844 and −0.243 for PC1, +0.690 and −0.409 for PC2, and +0.909 and −0.299 for PC3), two PCs are retained. The seven PCs are provided in Table 1. The PCA of the 141 bottles of sakes, after 3 months storage at room temperature was conducted as described above (Table 2).

Figure 2 shows PC1 score versus PC2 score plot for the sakes. The sakes produced by different sake-making technologies were well separated in this plot. The points for the sakes brewed with "*Kimoto yeast starter*" and "*Yamahaimoto*" were ranked above those for the sakes brewed with "*Sokujo-moto*". The point for one sake (No. 0105) brewed with "*Kimoto yeast starter*" ("*Honjozo*" specific class) was situated to the left side of the majority of points for those brewed with "*Kimoto yeast starter*" and "*Yamahaimoto*", specifically, "*Junmai Daiginjo*", "*Junmai Ginjo*", "*Special Honjozo*", "*Special Junmai*", and "*Junmai*". These results indicate that the sakes brewed with "*Kimoto yeast starter*" and "*Yamahaimoto*" have high scores for PC1 and PC2 and showed strong tastes in comparison with those brewed with "*Sokujo-moto*". In addition, the specific class of sake might be an important factor determining the taste of the sake among sakes that are produced using the same brewing method.

Figure 3 shows the effects of the D-Ala, D-Asp, and D-Glu concentrations in the 141 bottles of sake on the PC1 score. When below 50 μM, the concentration of D-Ala did not affect the score of PC1, whereas all of the sakes showed a high PC1 score at concentrations above 100 μM (Fig. 3a). Similar observations were found for the D-Asp (Fig. 3b) and D-Glu (Fig. 3c) concentrations with regard to PC1, with threshold concentrations for D-Asp and D-Glu were 33.8 and 33.3 μM, respectively, for an effect on the taste. Interestingly, other D-amino acids, such as D-Ser, D-Val, D-Asn, D-Gln, D-Arg, D-Lys, D-Tyr, D-His and D-Phe, did not show any correlation with the PC1 score (Figs. 4, 5, 6).

Table 4 Effects of addition of DL-Ala or L-Ala on taste and aroma of sake No. 4504

Ala concentration (μM)	Taste					Comprehensive evaluation		Aroma			
	Strong or mild	Bitter	Sour	Delicious (Umami)	Sweet	Maturation	Balance of taste	High or Low	Acid	Charcoal	Sweet
Blank											
0	4.0	5.0	5.0	5.0	5.0	5.0	2.0	5.0	0	2.0	0
L-Ala											
5	4.0	5.0	5.0	5.0	5.0	5.0	2.0	5.0	0	2.0	0
10	4.0	5.0	5.0	5.0	5.0	5.0	2.0	5.0	0	2.0	0
25	4.0	6.0	5.0	5.0	5.0	5.0	2.0	5.0	0	2.0	0
50	7.3	6.0	5.0	7.0	5.0	5.0	1.7	5.0	0	2.0	0
100	7.7	7.0	6.0	7.0	5.0	5.0	1.3	5.0	0	2.0	0
250	9.0	8.0	8.0	7.0	5.0	5.0	1.0	5.0	0	2.0	0
500	9.0	8.0	8.0	7.0	5.0	5.0	0.7	5.0	0	2.0	0
1000	9.0	8.0	8.0	7.0	5.0	5.0	0.3	5.0	0	2.0	0
2500	9.0	8.0	8.0	7.0	5.0	5.0	0.3	5.0	0	2.0	0
5000	9.0	8.0	8.0	8.0	5.0	5.0	0.0	5.0	0	2.0	0
DL-Ala											
10	4.0	5.0	5.0	5.0	5.0	5.0	2.0	5.0	0	2.0	0
20	4.0	5.0	5.0	5.0	5.0	5.0	2.0	5.0	0	2.0	0
50	5.3	6.0	5.0	5.7	5.0	5.0	1.7	5.0	0	2.0	0
100	7.0	7.0	7.0	6.3	5.0	5.0	0.7	5.0	0	2.0	0
200	9.0	7.0	7.0	6.3	5.0	5.0	0.7	5.0	0	2.0	0
500	9.0	7.0	8.0	7.0	5.0	5.0	0.7	5.0	0	2.0	0
1000	9.0	8.0	8.0	7.0	5.0	5.0	0.0	5.0	0	2.0	0
2000	9.0	8.0	8.0	7.0	3.7	5.0	0.0	5.0	0	2.0	0
5000	9.0	8.0	8.0	7.0	3.7	5.0	0.0	5.0	0	2.0	0
10000	9.0	8.0	8.0	7.0	1.0	5.0	0.0	5.0	0	2.0	0

Effects of storage on the D-amino acid concentrations and the taste of sake

Figure 7 shows the effects of the D-Ala, D-Asp and D-Glu concentrations in sake on the PC1 score after 3 months of storage at room temperature. The D-Ala, D-Asp and D-Glu concentrations increased markedly, with the concentrations ranging from 524.3, 66.9 and 132.0 μM to 1510.4, 322 and 341.1 μM for D-Ala, D-Asp and D-Glu, respectively. We previously reported that D-Ala, D-Asp and D-Glu were produced by lactic acid bacteria, such as *Lactobacillus sakei* NBRC 15893 and *Leuconostoc mesenteroides* NBRC 102480 isolated from "*Kimoto yeast starter*" (Gogami et al. 2011b). These results suggested that some bacteria, especially lactic acid bacteria, in sake produced D-Ala, D-Asp and D-Glu during storage and that these D-amino acids increased the PC1 score, conferred a strong the taste (*Nojun*).

Effects of the addition of DL-Ala or L-Ala on the taste and aroma of sake

Based on the results of the PCA of the D-amino acid analysis and the sensory evaluation of 141 bottles of sake, we found that D-Ala, D-Asp and D-Glu influenced the taste at high concentrations. To clarify the effects of these D-amino acids, we added these D-amino acids to select sakes and re-performed the sensory evaluation; however, the Japanese Ministry of Health, Labor and Welfare only permits the addition of DL-Ala to food. Thus, we chose three sakes (Nos. 2402, 4003 and 4504) that had low D-Ala concentrations and scores for the delicious attribute.

Table 3 shows the effects of the addition of DL-Ala or L-Ala on the taste and aroma of sake No. 4003. For all three sakes, the value for bitter taste gradually increased, depending on the increasing L-Ala concentration, after the addition of L-Ala. In the case of DL-Ala in the presence of

Table 5 Effects of addition of DL-Ala or L-Ala on taste and aroma of sake No. 2402

Ala concentration (μM)	Taste					Comprehensive evaluation		Aroma			
	Strong or mild	Bitter	Sour	Delicious (Umami)	Sweet	Maturation	Balance of taste	High or Low	Acid	Charcoal	Sweet
Blank											
0	4.0	4.0	5.0	2.3	5.0	5.0	2.0	10.0	0	2.0	0
L-Ala											
5	4.0	4.0	5.0	2.3	5.0	5.0	2.0	10.0	0	2.0	0
10	4.0	6.0	5.0	2.3	5.0	5.0	1.7	10.0	0	2.0	0
25	6.0	8.0	6.0	3.7	3.7	5.0	1.0	10.0	0	2.0	0
50	8.0	8.0	6.0	5.0	3.7	5.0	1.0	10.0	0	2.0	0
100	9.3	8.0	7.0	5.7	3.7	5.0	0.7	10.0	0	2.0	0
250	9.7	8.0	8.0	6.7	3.7	5.0	0.0	10.0	0	2.0	0
500	9.7	8.0	8.0	6.7	3.7	5.0	0.0	10.0	0	2.0	0
1000	9.7	8.0	8.0	6.7	3.7	5.0	0.0	10.0	0	2.0	0
2500	9.7	8.0	8.0	6.7	3.7	5.0	0.0	10.0	0	2.0	0
5000	9.7	8.0	8.0	6.7	3.7	5.0	0.0	10.0	0	2.0	0
DL-Ala											
10	6.0	5.0	6.0	3.7	3.7	5.0	1.7	10.0	0	2.0	0
20	8.0	5.0	7.0	3.7	3.7	5.0	1.3	10.0	0	2.0	0
50	10.0	5.0	8.0	5.0	3.7	5.0	1.0	10.0	0	2.0	0
100	10.0	7.0	8.0	5.7	3.7	5.0	0.7	10.0	0	2.0	0
200	9.7	8.0	8.0	5.7	3.7	5.0	0.0	10.0	0	2.0	0
500	9.7	8.0	8.0	5.7	3.7	5.0	0.0	10.0	0	2.0	0
1000	9.7	8.0	8.0	5.7	3.7	5.0	0.0	10.0	0	2.0	0
2000	9.7	8.0	8.0	5.7	3.7	5.0	0.0	10.0	0	2.0	0
5000	9.7	8.0	8.0	5.7	3.7	5.0	0.0	10.0	0	2.0	0
10000	9.0	8.0	8.0	6.3	3.7	6.0	0.0	10.0	0	2.0	0

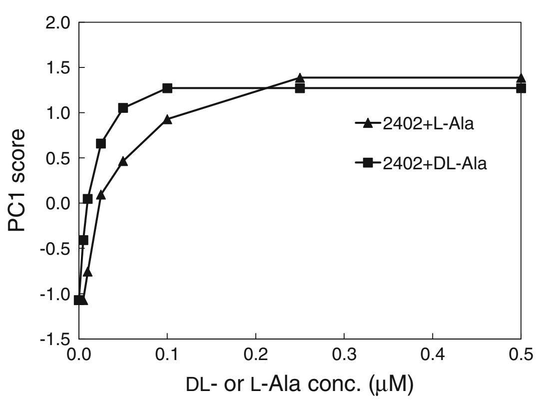

Fig. 8 The effects of the DL-Ala, and L-Ala concentrations added to sake No. 2402 on the PC1 score

the same concentration of L-Ala, the value for bitter taste is lower. For example, the value for bitter taste was 8.0 at 100 and 250 μM L-Ala in No. 4003, whereas the value was 7.0 at 200 and 500 μM DL-Ala. The same D-Ala effect was observed at 50 μM L-Ala and 100 μM DL-Ala in No. 4504 (Table 4), and from 10 μM to 50 μM L-Ala and 20 μM to 100 μM DL-Ala in No. 2402 (Table 5). The value for umami taste in the sensory evaluation was also affected when D-Ala was added to the three sakes (Tables 3, 4, 5). For example, the values of umami taste were 1.0, 3.0, 3.0 and 5.7 at 5, 10, 25 and 50 μM L-Ala, respectively, in No. 4003, whereas the values were 4.3, 6.3, 6.3 and 6.3 at 5, 10, 25 and 50 μM DL-Ala, respectively (Table 3). Similar effects were also observed for No. 4504 (Table 4) and No. 2402 (Table 5). These results suggested that, when added to the sakes, both D- and L-Ala increased the value for umami taste in the sensory evaluation, with the effect of D-Ala being much stronger than that of L-Ala. The addition of 50–5,000 μM DL-Ala did not effect on the aroma of three sakes at all (Tables 3, 4, 5). Figure 8 shows the effects of adding different concentrations of DL-Ala and L-Ala on the PC1 score of sake No. 2402. The PCA of various concentrations of DL-Ala and L-Ala added to No. 2402 and the sensory evaluations obtained for the 141 bottles of sake revealed that DL-Ala increased the PC1 score (taste and total balance of sake) more than L-Ala; however, when the concentration exceeded approximately

0.2 μM, the PC1 score after the addition of DL-Ala was lower than that after the addition of L-Ala addition. Accordingly, the addition of 5–200 μM DL-Ala effectively increased the taste and total balance, whereas the excessive addition of DL-Ala (more than 200 μM) decreased these parameters. The quantitative analysis of L-amino acids, organic acids and alcohols in sake has been performed using gas chromatography−mass spectrometry (GC–MS) and HPLC, and the relationship between the concentrations of these ingredients and the taste of sake has been studied using principal component analysis (Iwano et al. 2004, 2005). L-Ala, L-Arg, L-Glu, and L-Asp were considered to have a slight effect on the taste of sake (Iwano 2006), but the authors did not consider the fact that their samples contained both amino acid enantiomers, and accordingly, their data should perhaps be re-evaluated. In the present study, we clarified the relationship between the D-amino acid concentration and the taste of sake. To our knowledge, this is first study concerning the effects of D-amino acids on the taste of food. D-Asp in food was reported to have a skin enhancement effect (http://www.shiseido.co.jp/corp/ir/report/s1009jig/strategy04.html), but other physiologic functions of D-amino acids in food remain unknown. We are currently studying potential effects using various biologic systems.

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Conflict of interest The authors declare that they have no conflict of interest.

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