

Prediction of the bitterness of single, binary- and multiple-component amino acid solutions using a taste sensor

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Abstract

The purpose of this study was to develop a quick, quantitative, prediction method for the determination of the bitterness of solutions containing one or more of five amino acids (L-isoleucine, L-leucine, L-valine, L-phenylalanine, and L-tryptophan), using an artificial taste sensor. The bitterness of various solutions containing different concentrations (1, 3, 10, 30, and 100 mM) of five amino acids, singly and in combination, was estimated using a multichannel taste sensor and compared with the results of human gustatory sensation tests with nine volunteers. The relative response electric potential patterns were similar for all five amino acids. Large sensor outputs were observed in channels 1–4 (which are negatively charged) while there were no responses in channels 5–8 (positively charged). The sensor output for channel 1, which was the largest output value, was used for prediction of bitterness. The change of membrane potential caused by adsorption (CPA), which corresponds to aftertaste, could not be used as an explanatory variable since the adsorption of the amino acids to the sensor membrane was weak and CPA values were small. The bitterness intensity scores for single, binary, and multi-component amino acid solutions, could be easily predicted on the basis of the sensor output value of channel 1 using regression analysis. Principal component analysis of the sensor output data suggested that the sourness, astringency and/or smell of the solutions also played a role in the perception of bitterness.

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1. Introduction

The sense of taste provides animals with valuable information about the nature and quality of food. Mammals can recognize and respond to a wide range of chemical entities, including sugars, salts, acids, and toxic substances (Lindemann,

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1996). Almost all animals recognize and refuse to eat bitter-tasting substances.

Some amino acids taste sweet and other amino acids taste delicious (umami in Japanese) to humans, but many amino acids, such as tryptophan and isoleucine, taste extremely bitter, especially when present in highly concentrated solutions. Commercially available elemental diets contain high concentrations of branched-chained amino acids such as isoleucine, leucine, and valine, as well as aromatic amino acids such as tryptophan and phenylalanine. Patients suffering from hepatic diseases must sometimes take these elemental diets for a long period, and their bitterness is not only unpleasant but also reduces compliance. In the present study, therefore, we focused on bitterness of the above five amino acids.

A taste sensor, an 'electric tongue' with global selectivity, has been developed by Toko. It comprises several kinds of lipid/polymer membrane which are able to transform information about substances producing taste into electrical signals (Hayashi et al., 1990; Iiyama et al., 1996; Fukunaga et al., 1996; Toko, 1998; Takagi et al., 2001). The sensor output has been shown to produce similar patterns for groups of chemical substances with similar tastes. Thus, the taste of various foodstuffs can be expressed quantitatively using the sensor.

In our studies using the taste sensor, we have used quinine as the standard for bitterness. We have evaluated the bitterness of various medicines and suggested that the sensor could be used to obtain quantitative predictive data on the bitterness of commercial medicines (Uchida et al., 2001). In the present study, our goal was to see whether the sensor could be used to predict the bitterness of solutions containing one or more amino acids. In a previous report (Kikkawa et al., 1993), the sensor was used to characterize several amino acids with respect to taste (sourness, saltiness, sweetness, bitterness, and umami), and to examine differences in the taste patterns produced by these amino acids. However, that study did not include the branched-chain amino acids which are included in elemental diets. In the present study therefore, we used the taste sensor to evaluate the bitterness of various single, binary, and multiple-component

amino acid solutions, incorporating the amino acids commonly included in elemental diet formulations. We describe below a quick method for predicting the bitterness of multiple-component amino acid solutions using the taste sensor.

2. Method

2.1. Materials

Five amino acids, i.e. L-isoleucine (Ile), L-leucine (Leu), L-valine (Val), L-phenylalanine (Phe), L-tryptophan (Trp) were purchased from Nacalai Tesque Co. (Kyoto, Japan). They were dissolved and diluted to form 1, 3, 10, 30 and 100 mM solutions with 10 mM KCl. Quinine hydrochloride was purchased from Sigma Chemical Co. (St. Louis, MO, USA), dissolved, and diluted to produce a 0.10 mM solution with 10 mM KCl. All other reagents were of special reagent grade.

2.2. Sensor measurement and data analysis

The taste-sensing system SA402 of Intelligent Sensor Technology Co., Ltd., Atsugi, Japan, was used to measure the electric potential of various concentrations of amino acid solutions as shown in Fig. 1. The electrode set was attached to a mechanically controlled robot arm. The detecting sensor part of the equipment consists of eight electrodes composed of lipid/polymer membranes. The lipid components of the sensor used in the present study are the same as those described in a previous paper (Uchida et al., 2000). Each lipid was mixed in a test tube containing poly(vinylchloride) and dioctylphenylphosphonate as a plasticizer, dissolved in tetrahydrofuran, and dried on a glass plate at 30 °C to form a transparent thin film, almost 200 µm thick. The electrodes consisted of an Ag wire whose surface was plated with Ag/AgCl, with an internal cavity filled with 3 M KCl solution. The difference between the electric potential of the working electrode and the reference electrode was measured by means of a high-input impedance amplifier connected to a computer.

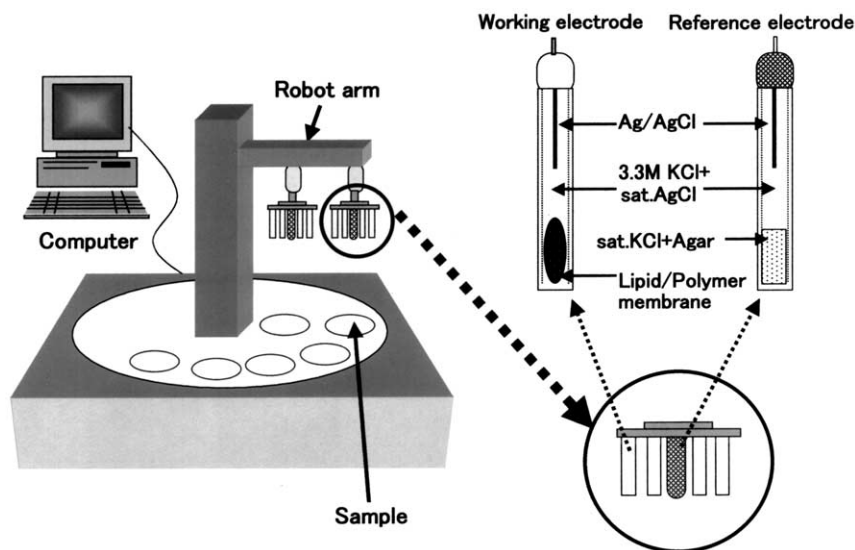


Fig. 1. The Multichannel Taste-sensing System (SA402) used in the present study.

Samples consisting of various concentrations of amino acids in 10 mM KCl solution were used in the study. Fresh 30 mM KCl solution containing 0.3 mM tartaric acid (corresponding to saliva) was used as the reference sample (V_r) and also to rinse the electrodes after every measurement. The method used to measure the sensitivity and the selectivity of adsorption of the samples is summarized in Plate 1. The electrode is first dipped into the reference solution (V_r) and then into the sample solution (V_s). The relative sensor output is represented as the difference ($V_s - V_r$) between the potentials of the sample and the reference solution. When the electrode is dipped into the reference solution again, the new potential of the reference solution is defined as V_r' . The difference ($V_r' - V_r$) between the potentials of the reference solution before and after sample measurement is defined as CPA (change of membrane potential caused by adsorption) and corresponds to after-taste. Each measuring time was set 30 s, and the electrodes were rinsed after each measurement. S-PLUS 2000J (Mathematical Systems, Inc., Tokyo, Japan) was used for regression analysis. In the present study, only the relative sensor output in channel 1 was used to predict bitterness, since the adsorption of the amino acids to the membrane

surface was expected to be weak and CPA values were expected to be small.

Finally, relative sensor output values and CPA values were used to predict the bitterness and aftertaste of single, binary, and multi-component solutions with measurable adsorption (i.e. quinine solutions, and binary solutions consisting of quinine and an amino acid).

2.3. Gustatory sensation test

The gustatory sensation tests were performed with human volunteers according to a previously described method (Indow, 1966; Katsuragi et al., 1997). The standard quinine hydrochloride concentrations used were 0.01, 0.03, 0.10, 0.30 and 1.00 mM and the corresponding bitterness score were defined as 0, 1, 2, 3 and 4, respectively. Before testing, the volunteers ($n = 9$) were asked to keep the above standard quinine solutions in their mouths, and were told the concentrations and bitterness scores for each solution. After tasting a 0.3 mM sample of test drug solution, they were asked to give the sample a bitterness score. All samples were kept in the mouth for 15 s. After tasting the sample, subjects gargled well and waited for at least 20 min before tasting the next sample.

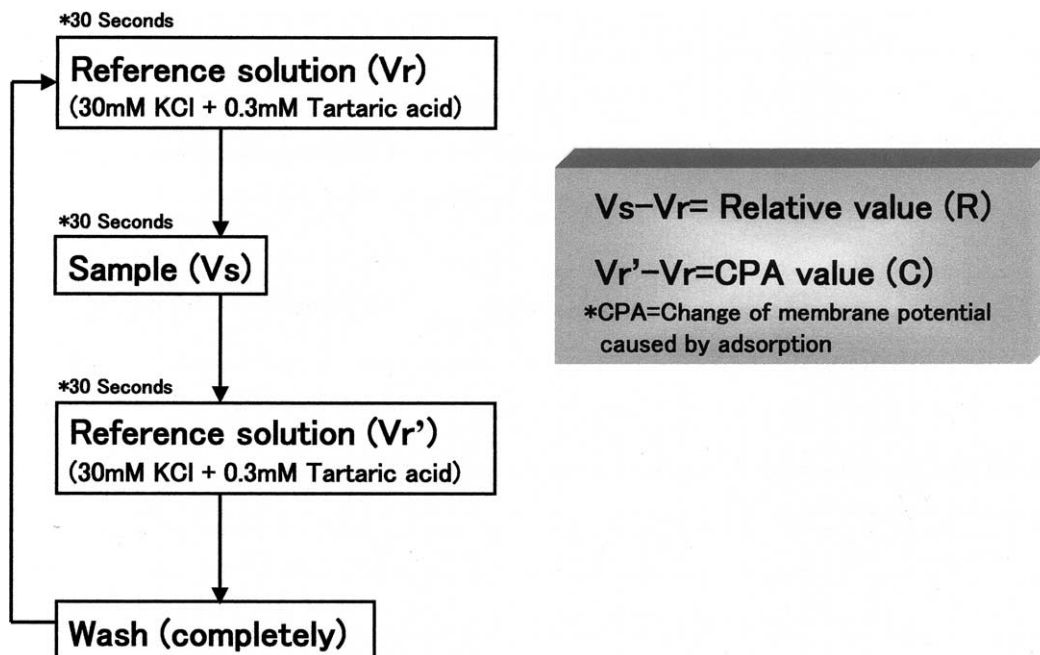


Plate 1. Measuring procedure in this study.

3. Results and discussion

3.1. Bitterness prediction for single-component amino acid solutions

The relationship between the sample concentration and bitterness score as evaluated by gustatory sensation tests for five single-component amino

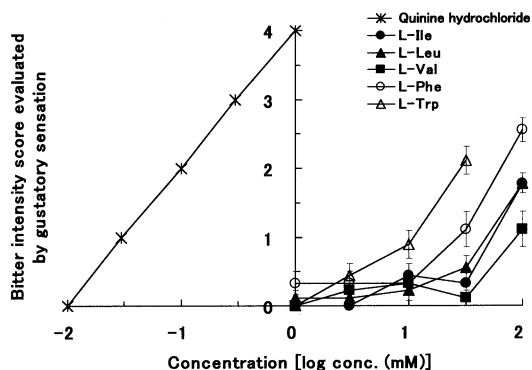


Fig. 2. The relation between the concentration of the sample solution and the bitterness scores obtained in human gustatory sensation tests for solutions of five different amino acids and quinine hydrochloride (standard) (error bar represents S.E.).

acid solutions is shown in Fig. 2. No measurements could be made at 100 mM Trp due to solubility problems. As shown in Fig. 2, highly concentrated amino acid solutions show almost the same levels of bitterness as quinine hydrochloride. The aromatic amino acids Phe and Trp were more bitter than the branched-chain amino acids, Ile, Leu, and Val. This supports the earlier findings of Wieser and Belits (1975). The greater bitterness of aromatic amino acids such as Trp, and Phe, seems to be due to the functional aromatic group. Kurihara et al. (1994), in a review of the receptor mechanisms of bitter substances, noted that bitter alkaloids such as quinine or strychnine carry a comparatively large positive charge inside the molecule which makes the molecule hydrophobic and thus easily bound to the receptor site. Aromatic amino acids such as Trp and Phe also have a hydrophobic structure inside the molecule. Therefore, from the standpoint of partition of these drugs to the surface of the tongue, it is likely that they will be more bitter than the branched-chain amino acids.

Fig. 3a and b show the response electric potential patterns of relative output value and

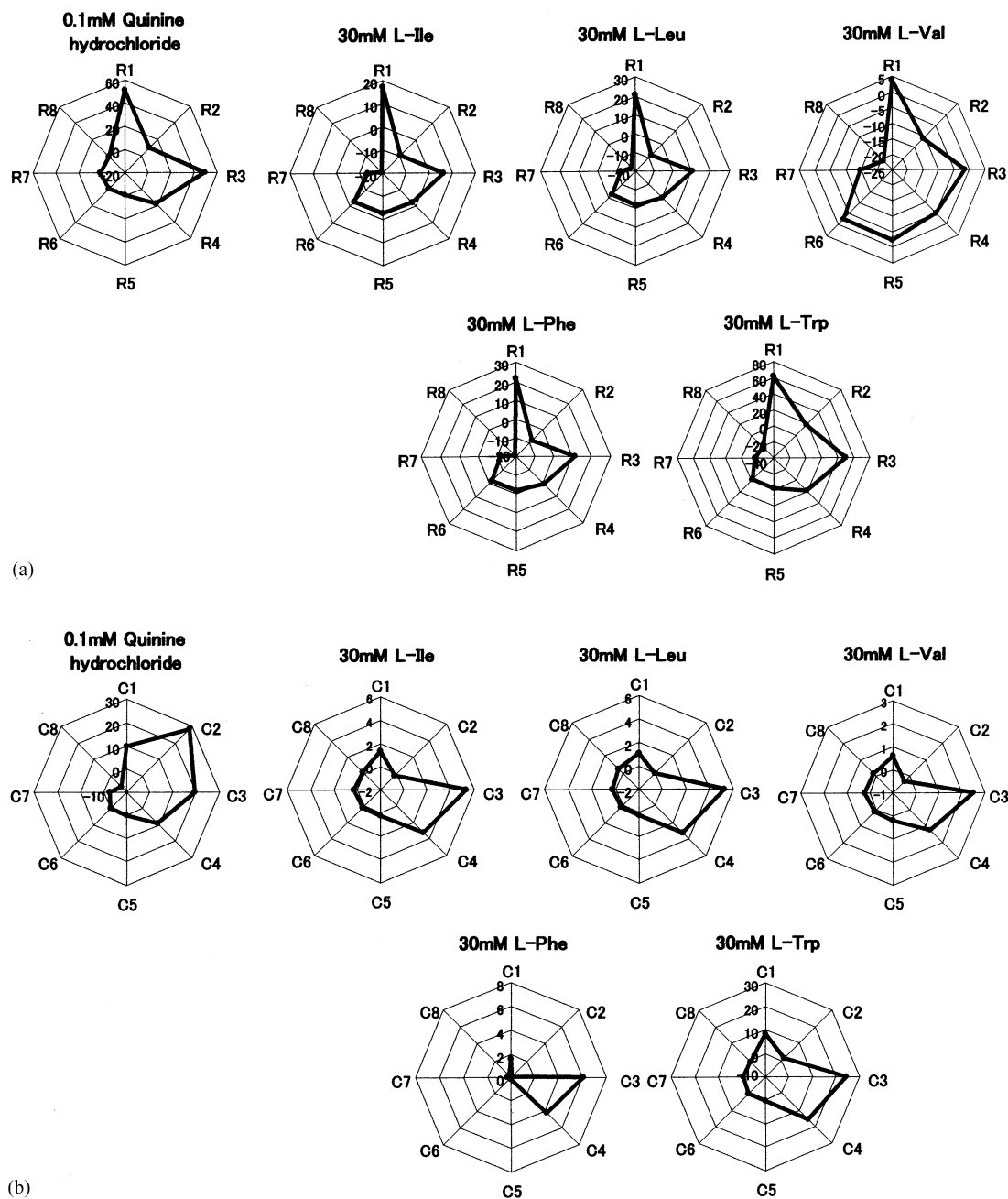


Fig. 3. Sensor response output electric potential patterns for amino acids and quinine hydrochloride. (a) Relative value; (b) CPA value. For detailed explanation of CPA, see text.

CPA value, respectively. The CPA, which is defined as the change of membrane potential caused by adsorption, corresponds to the after-

taste. As shown in Fig. 3b, 0.1 mM quinine hydrochloride solution shows a comparatively large CPA value (almost 30 mV in channel 2),

whereas the amino acid solutions all show small CPA values, with only Trp solutions showing CPA values over 10 mV. This indicates that the adsorption of the amino acids to the sensor membrane surface is weak, as could be predicted on the basis of their hydrophobicity, which is much less than that of quinine. As shown in Fig. 3a, the relative response electric potentials caused by the amino acids were comparatively large in channels 1–4 (which have a negative charge), while there was no response in channels 5–8 (which have a positive charge). The electric potential pattern of all five amino acids was similar (and also quite similar to that of quinine hydrochloride), showing the largest values for channel 1. Therefore, it was decided to use the relative response electric potentials in channel 1 in the regression analysis.

For prediction of bitterness of single-component amino acid solutions (Ile, Leu, Val, Phe and Trp) of various concentrations (1, 3, 10, 30, and 100 mM), the sensor output values in channel 1 and gustatory sensation data were used in a regression analysis using S-PLUS 2000J (Mathematical Systems, Inc.). Data from 24 points were used in the calculation (there were no data from 100 mM Trp); the results are shown in Fig. 4. A comparatively good correlation ($r^2 = 0.704$, $P < 0.001$) was

obtained between the estimated bitterness scores and the results of the human gustatory tests for single-component amino acid solutions of the five amino acids tested. These data demonstrated good predictability of the taste sensor.

3.2. Bitterness prediction for binary amino acid solutions

For binary amino acid solutions, three methods were proposed for prediction of bitterness scores. As an example, we will use the prediction of the bitterness of a mixture of 30 mM of Ile and Leu.

3.2.1. Method 1

We can use data from all points obtained with Ile (1, 3, 10, 30, 100 mM) and Leu (1, 3, 10, 30, 100 mM) plus the 30 mM KCl solution containing 0.3 mM tartaric acid (control solution; corresponding to saliva), i.e. 11 points, to make our prediction (sensor output and gustatory sensation data).

3.2.2. Method 2

We can use data from 30 mM Ile, 30 mM Leu, and the control solution, i.e. only three data points. In this way, we can predict the bitterness of 10 kinds of 30 mM binary amino acid solutions

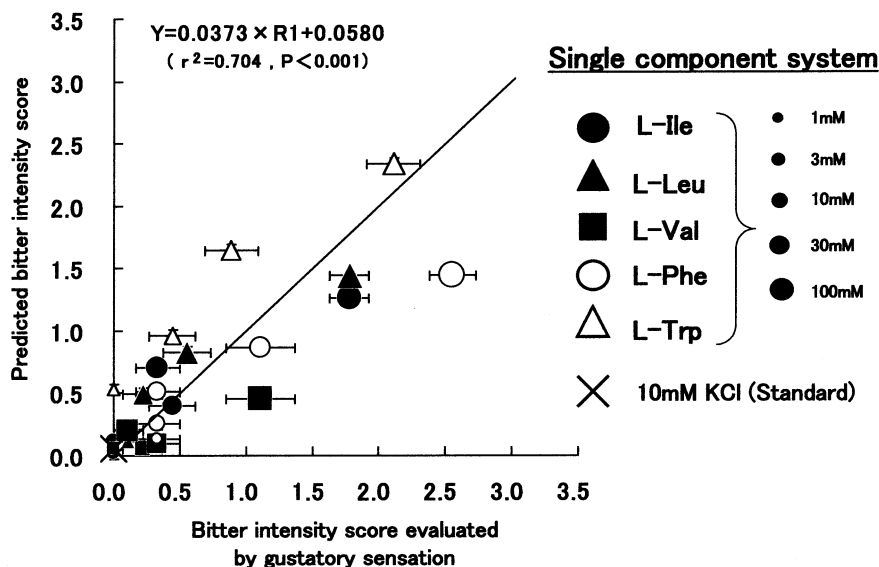


Fig. 4. The effect of concentration on the relation between the concentration of the sample solution and the bitterness scores obtained in human gustatory sensation tests for five different amino acids and quinine hydrochloride (error bar represents S.E.).

(Ile+Leu, Ile+Val, Ile+Phe, Ile+Trp, Leu+Val, Leu+Phe, Leu+Trp, Val+Phe, Phe+Trp, Val+Trp) using corresponding coupled 30 mM amino acid solution data plus data from the control solution.

3.2.3. Method 3

Thirdly, we can derive one regression equation from the data from the 30 mM solutions of all five amino acids plus the control solution (six data points). We can then predict the bitterness scores of ten kinds of combined solution, as above, using the one regression equation.

To investigate these three methods, discrepancies between the bitterness scores obtained in human gustatory sensation tests and the predicted values obtained using each of the three methods were evaluated. The sum total of the absolute values of the deviation of predicted value from obtained value were calculated for ten kinds of binary solution using the three methods mentioned above. The results, summarized in Table 1, show

that there were no great differences between the three methods. A typical example, showing the relation between the gustatory sensation data and the predicted bitterness scores obtained using method 3, is shown in Fig. 5. The derived regression equation was $Y = 0.0351 \times R1 - 0.0253$ ($r^2 = 0.928$, $P < 0.005$), where R1 was the relative value observed in channel 1. Good correlation was observed between predicted bitterness intensity based on the above equation and gustatory sensation result shows good. The calculated regression equation was $Y = 1.111X + 0.119$ ($r^2 = 0.776$, $P < 0.001$), where Y and X mean the predicted and observed bitterness score, respectively. So, the observed gustatory bitterness and the predicted bitterness calculated by the above equation, were almost located near the diagonal line in the graph, and it was demonstrated that the bitterness of binary amino acid solutions could be estimated with good accuracy using the taste sensor. For convenience, method 2 or 3 is recommended.

Table 1

Regression equation for predicting bitter intensity score, and deviation of gustatory sensation from predicted bitter intensity score

Method	Components of each sample	(1)	(2)	(3)
Method 1	L-Ile+L-Leu	0.0473	−0.0944	0.38
	L-Ile+L-Val	0.0480	0.0674	
	L-Ile+L-Phe	0.0589	−0.0815	
	L-Ile+L-Trp	0.0335	−0.0698	
	L-Leu+L-Val	0.0414	0.0724	
	L-Leu+L-Phe	0.0552	−0.0949	
	L-Leu+L-Trp	0.0344	−0.1206	
	L-Val+L-Phe	0.0611	0.0642	
	L-Val+L-Trp	0.0272	0.0969	
	L-Phe+L-Trp	0.0355	0.0332	
Method 2	L-Ile+L-Leu	0.0244	−0.0126	0.35
	L-Ile+L-Val	0.0185	0.0182	
	L-Ile+L-Phe	0.0423	−0.0662	
	L-Ile+L-Trp	0.0356	−0.1178	
	L-Leu+L-Val	0.0266	0.0050	
	L-Leu+L-Phe	0.0401	−0.0106	
	L-Leu+L-Trp	0.0350	−0.0676	
	L-Val+L-Phe	0.0529	−0.0397	
	L-Val+L-Trp	0.0346	−0.0092	
	L-Phe+L-Trp	0.0333	0.1541	
Method 3	All sample of binary component system	0.0351	−0.0253	0.34

(1) Slope of regression equation for predicting bitter intensity score. (2) Intercept of regression equation for predicting bitter intensity score. (3) Deviation of gustatory sensation from predicted bitter intensity score: $(\Sigma |\text{Gustatory Sensation} - \text{Predicted}|)/10$. All amino acids were prepared 30 mM.

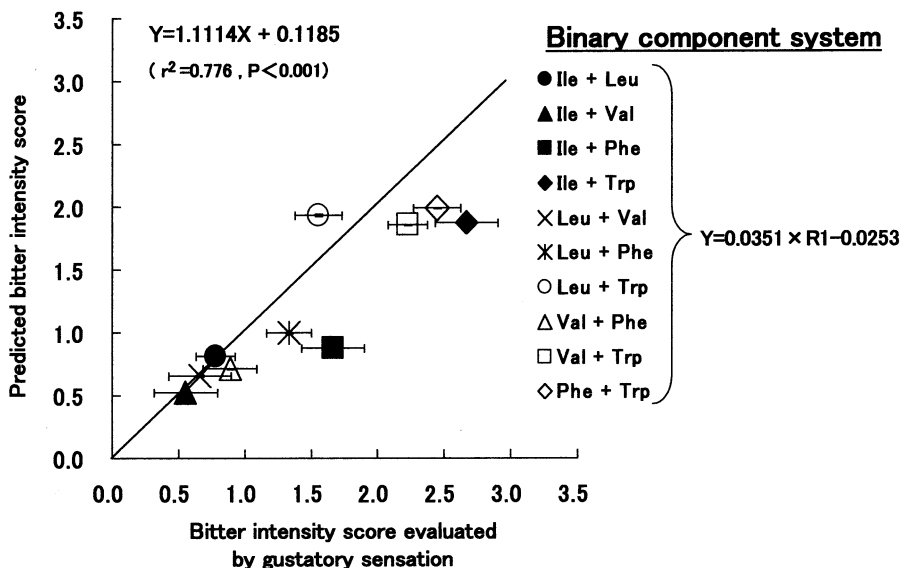


Fig. 5. Comparison between predicted and gustatory sensation bitterness scores obtained using Method 3 for binary solutions of amino acids (error bar represents S.E; all amino acids 30 mM).

3.3. Bitterness prediction for multiple-component amino acid solutions

For the bitterness prediction of multi-component amino acid solutions, we prepared amino acid solutions imitating two commercially available elemental diets, Aminoleban EN[®] and ELENTAL[®]. Thus, the amino acid components and concentrations in Solutions A and B, are the same as those described in the package inserts of Aminoleban EN[®] and ELENTAL[®], respectively (summarized in Table 2). Single amino acid samples at concentrations immediately above and below the concentration in the Solution were used to predict the bitterness scores of Solutions A (four components) and B (five components). For example, the concentrations of Ile, Leu, Val, and Trp in Solution A are 73.2, 77.7, 68.4 and 1.80 mM, respectively. We therefore used 30 and 100 mM samples for Ile, Leu, and Val, and 1 and 3 mM samples for Trp, plus data from the control solution, for the prediction of bitterness.

Fig. 6 shows the relation between the predicted and observed gustatory sensation bitterness scores for a multiple-component solution resembling a commercial elemental diet. The observed gustatory

bitterness and the predicted bitterness (calculated using the equation $Y = 0.0456 \times R1 - 0.127$ for Solution A and $Y = 0.0232 \times R1 + 0.0703$ for Solution B) were very similar, since both points were located near the diagonal line in the graph. As expected, Solution A, imitating Aminoleban EN[®], and containing higher concentrations of the amino acids, showed a greater bitterness. Thus, for multiple-component amino acid solutions, bitterness could be estimated with good accuracy using the taste sensor.

3.4. Principal component analysis of sensor data

Finally, we performed a principal component (PC) analysis of the data obtained from the taste sensor for all single, binary, and multi-component amino acid solutions, quinine solutions, and binary solutions consisting of quinine plus an amino acid. Principal component analysis is a multivariate analytical method, which reduces the dimensional space without losing any information. We used principal component analysis to estimate the largest and second largest relative contribution factors (PC1 and PC2) using all sensor data. The results are shown in Fig. 7.

Table 2

Compositions of sample solution imitating commercial amino acid nutritions (multiple component system)

	Molecular weight	The content per package (50 g)	Concentration when dissolving in the water of 200 ml and taking (mM)	Standard sample solution to predict bitter intensity score (mM)
<i>Sample A solution imitating Aminoleban EN®</i>				
L-Leu	131.17			30,100
		2.037	77.65	
L-Ile	131.17			30,100
		1.923	73.28	
L-Val	117.15			30,100
		1.602	68.37	
L-Trp	204.21			1,3
		0.074	1.80	
L-Phe	165.19			
		0.000	0.00	
		The content per package (80 g)	Concentration when dissolving in the water of 300 ml and taking (mM)	
<i>Sample B solution imitating ELENTAL®</i>				
L-Leu	131.17			10,30
		0.899	22.85	
L-Ile	131.17			10,30
		0.642	16.31	
L-Val	117.15			10,30
		0.701	19.95	
L-Trp	204.21			1,3
		0.151	2.46	
L-Phe	165.19			10,30
		0.871	17.58	

The relative contributions of PC1 and PC2 are 85 and 13%, respectively. The factor PC1 can be assumed to represent the intensity of bitterness. When the data are read along this axis in Fig. 7, the ranking of bitterness concurs with that obtained from human gustatory tests: single amino acids < binary amino acids < quinine plus amino acid. Of the solutions containing quinine plus an amino acid, the combination quinine plus Trp, is the most bitter. This supports our assumption that PC1 represents bitterness.

We have not yet determined exactly what is represented by PC2, which contributed approximately 13% to the principal component analysis. PC2 seems to be a combination of several factors, including sourness, smell, and/or astringency, which are involved in the perception of bitterness on the sensor membranes. This is supported by the fact that our human volunteers sometimes com-

mented that they experienced sourness, smell or astringency with the more highly concentrated amino acid solutions (for example, Solution A), although we did not specifically enquire about sensations of sourness, smell or astringency in our gustatory sensation tests. In general, amino acids are more astringent than quinine hydrochloride, and L-Trp is the most astringent of the five amino acids used in our pilot study (data not shown). If the data are read along the vertical axis, the ranking according to PC2 is in the order: quinine = quinine plus amino acids except Trp < quinine plus Trp < Trp < Solution A. This is in accordance with the finding that all volunteers agreed that Solution A was the most astringent or smelly of the samples. Some volunteers reported sourness with Solution A, to a greater extent than with Solution B (data not shown).

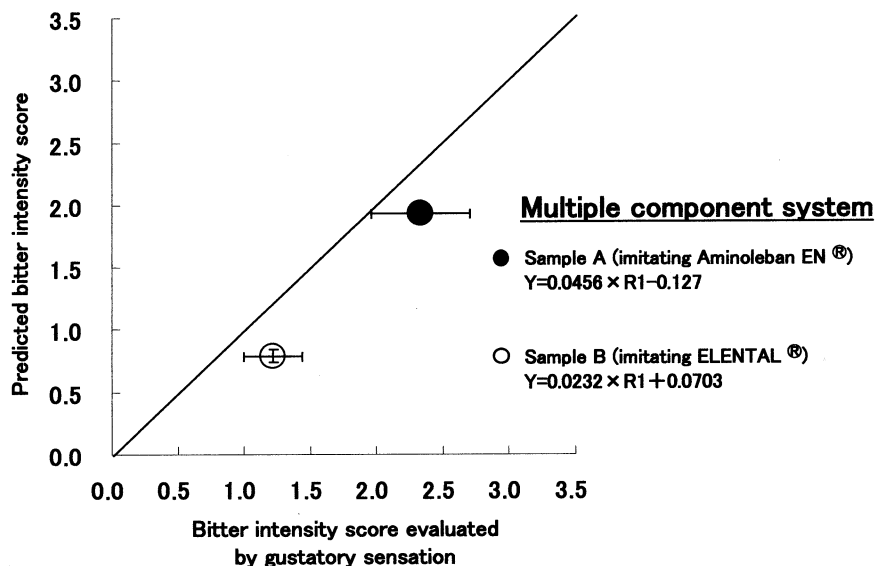


Fig. 6. Comparison between predicted and gustatory sensation bitterness scores for multiple-component solutions of amino acids (error bar represents S.E.).

In conclusion, although we could not define the exact significance of PC2, it seems to represent other factors (sourness, smell, or astringency) which contribute to the perception of bitterness

on the sensor membranes. Quinine has a (positively charged) amino residue, which seems to be responsible for the initiation of the perception of bitterness. Amino acids, on the other hand, have

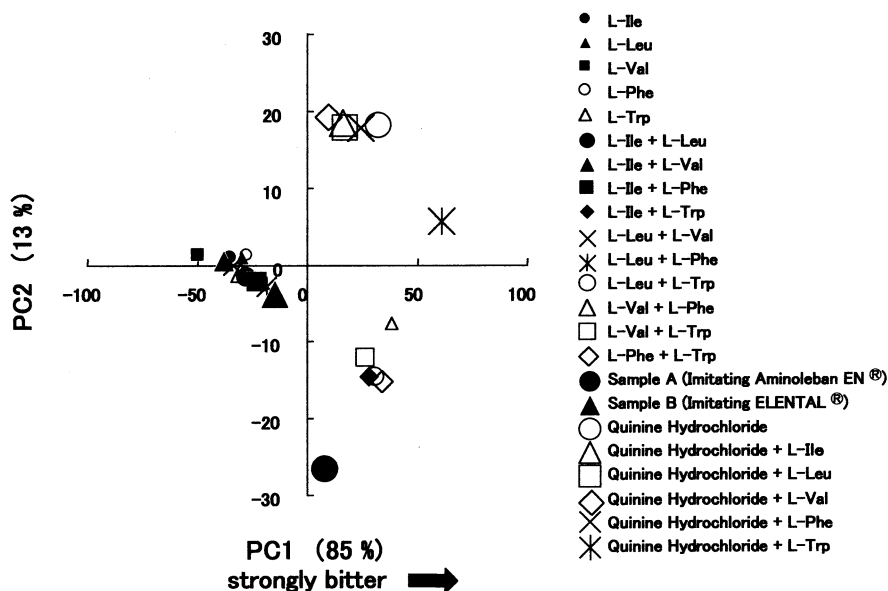


Fig. 7. Principal Component (PC) analysis of sensor output values for single, binary, and multi-component solutions of amino acids and quinine hydrochloride (error bar represents S.E.; all amino acids 30 mM, quinine sample 0.1 mM). The relative contributions of PC1 and PC2 were calculated to be 85 and 13%, respectively. For further explanation, see text.

both (positively charged) amino groups and (negatively charged) carboxyl groups within the molecule. Of course, the amino group may contribute positively to bitterness perception with respect to the sensor output while the carboxyl group may contribute negatively. The carboxyl group may also be a candidate for interference with the positive charge of bitter compounds such as quinine. We intend to investigate the significance of PC2 in a further study.

4. Conclusions

The bitterness of single, binary and multi-component amino acid solutions, could easily be predicted using regression analysis of data derived from the relative sensor output value obtained by channel 1 of the artificial taste sensor. The data produced correlated well with gustatory sensation test results.

The mechanism of perception of bitterness by taste receptors has been the subject of much recent discussion (Keast and Breslin, 2002; Nelson et al., 2002; Tamura et al., 1990), and several studies have shown that the action potential and Ca^{2+} levels in the taste cells play an important role in the perception of bitterness (Kashiwayanagi et al., 1981; Kumazawa et al., 1986). Recently the cloning of the bitterness receptor of mammals has also been reported (Chandrashekar et al., 2000). This information could be used to produce a more evidence-based design of membrane components in the taste sensor.

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