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Measurement of *Umami* Substances Using Multichannel Taste Sensor with Lipid Membranes

Satoru Iiyama, Yasuji Iida and Kiyoshi Toko[1]*

Department of Industrial Chemistry, Faculty of Engineering,
Kinki University in Kyushu, Iizuka 820, Japan
[1]Graduate School of Information Science and Electrical Engineering,
Kyushu University, Fukuoka 812, Japan

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Interaction between taste substances (i.e., taste qualities) was studied using a multichannel taste sensor with lipid membranes. It was revealed that four basic taste substances, sour, salty, bitter and sweet, mutually suppressed the increase in stimulation. *Umami* substances also caused suppression, but the mechanism of suppression differed to a large extent from those of the others; they markedly changed the response patterns of other taste substances. This result agrees with the neurophysiological and psychological views that *umami* is the fifth independent taste.

1. Introduction

It seems that there is a general agreement on the existence of five basic taste qualities for humans. The first is sourness produced by hydrogen ions in, for example, HCl, acetic acid and citric acid. The second is saltiness produced mainly by NaCl. The third is bitterness. Quinine, caffeine and $MgCl_2$ produce bitterness. The fourth is sweetness due to such compounds as sucrose, glucose, and aspartame. The last is *umami*, which is the Japanese term for implying deliciousness. Monosodium glutamate (MSG), contained mainly in seaweeds, disodium inosinate (IMP) in meat and fish and disodium guanylate (GMP) in mushrooms give *umami* taste.(1–3)

These tastes can be measured using a taste sensor which responds to different taste qualities by means of unique patterns of output signals for each taste quality.(4–7) The

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*To whom all correspondence should be addressed.

sensor uses lipid membranes as transducers of taste substances and a computer as a data analyzer. The transducer plays the role of transforming the taste information generated by chemical substances into electric potentials of the lipid membranes. The output of the sensor gives not the amount of specific taste substances but the taste quality and intensity. Different outputs of electric patterns were obtained for different taste groups such as sourness and saltiness. In contrast, similar patterns were obtained for taste substances in the same group such as MSG, IMP and GMP which have an *umami* taste, and NaCl, KCl and KBr which have a salty taste.

The tastes of astringent substances and amino acids were studied using a taste sensor.(8,9) It was shown that astringency is a taste similar to bitterness and amino acids that show bitterness, such as L-tryptophan, can be distinguished from those that show sweetness, such as L-alanine. So far, the taste sensor has been applied to many foodstuffs such as mineral water,(10) beer,(5,11) sake,(12) milk(13) and soybean paste.(14) Therefore, the taste of foodstuffs can be quantitatively discussed using the taste sensor which provides an objective scale for human sensory expression.

Interaction among taste qualities is one of the interests in the study of taste sensation. Several sensory tests were carried to investigate this. Pangborn reported that, in general, all compounds, such as sucrose, citric acid, NaCl and caffeine, depress each other's intensity.(15) However, this does not always agree well with the experimental results.(16,17) Furthermore, no investigation of taste interaction using an instrumental apparatus has ever been reported.

In this study, a taste sensor was applied to understand the interaction among taste qualities in situations where two kinds of taste substances are included together in a solution. From the experimental results, it was revealed that *umami* substances significantly depressed the response patterns of other taste substances.

2. Materials and Methods

2.1 *Taste substances*

Commercial products were employed without further purification. Sucrose, HCl, NaCl, quinine hydrochloride, NaOH, $NaHCO_3$, MSG and sodium aspartate (Asp) were obtained from Wako Pure Chem. Ind., and IMP and GMP from Tokyo Kasei Org. Chem. All substances were dissolved in 1 mM KCl solution.

2.2 *Lipid membranes*

The taste sensor with a multichannel electrode used in this study is similar to that previously reported.(4,7,8) The lipids are abbreviated as follows: dioctyl phosphate, C; trioctyl methylammonium chloride, T; oleyl amine, N; decyl alcohol, DA; oleic acid, OA. Lipid membranes such as C:T = 9:1, C:T = 3:7, and C:T = 5:5 imply a mixture of two lipids in the ratio showing the molar concentration. The membranes of C:T = 3:7, T, and N are positively charged, whereas those of C:T = 9:1, C and OA are negatively charged. The membrane of DA is somewhat negatively charged owing to the presence of the plasticizer.(18) The membrane of C:T = 5:5 has almost zero electric charge. These lipids are indicated by the channel number (Table 1).

Table 1
Lipid membranes used in the taste sensor. Abbreviations are detailed in the text.

Channel	Lipid membrane
1	C:T = 3:7
2	T
3	C:T = 5:5
4	N
5	C
6	C:T = 9:1
7	DA
8	OA

2.3 *Measurements using taste sensor*

The electric potential across the membrane was detected by means of the Ag·AgCl electrode in the hole filled with 100 mM KCl and a reference electrode (TOA, HS205C). The construction of this measuring system is as follows: Ag·AgCl electrode in 100 mM KCl solution | membrane | reference electrode in the taste solution. Taste substances changed the membrane potential, and then the electric signal from each membrane was converted into a digital code using a digital voltmeter (ADVANTEST, R6551) through a high-input impedance amplifier and a handmade eight-channel scanner, and recorded in a computer (NEC, PC-9801). The sensor output is the response pattern composed of the eight electric potentials from the above lipid membranes. The response of the sensor toward each taste substance was measured relative to 1 mM KCl solution.

2.4 *Principal component analysis*

Data obtained using the taste sensor were further studied by means of a principal component analysis. In the present case, the original data were expressed in the eight-dimensional space comprised of the outputs of the eight kinds of membranes. They were visualized on the two-dimensional plane using principal component analysis, which is very effective in reducing the dimensional space with minimum loss of information.(19)

3. Results

3.1 *Interaction among sour, salty, bitter and sweet substances*

Prior to the study of interaction among taste substances, let us outline the responses of the taste sensor toward each taste substance. Figure 1 shows the response of the negatively charged C membrane (ch. 5) toward the five taste substances HCl (sour), NaCl (salty), quinine hydrochloride (bitter), sucrose (sweet) and MSG (*umami*).

Sodium ions of NaCl affect the negatively charged membrane and reduce its negative charge by means of the shielding effect, thus increasing the membrane potential. The threshold concentration for Na^+ is about 1 mM. In addition, hydrogen ions of sour

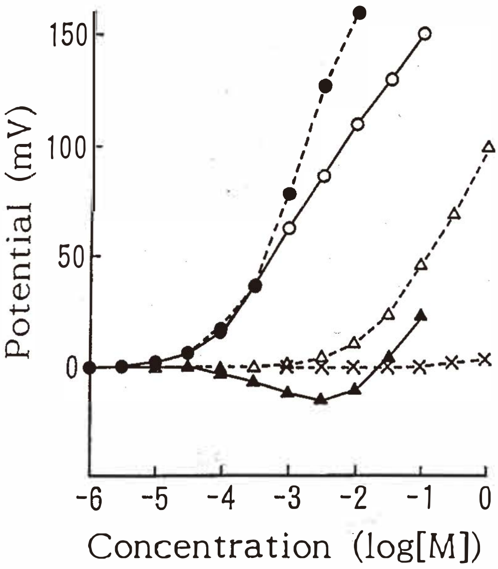

Fig. 1. Responses of the C membrane toward five basic taste substances. ○ HCl, △ NaCl, ● quinine hydrochloride, × sucrose, ▲ MSG.

substances are bound to the functional group of lipids, and quinine of bitter substances is adsorbed onto the hydrophobic part of lipid membranes, as quantitatively shown using the electrochemical theory.(18) The threshold concentrations for both substances are lower than that for NaCl. The effect of MSG on this lipid membrane is biphasic. The decrease and the subsequent increase of the membrane potential could be attributed to the adsorption of glutamate(20,21) and the shielding effect due to Na^+, respectively. According to the response patterns, the glutamate portion of MSG seems to be adsorbed by negatively charged membranes such as C, C:T = 9:1, DA, OA and the electrically neutral membrane of C:T = 5:5. The common negative ion in sour, salty and bitter substances is Cl^-, which decreases the electric potential of the positively charged membranes. Cl^- has only the shielding effect on the membranes, so the threshold concentration for Cl^- is about 1 mM. In fact, HCl and quinine hydrochloride, which are usually employed at low concentrations, have little effect on positively charged membranes.

Since the electrically neutral membrane of C:T = 5:5 shows no response to NaCl, this membrane responds only toward the glutamate portion of MSG; i.e., the response potential decreases monotonically with increasing MSG concentration.(21,22) This is very important in order to characterize the *umami* taste, as shown later.

Interaction among HCl, NaCl, quinine hydrochloride and sucrose was investigated using the taste sensor. Figure 2 shows an example of interaction between HCl and NaCl, where the effects of both substances on the electric potential of the membrane are

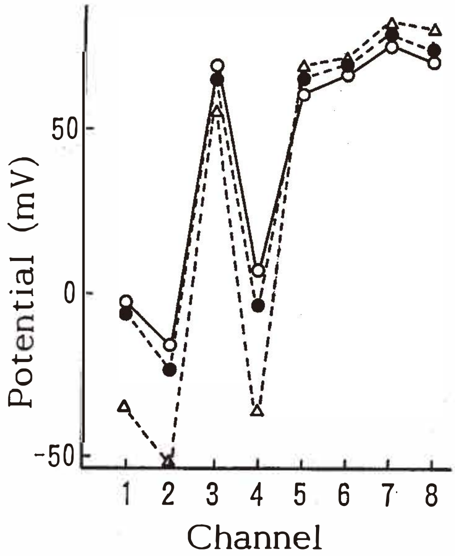

Fig. 2. Interaction between HCl and NaCl. ○ 1 mM HCl, ● 1 mM HCl + 10 mM NaCl, △ 1 mM HCl + 100 mM NaCl.

considerably depressed. The potential changes in the C membrane (ch. 5) induced by 1 mM HCl and 100 mM NaCl are 60 mV and 45 mV, respectively, as shown in Fig. 1, but that of the mixture of the two is only 6 – 8 mV, as shown in Fig. 2. The other negatively charged membranes of channels 6 – 8 exhibit the same phenomena. The response potentials in the situation of coexisting HCl and NaCl do not become the simple sum of the two responses to 1 mM HCl and 100 mM NaCl, although the response becomes slightly higher. On the other hand, the positively charged membranes, such as channels 1 – 4, significantly changed their potentials. This is mainly because 1 mM HCl originally has little effect on these membranes.

Other combinations of taste substances exhibited a similar tendency. The response potentials in the coexistent situation were lower than the simple sum of the two individual response potentials, and were slightly larger values than the individual response.

3.2 *Effect of MSG*

Interaction between HCl and MSG is shown in Fig. 3. The type of interaction differs from those observed among taste substances such as HCl, NaCl, quinine hydrochloride and sucrose. MSG markedly changed the response pattern which was obtained with HCl. MSG itself decreased the membrane potential, as shown in Fig. 1; therefore, a decrement is expected. However, its magnitude is unexpectedly large. In the presence of 1 mM HCl, 1 mM MSG decreased the electric potential of the C membrane (ch. 5) by 35 mV, whereas it

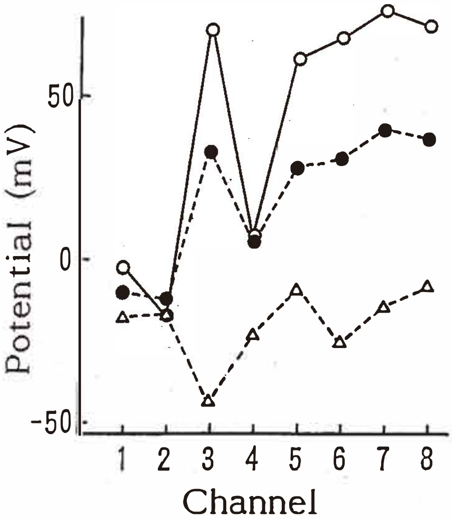

Fig. 3. Interaction between HCl and MSG. ○ 1 mM HCl, ● 1 mM HCl + 1 mM MSG, △ 1 mM HCl + 10 mM MSG.

decreased by only 10 mV without HCl (Fig. 1). The change in the response pattern caused by MSG is most prominent at the C:T = 5:5 membrane (ch. 3). This membrane reflects the effect of MSG on HCl most significantly.

The effects of MSG on quinine hydrochloride and NaCl are shown in Figs. 4 and 5, respectively. The concentrations of the two substances were chosen such that the responses of negatively charged membranes could reach a level similar to that of 1 mM HCl. In both combinations, MSG decreased the electric potential of the C:T = 5:5 membrane (ch. 3) most significantly and then those of the negatively charged membranes. Here, too, the electrically neutral membrane behaves as the membrane most sensitive to the effect of MSG on other taste qualities.

This naturally leads to the conclusion that MSG suppresses the other tastes. MSG decreased the original intensities shown by the coexisting taste substances and changed the response patterns of the other tastes.

3.3 *Effect of umami substances other than MSG*

Other *umami* substances such as Asp, IMP and GMP are also popular. Figure 6 shows the interaction between HCl and IMP measured using a taste sensor. IMP exhibits a result similar to that obtained with MSG. However, it also significantly decreased the potential of the N membrane (ch. 4). This membrane is quite sensitive to alkalis because its hydrophilic portion is a protonated ammonium group. In fact, the pHs of MSG, Asp, IMP and GMP at 100 mM were 6.8, 6.7, 7.6 and 7.8, respectively; nucleotides are weakly alkaline.

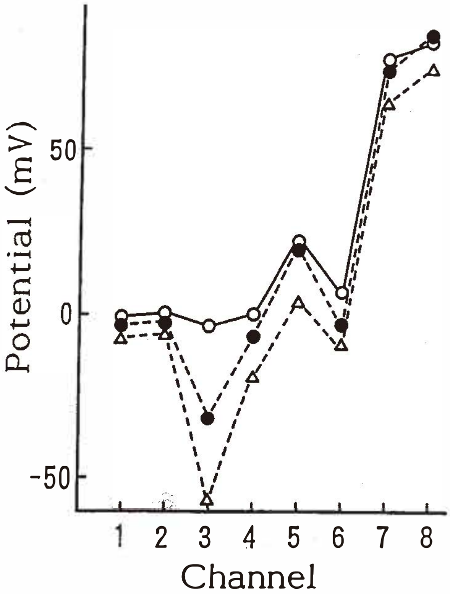

Fig. 4. Interaction between quinine hydrochloride and MSG. ○ 0.1 mM quinine, ● 0.1 mM quinine + 1 mM MSG, △ 0.1 mM quinine + 10 mM MSG.

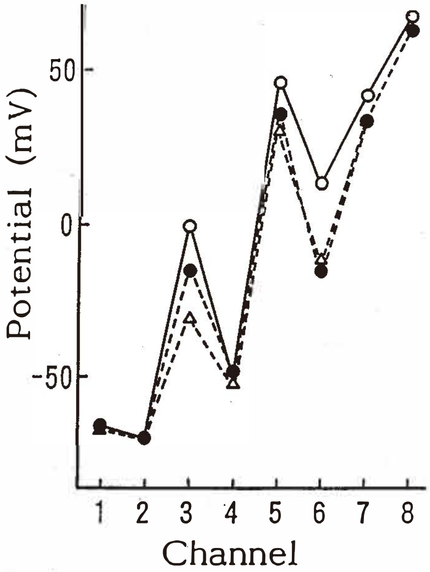

Fig. 5. Interaction between NaCl and MSG. ○ 100 mM NaCl, ● 100 mM NaCl + 1 mM MSG, △100 mM NaCl + 10 mM MSG.

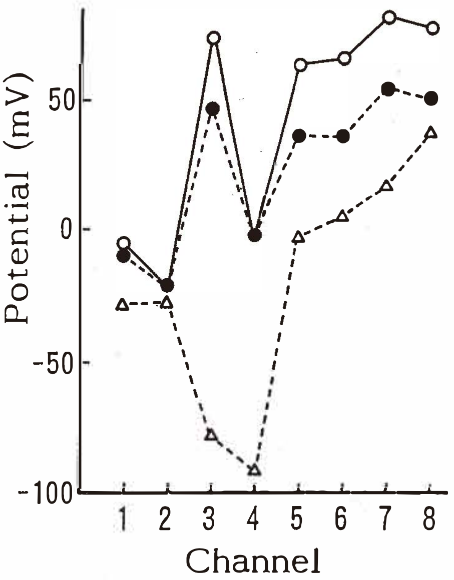

Fig. 6. Interaction between HCl and IMP. ○ 1 mM HCl, ● 1 mM HCl + 1 mM IMP, △ 1 mM HCl + 10 mM IMP.

If the effect of alkaline substances were to resemble that of *umami* substances in the response patterns of the taste sensor, it would be difficult to distinguish between them. For this reason, 3 mM of alkaline (NaOH, $NaHCO_3$) or *umami* (MSG, Asp, IMP, GMP) substances were mixed with 1 mM HCl, 0.1 mM quinine hydrochloride or 100 mM NaCl. After the measurements using the taste sensor, a principal component analysis of these data was carried out. The contribution rates were 54.5%, 28.9%, 12.5% and 2.5% for PC1, PC2, PC3 and PC4, respectively. Each vector was composed of the following responses: PC1 (reflecting the response of the N membrane), PC2 (reflecting the sum of the responses of negatively and positively charged membranes), PC3 (reflecting the difference between the responses of negatively and positively charged membranes) and PC4 (reflecting the response of C:T = 5:5 membrane).

The plot of PC4 vs PC1 is shown in Fig. 7. MSG and Asp are clearly distinguished from NaOH, although IMP and GMP are close to $NaHCO_3$. This is because PC1 and PC4 express the typical responses of the N membrane and the C:T = 5:5 membrane to alkaline and *umami* substances, respectively, as mentioned above.

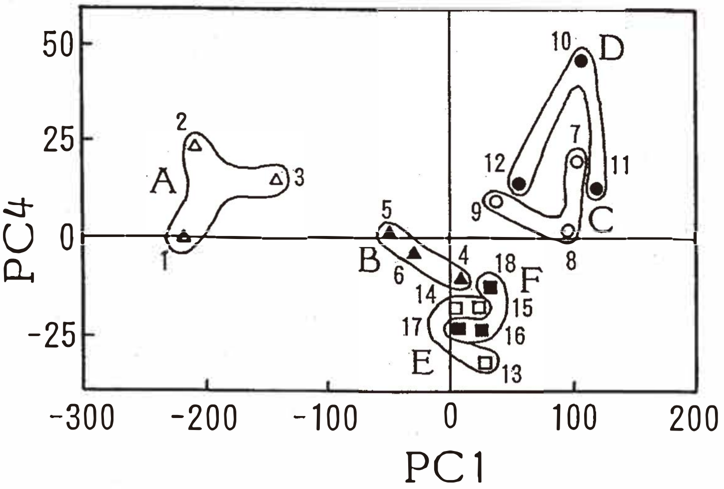

Fig. 7. Principal component analysis. 1) 1 mM HCl + 3 mM NaOH; 2) 0.1 mM quinine + 3 mM NaOH; 3) 100 mM NaCl + 3 mM NaOH; 4) 1 mM HCl + 3 mM $NaHCO_3$; 5) 0.1 mM quinine + 3 mM $NaHCO_3$; 6) 100 mM NaCl + 3 mM $NaHCO_3$; 7) 1 mM HCl + 3 mM MSG; 8) 0.1 mM quinine + 3 mM MSG; 9) 100 mM NaCl + 3 mM MSG; 10) 1 mM HCl + 3 mM Asp; 11) 0.1 mM quinine + 3 mM Asp; 12) 100 mM NaCl + 3 mM Asp; 13) 1 mM HCl + 3 mM IMP; 14) 0.1 mM quinine + 3 mM IMP; 15) 100 mM NaCl + 3 mM IMP; 16) 1 mM HCl + 3 mM GMP; 17) 0.1 mM quinine + 3 mM GMP; 18) 100 mM NaCl + 3 mM GMP. A, NaOH; B, $NaHCO_3$; C, MSG; D, Asp; E, IMP; F, GMP.

4. Discussion

The present work is a first attempt at studying the characteristics of *umami* taste using an artificial sensing device. The taste sensor can easily distinguish between the qualities of the five basic tastes, as well as some kinds of beverages such as beer, coffee, mineral water and milk. Interaction of taste substances was studied using the taste sensor, because the interaction has previously been investigated mainly by means of sensory tests. According to the results of the tests, most of the taste substances seem to suppress each other's intensities, although several conflicting conclusions have been obtained,(15–17) *e.g.*, saltiness enhances the strength of sweetness. It was revealed, using the taste sensor, that the four basic taste substances, such as HCl, NaCl, quinine hydrochloride and sucrose, suppress the responses of the sensor toward other taste stimuli. When different taste qualities were mixed, the response of the sensor was lower than that of the sum of each response. From these results alone, however, it may be difficult to discuss the change in each taste strength because in this coexisting situation, individual taste qualities cannot be separated in a straightforward manner based on the electric response pattern.

Umami substances also suppressed the membrane response, but the manner of suppression clearly differed from those other of taste substances. MSG markedly decreased the responses to HCl, quinine hydrochloride, NaCl and sucrose, and changed their response patterns. For example, when MSG and HCl were mixed, the response of the sensor was much lower than the response to HCl alone.

MSG can be considered to be adsorbed onto the lipid membranes, because it decreases the electric potential of the negatively charged membranes (Fig. 1), as was also shown previously by measuring the amount of MSG adsorbed on a lipid membrane.(20) It could be most effectively adsorbed onto the C:T = 5:5 membrane, which has zero electric charge, as estimated from the electric potential response patterns (Figs. 3 – 5). The threshold concentration is not very low, and hence the adsorption may be weaker than in the case of quinine hydrochloride which penetrates into the hydrophobic portion of membranes. The adsorption of MSG seems to be increased by the existence of other taste substances, since the potential reduction induced by MSG becomes more prominent (Fig. 3).

IMP and GMP are somewhat alkaline, whereas MSG and Asp are neutral. The pH of $NaHCO_3$ resembles those of nucleotides, and hence $NaHCO_3$ may have slight *umami* effect. However, it cannot be used in cooking owing to its instability in heat. The difference between *umami* substances and alkaline substances was clearly shown by principal component analysis (Fig. 7).

Several possibilities of the taste reception mechanism have been proposed for even a single taste quality such as bitterness.(23–26) The taste sensor will help in the clarification of this mechanism.

Other types of taste sensors(27,28) will also be effective for studying the taste interaction and making comparisons with the present result. In the present study, we did not characterize each taste from the electric potential response pattern in the situation of two coexisting taste qualities. To do so, it may be necessary to deduce the property of the response pattern for each taste quality in this coexisting situation, where the pattern is greatly different from the original pattern for each individual taste quality. Nevertheless, the present study is worthwhile as a first trial of investigating the interactions between taste qualities using an electronic sensing device. Quantification of the change in each taste quality in a coexisting situation is proposed as a future task. Sensory tests are indispensable for this purpose, because they directly evaluate taste qualities. On the other hand, the taste sensor does not directly express taste qualities, but its sensitivity, durability and reproducibility are superior to those of humans. In consequence, studies using the taste sensor will become more important and will facilitate sensory tests.

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