Development of Sensor with High Selectivity for Saltiness and Its Application in Taste Evaluation of Table Salt

Ronggang Chen[1,2,*], Ikezaki Hidekazu[1] and Kiyoshi Toko[2]

[1]Intelligent Sensor Technology, Inc.,
5-1-1 Onna, Atsugi-shi, Kanagawa 243-0032, Japan
[2]Information Science and Electrical Engineering, Kyushu University,
744 Motooka, Nishi-ku, Fukuoka 819-0395, Japan

Previous research has focused on using taste sensors to evaluate very low concentrations of minerals in table salt that cannot be detected with the senses, indicating potential applications in salt quality control. However, the poor selectivity of taste sensors for salt and the inability to evaluate the total taste due to the presence of other tastes, such as bitterness, besides saltiness have become new research topics. Our work aims to improve the selectivity of sensors for saltiness by optimizing the sensor components for foodstuff applications. Furthermore, we have evaluated saltiness with a high correlation to human sensory saltiness evaluation scores by a new analysis method using saltiness sensors with positively and negatively charged membranes. We hope that the optimized saltiness sensors and new analysis method can be used in evaluating the taste of salt in the general foodstuff industry.

1. Introduction

The general foodstuff industry uses table salt from both domestic and overseas producers; the contents of chemicals, including $NaCl$, $MgCl_2$, $CaSO_4$, and KCl, in salt vary according to the production method, causing large differences in taste. Since $MgCl_2$, $CaCl_2$, and KCl, have bitter and salty tastes, solutions with high levels of these chemicals are called *nigari* in Japan.[1] We have reported a previous work on evaluating trace concentrations of *nigari* components, such as $MgCl_2$ and $CaCl_2$, in table salt with good reproducibility and possible applications to food quality control.[2,3]

Previous research has used adsorption data from artificial lipid-membrane sensors similar to those used to evaluate hydrophobic bitter materials, such as quinine hydrochloride, to evaluate Mg^{2+} and Ca^{2+} ions in *nigari* components. Owing to the high selectivity of the sensor for Mg^{2+} and Ca^{2+}, trace concentrations can be detected

*Corresponding author: e-mail: chin.eigou@insent.co.jp

with a high sensitivity at any NaCl concentration.[2,3]   However, it is commonly observed that bitterness cannot be tasted in salt solutions containing trace amounts of bitter components. Consequently, we consider that bitterness is suppressed either by a certain form of interaction between the *nigari* components and NaCl, or between the *nigari* components, NaCl, and the bitterness receptors in the human tongue. Strongly hydrophobic materials, such as quinine hydrochloride, are considered to be adsorbed onto the sensor lipid membrane, changing the membrane potential, but metallic ions, such as Mg^{2+} and Ca^{2+}, are thought to react through a certain mechanism other than adsorption. As a consequence, current saltiness sensors have difficulty evaluating the tastes of salty foodstuffs that include bitterness caused by *nigari* components.

The current work investigates how to improve selectivity for saltiness by optimizing the sensor components. We measured various salty materials focusing on their anion and cation structures using sensors with positively and negatively charged membranes with a high selectivity for saltiness, and evaluated the strength of and qualitative differences in saltiness. In addition, we measured samples including *nigari* components using sensors with positively and negatively charged membranes, and estimated the vertical distance between the response curves and the NaCl curve as an index of bitterness. The results suggest that it is possible to evaluate the tastes of both the NaCl and *nigari* components in table salt. Furthermore, the analysis results obtained with the saltiness sensors closely approximated the results of human sensory evaluation scores for salty materials, suggesting that these sensors can be used to accurately assess the saltiness of various foodstuffs.

2.   Materials and Methods

2.1   *Measurement setup*

Measurements were performed using the TS-5000Z Taste Sensing System of Intelligent Sensor Technology Inc., Japan. The system is composed of a sensor unit and a management server computer. Up to 8 sensors can be connected to the unit. Figure 1 shows a diagram of the taste sensor and taste-sensing system. The sensor surface is composed of an attached artificial lipid membrane containing an artificial lipid that responds to basic tastes plus a plasticizer. This artificial lipid membrane reacts with taste substances based on electrostatic and hydrophobic interactions, and the electric potential of the lipid membrane changes in the same way as that of the taste receptors of living organisms. This potential change is detected as the sensor output and evaluated as taste information by the computer.[4–6]   A Ag/AgCl electrode including a saturated solution of KCl was used as the reference electrode. A solution of 3.3 M KCl and saturated AgCl was used as the inner solution of the lipid-membrane sensor and reference electrode. Before measurement, these electrodes were preconditioned for 2 days in a reference solution containing 30 mM KCl and 0.3 mM tartaric acid. Table 1 shows the components of the taste sensors used in this work. Since the lipid membrane was either positively or negatively charged depending on the lipid type, we describe the sensors as either positively or negatively charged membrane sensors in this paper.
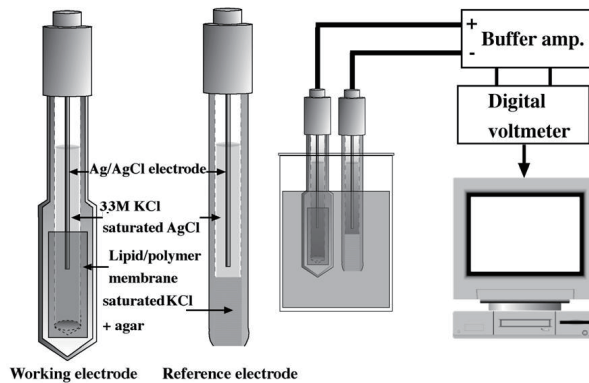
Fig. 1.   Diagram of taste sensor and taste-sensing system.

Table 1
Components of lipid membrane.

Ch.	Lipid	Plasticizer	Charge
1	phosphoric acid di-*n*-decyl ester (PADE)	1000 μl di-*n*-octyl phenylphosphonate (DOPP)	negative
2	sodium hexadecyl sulfate (SHS)	1000 μl bis(1-butylpentyl) adipate (BBPA)	negative
3	400 mg tetradodecyl ammonium bromide 315 mg 1-hexadecanol	3850 mg di-*n*-octyl phenylphosphonate (DOPP)	positive

2.2   Test methods and samples

2.2.1 Measurement method

The reference solution was measured first (V_r) before measuring the sample (V_s) and $V_s - V_r$ was used as the sensor output.

2.2.2 Evaluating saltiness selectivity of saltiness sensor

Table 2 shows the five representative taste sample materials measured to evaluate the sensor selectivity.

2.2.3 Taste evaluation of salty materials

To evaluate the concentration dependence of various salty materials, solutions of NaCl, KCl, $MgCl_2$, $CaCl_2$, Na_2SO_4, and NaH_2PO_4 at different concentrations shown in Table 3 were measured. In addition, to investigate the bitterness of *nigari* components, the NaCl, KCl, $MgCl_2$, and $CaCl_2$ mixtures listed in Table 4 were measured.

2.2.4 Sensory score evaluation

As mentioned previously, the *nigari* components of the salty materials, such as KCl, $MgCl_2$ and $CaCl_2$, are both salty and bitter tasting. Moreover, the bitter taste of these *nigari* components varies greatly depending on the NaCl concentration. To evaluate the saltiness of materials including these *nigari* components, panels of two healthy

Table 2
Components of sample solutions for basic tastes.

Sample	Components
Reference solution	30 mM KCl + 0.3 mM tartaric acid
Salty sample	300 mM KCl + 0.3 mM tartaric acid
Sour sample	30 mM KCl + 3 mM tartaric acid
Umami (savoriness) sample	30 mM KCl + 0.3 mM tartaric acid + 10 mM sodium hydrogen L-glutamate monohydrate
Bitter sample (cation)	30 mM KCl + 0.3 mM tartaric acid + 0.1 mM quinine hydrochloride
Bitter sample (anion)	30 mM KCl + 0.3 mM tartaric acid + 0.01 vol% humulone derivatives
Astringent sample	30 mM KCl + 0.3 mM tartaric acid + 0.05 wt% tannic acid

Table 3
Samples for saltiness evaluation.

Sample*	Components
Samples 1–8	0, 1, 3, 10, 30, 100, 300, 1000 mM NaCl
Samples 9–16	0, 1, 3, 10, 30, 100, 300, 1000 mM KCl
Samples 17–21	0, 0.3, 1, 3, 10 mM $MgCl_2$
Samples 22–26	0, 0.3, 1, 3, 10 mM $CaCl_2$
Samples 27–32	0, 1, 3, 10, 30, 100 mM Na_2SO_4
Samples 33–38	0, 1, 3, 10, 30, 100 mM NaH_2PO_4

*All samples included 1 mM KCl as the supporting electrolyte.

Table 4
Salty materials in samples.

Sample[*1]	Components[*2]	
	Component A	Component B
Samples 1–3	0 mM NaCl	10 mM KCl, 10 mM $MgCl_2$, 10 mM $CaCl_2$
Samples 4–6	1 mM NaCl	10 mM KCl, 10 mM $MgCl_2$, 10 mM $CaCl_2$
Samples 7–9	3 mM NaCl	10 mM KCl, 10 mM $MgCl_2$, 10 mM $CaCl_2$
Samples 10–12	10 mM NaCl	10 mM KCl, 10 mM $MgCl_2$, 10 mM $CaCl_2$
Samples 13–15	30 mM NaCl	10 mM KCl, 10 mM $MgCl_2$, 10 mM $CaCl_2$
Samples 16–18	100 mM NaCl	10 mM KCl, 10 mM $MgCl_2$, 10 mM $CaCl_2$

[*1]All samples included 1 mM KCl as the supporting electrolyte.
[*2]All samples were composed of two types of salty material (components A and B). For example, Sample 1 was [0 mM NaCl + 10 mM KCl] and Sample 2 was [0 mM NaCl + 10 mM $MgCl_2$].

women and two healthy men evaluated the nine samples listed in Table 5 to correlate the taste of *nigari* components, such as NaCl, KCl, $MgCl_2$, and $CaCl_2$, with changes in the strength of the bitter taste between *nigari* components. Each panel of tasters evaluated the strength of the bitter taste by holding a sample in the mouth for 10 s. To simplify evaluation of differences between samples, each test was performed once by each group. Furthermore, six sample combinations were selected to test for differences in the strength of the bitter taste of the *nigari* components as well as the correlation between NaCl and

Table 5
Components of sensory score evaluation samples.

Sample	Components
1	10 mM KCl
2	10 mM KCl + 10 mM NaCl
3	10 mM KCl + 100 mM NaCl
4	10 mM $MgCl_2$
5	10 mM $MgCl_2$ + 10 mM NaCl
6	10 mM $MgCl_2$ + 100 mM NaCl
7	10 mM $CaCl_2$
8	10 mM $CaCl_2$ + 10 mM NaCl
9	10 mM $CaCl_2$ + 100 mM NaCl

the strength of the bitter taste of the *nigari* components by performing six rounds of sensory evaluation tests, as shown in Fig. 11. Tasters rinsed their mouths between tests. The bitterness score (bitterness strength) was ranked on a scale of 3 with 3 being the most bitter and 1 being not bitter at all.

3. Results and Discussion

3.1 *Optimizing plasticizers and lipids*

Previously described saltiness sensors have been composed of the plasticizer di-*n*-octyl phenylphosphonate (DOPP) and the artificial lipid phosphoric acid di-*n*-decyl ester (PADE); Fig. 2(a) shows the molecular structure. Since they are negatively charged, they respond to cations, such as Na^+ and K^+.[2,3] However, obtaining a response to acidic and salty bitter materials indicates a low selectivity for salt, creating problems with achieving a high-accuracy method of evaluating the taste of salt in actual daily foodstuffs. Consequently, this work focused on optimizing the components of a saltiness sensor that would not be affected by either sour or bitter materials.

The response to sour materials depends on changes in pH, which cause changes in the levels of dissociation of PADE and impurities (such as monoester phosphate)[6] in the commercial DOPP plasticizer used in lipid membranes. Consequently, we must select an artificial lipid and a plasticizer that do not dissociate in the pH range of general foodstuffs from 2 to 8. As a result, we used bis(1-butylpentyl) adipate (BBPA) as a new plasticizer with no monoester impurities. To evaluate the plasticizer response to basic tastes, we fabricated a sensor composed of BBPA plasticizer without artificial lipids and measured the samples listed in Table 2. The results are shown in Fig. 3. For comparison, the same measurements were performed using a DOPP-only membrane sensor.

Figure 3 shows that the DOPP-membrane sensor is more responsive to sour samples than salty samples, whereas the BBPA-membrane sensor is more responsive to salty samples than sour samples. From these results, BBPA is clearly considered to be a better plasticizer for a saltiness sensor than DOPP.

However, Fig. 3 shows that the DOPP- and BBPA-membrane sensors have about the same high response to the bitter sample (cation), suggesting that the saltiness selectivity
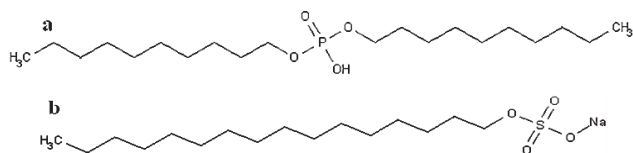
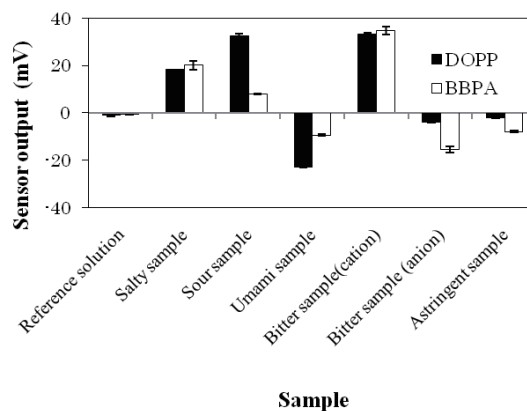Fig. 2.   Molecular structures of PADE and SHS: (a): PADE and (b): SHS.

Fig. 3.   Responses of sensors with different plasticizers to the sample solutions for basic tastes.

of these sensors might be affected by bitter materials. Sensitivity and selectivity to bitter materials are based greatly on the charge density of taste sensors, depending on the degree of lipid incorporation into the membrane.[8,9] At higher lipid concentration in the membrane, even if the bitter substance adsorbs on the membrane and then causes a decrease in the charge density, it cannot cause change in the membrane potential owing to the extremely high density of the membrane. This finding suggests that the sensor with higher charge density has lower sensitivity to bitter materials, consequently leading to higher selectivity to salty substances. Therefore, to reduce the impact of both the presence of bitter samples (cation) and the changes in the pH of samples, we need to fabricate a negatively charged lipid membrane with a high charge density. To meet this need, we fabricated different sensors consisting of different amounts of the lipid "sodium hexadecyl sulfate (SHS)," which has a higher dissociation than the lipid PADE, and the BBPA plasticizer (molecular structure shown in Fig. 2(b)). For comparison, sensors were also fabricated with different amounts of PADE and the plasticizer BBPA. These sensors with the two types of lipid were measured with the samples listed in Table 2. The test results are shown in Fig. 4.

   Figure 4(a) shows that the sensitivity to salty samples increases as PADE content increases, but the sensitivity to other bitter samples (cation), which affects salt sensitivity, also increases. Conversely, Fig. 4(b) shows that the sensitivity to salty samples increases as the amount of SHS increases, but the sensitivity to other samples is almost zero. From
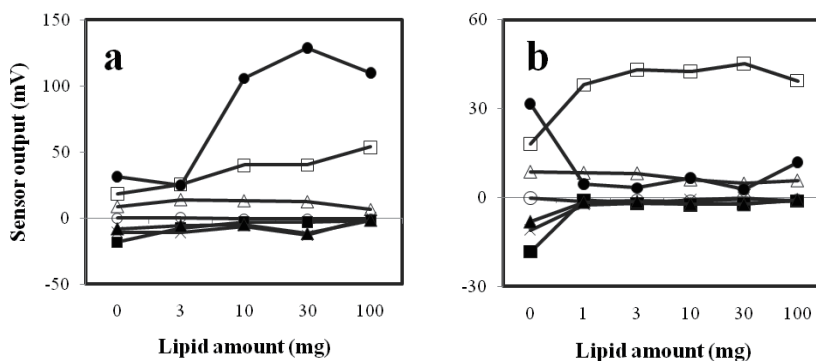
Fig. 4. Responses of sensors with different lipids to the sample solutions for basic tastes: (a): PADE and (b): SHS (○: reference sample; □: salty sample; △: sour sample; ×: *umami* sample; ●: bitter sample (cation); ■: bitter sample (anion); ▲: astringent sample).

these results, the sensor membrane composed of the BBPA plasticizer and SHS lipid is considered to be greatly improved in terms of the selectivity for salty materials.

These results are explained by the dissociation and hydrophobicity of the lipid. Figure 5 shows the effect of pH on the ion fraction ratio of SHS and PADE in a solution using "Marvin Sketch," which is a chemistry software designed by ChemAxon Corp, Hungary. Marvin Sketch can calculate the microspecies ratio of all proton gaining or losing atoms on the basis of the partial charge in distribution, and the octanol/water partition coefficient. This result indicates that while SHS is mostly dissociated in the range of pH from 0 to 8, PADE increases the level of dissociation in the range of pH from 0 to 4 as pH increases, indicating that SHS is practically unaffected by pH changes in the range of pH from 0 to 8. In contrast, PADE is affected in the range of pH from 0 to 4. The difference in the two molecules in terms of response to pH is because the SHS has the sulfonate group, which dissociates completely in an aqueous solution, while the PADE contains the phosphate group, which dissociates partially in an aqueous solution. Dissociation in a solution and dissociation in a membrane are expected to be naturally different, but the calculation results show that the effect is clear, as shown in Fig. 5. For these reasons, SHS is better at suppressing the effect of pH than PADE.

Next, we used Marvin Sketch to calculate Log P as an index of the hydrophobicity of the artificial lipids. The results of 3.82 for SHS and 6.2 for PADE show that SHS has higher hydrophilicity in a solution at pH 3.5. From these results, we consider that SHS forms a hydrophilic membrane surface more easily owing to self-organization in an aqueous solution. On the basis of this observation, we assume that increasing the hydrophilicity of the SHS sensor membrane surface suppresses the adsorption of hydrophobic materials, such as the bitter sample.

However, because SHS is more hydrophilic than PADE, there is a possibility of a slight dissolution of SHS during measurement causing variations in sensitivity. As
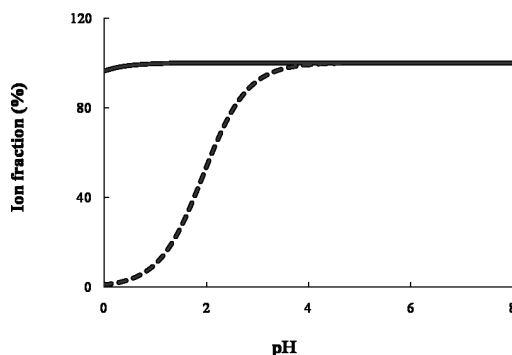
Fig. 5.   Ion fraction of the lipids in the membrane in solution with change in pH (curve: SHS ions; broken line: PADE; calculated using Marvin Sketch).

shown in Fig. 4(b), when SHS is 3 mg or less, a decrease in saltiness sensitivity can be expected; thus, the optimum SHS content is 30 mg. In the rest of this paper, this sensor is described as a "negatively charged saltiness sensor."

3.2   *Identifying salty materials using new analysis methods for sensitivity of positively and negatively charged saltiness sensors*

Salty materials have both anion and cation structures, and their tastes are considered to be determined by the combination of these two ion types. We investigated the taste evaluation of salty materials using two sensors with a high selectivity for anionic and cationic salty materials. To evaluate cations, we used the negatively charged saltiness sensor fabricated as described in §3.1; anions were evaluated as described in Table 1 of §3, where a saltiness sensor with a fixed positively charged lipid is described from hereon as a "positively charged saltiness sensor." Figure 6 shows the response of this sensor to the basic tastes in Table 2. From Fig. 6 it is clear that the positively charged saltiness sensor responds only to the salty sample and has both high sensitivity and high selectivity for anionic salty materials.

We measured the samples listed in Table 3 to evaluate the dependence of these two sensors on the concentration of each salty material. The results in Fig. 7 indicate that the negatively charged saltiness sensor responds equally to the Na^+ ions in the different salty materials, but its response to other cations varies. This observation suggests that this sensor can identify cations and is highly sensitive to Ca^{2+}, Mg^{2+}, K^+, and Na^+ in this order. It is well known that the ion exchange resin with sulfonic acid responds to cations in the following order: Ca > Mg >> K > Na > H. This implies that the response of this negatively charged saltiness sensor may be similar to that of the ion exchange resin. This is thought to be due to differences in ion radius and electronegativity between cations and ions, although we are still not absolutely sure.[10,11] In the same way, Fig. 7(b) shows that the positively charged saltiness sensor responds equally to the Cl^- ions in the different salty materials, but its response to other anions varies. This observation suggests that this sensor can discriminate between different anion types.
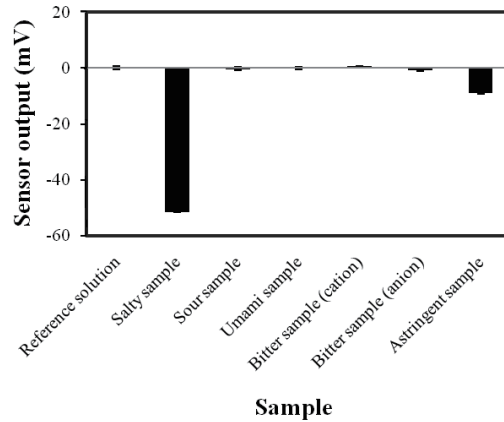
Fig. 6.   Response of positively charged saltiness sensor to the sample solutions for basic tastes.
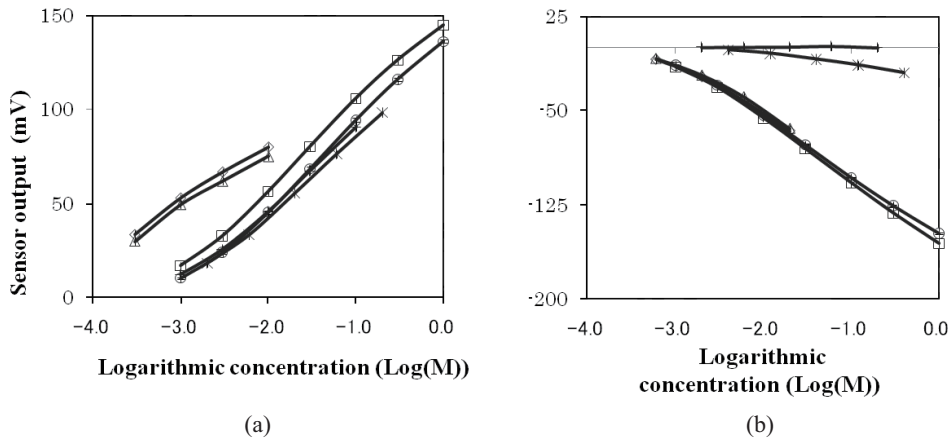

(a)   (b)

Fig. 7.   Concentration dependence of responses of the saltiness sensors to salty materials: ○: NaCl; □: KCl; △: $MgCl_2$; ◇: $CaCl_2$; ×: Na_2SO_4; +: NaH_2PO_4 (left: negatively charged saltiness sensor; right: positively charged saltiness sensor).

Figure 7(b) shows that this positively charged saltiness sensor can also be used to evaluate samples containing SO_4^{2-} and PO_4^{3-} ions, but considering the components of currently marketed table salt and comparing SO_4^{2-} and PO_4^{3-} with Cl^-, since the number of the former ions is much smaller than that of the latter ions, it seems most probable that the Cl^- anions have the greatest impact on the actual saltiness of table salt. As a result, we limited the measured salty materials in the examined samples to NaCl, KCl, $MgCl_2$, and $CaCl_2$. In addition, to simplify the observation of the two-dimensional data for anions and cations, the sensitivity data for the two types of sensor obtained from the results presented in Fig. 7 were plotted as a 2D scatter plot (Fig. 8). In this figure, the
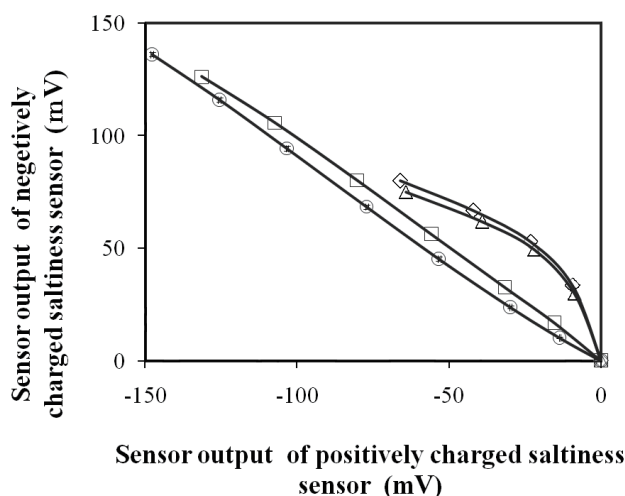
Fig. 8. Scatter plot of responses of positively and negatively charged saltiness sensors to anions and cations of salty materials: ○: NaCl; □: KCl; △: MgCl$_2$; ◇: CaCl$_2$.

curves for KCl, MgCl$_2$ and CaCl$_2$ are compared with the curve for NaCl, and the results indicate a clear discrimination from NaCl on the right side. In other words, these results show that irrespective of the concentration of the salty materials, it is possible to identify NaCl as the main component of table salt as well as KCl, MgCl$_2$, and CaCl$_2$ as the *nigari* components. Moreover, if we consider that the orthogonal distance between the curve for NaCl and the curve for each salty material indicates the difference in taste quality, KCl has a taste that is closest to that of NaCl, and CaCl$_2$ shows the largest difference in taste.

Next, to determine how NaCl, KCl, MgCl$_2$ and CaCl$_2$ included in table salt affect its taste, we measured the samples listed in Table 4 using the two types of saltiness sensor. The results are shown in Fig. 9. The outputs of the mixed solutions of NaCl and the other *nigari* components are plotted against the response curves for samples with NaCl and added *nigari* components. To make it easier to understand the taste of the different salty materials, we calculated the orthogonal distances between the NaCl curve and the curves for the *nigari* components, as shown by the arrows in Fig. 9. These analysis results are shown in Fig. 10. When the vertical axis is zero, the sample has a taste closest to that of NaCl. As the value increases, the taste of the sample differs from that of NaCl. From Fig. 10, when the concentration of NaCl on the horizontal axis is zero, a comparison of the tastes of the three *nigari* components shows that CaCl$_2$ has a taste most different from the salty taste of NaCl, indicating that it also can be evaluated as a bitter taste. Furthermore, irrespective of the type of *nigari* component, increasing the concentration of NaCl makes each *nigari* component taste more like NaCl. This result can be empirically considered to have a good correlation with the sensory evaluation scores. Therefore, the values on the vertical axis are described as the bitterness strength using the saltiness sensor.
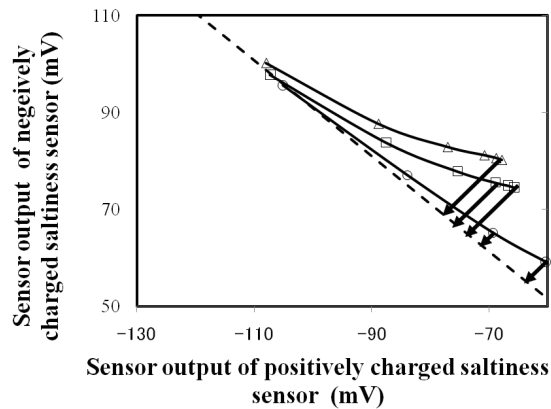
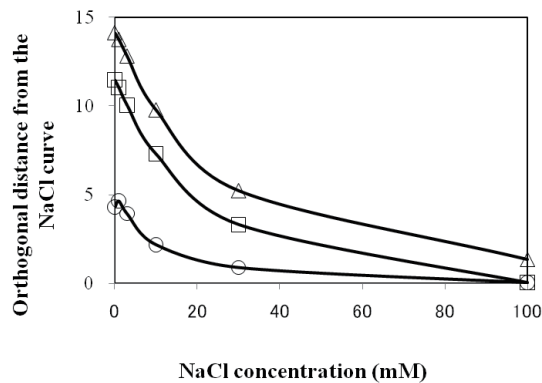Fig. 9. Response with addition of *nigari* components (dashed line: NaCl, ○: KCl + NaCl, □: MgCl$_2$ + NaCl, △: CaCl$_2$ + NaCl).

Fig. 10. Changes in orthogonal distance from the NaCl curve in response with addition of *nigari* components (○: KCl + NaCl, □: MgCl$_2$ + NaCl, △: CaCl$_2$ + NaCl).

3.3 *Correlation with sensory evaluations*

From the results in Fig. 10, the response results from the two types of taste show that the taste of table salt varies greatly depending on the included *nigari* components. To evaluate the validity of these results, sensory evaluation tests were performed for the samples listed in Table 5. Figure 11 shows a scatter plot of the results from Fig. 10 and the sensory evaluation tests. This figure shows that when no NaCl is added, the bitterness ranking of the three *nigari* components obtained from the sensor is CaCl$_2$ > MgCl$_2$ > KCl, which is very similar to the human sensory evaluation scores of CaCl$_2$ ≈ MgCl$_2$ > KCl. From Fig. 11, at 100 mM NaCl, the data from both the sensory evaluations and sensors indicate that MgCl$_2$ and KCl are both weakly bitter and only CaCl$_2$ is relatively bitter. We consider that this result is due to the fact that the increasing NaCl concentration reduces the sensitivity to bitterness, making MgCl$_2$ and KCl less bitter.
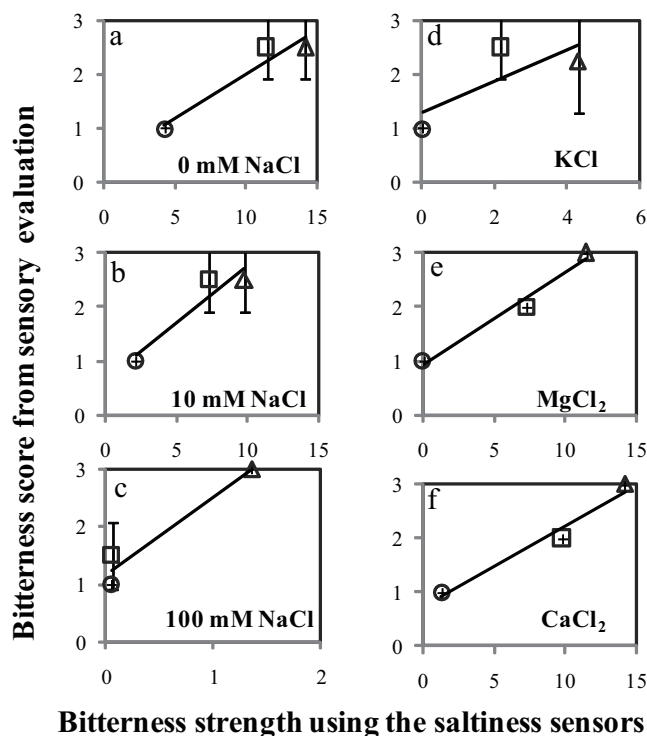
Fig. 11. Relationship between bitterness strength from the sensors and sensory evaluation scores with different *nigari* components (Figs. (a), (b), (c): effect of types of *nigari* components on the bitterness strength and bitterness score, ○: KCl, □: MgCl₂; △: CaCl₂; Figs. (d), (e), (f): effect of NaCl concentration on the bitterness strength and bitterness score, ○: 100 mM NaCl, □: 10 mM NaCl, △: 0 mM NaCl). Data on horizontal axis are obtained from Fig. 10.

Figure 11(d) shows that when comparing three samples with NaCl concentrations of 0, 10, and 100 mM at the same KCl concentration, the sensor data gives a bitterness ranking of KCl (0 mM NaCl) > KCl (10 mM NaCl) > KCl (100 mM NaCl), which is very similar to the human sensory evaluation ranking of KCl (0 mM NaCl) ≈ KCl (10 mM NaCl) > KCl (100 mM NaCl). The error bars on the KCl (0 mM NaCl) and KCl (10 mM NaCl) sensory evaluations are large, possibly explaining the small difference in taste score. Furthermore, Figs. 11(e) and 11(f) show that increasing the NaCl concentration reduces the bitterness in both the sensor measurements and sensory evaluations. These results show a high correlation between the results obtained from the sensory evaluations and the sensor outputs. In other words, at a fixed NaCl concentration, the bitterness ranking is CaCl₂ > MgCl₂ > KCl, and the bitterness of each bitterness component tends to decrease as NaCl concentration increases. These results suggest that the two types of saltiness sensor can be used to evaluate both saltiness and other tastes, such as bitterness.

4.   Conclusions

This research suggests that it is possible to evaluate the tastes of both NaCl and other *nigari* components in table salt by optimizing the sensor composition to improve selectivity for saltiness and performing analysis using the outputs of two salt sensors. Moreover, a comparison with the results of human sensory evaluations shows good agreement, suggesting that the above-described saltiness sensors and analysis methods offer an accurate method for evaluating the various tastes of table salt.

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