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Measurement of Fish Freshness Using Potentiometric Gas Sensor

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A system of potentiometric gas sensors was used to measure the odor emitted from decomposing fish to evaluate fish freshness. An oxidation-reduction gas sensor has been developed by us for the emitted gas measurement. We have evaluated the basic responses of multiple sensor systems comprising an oxidation-reduction gas sensor developed by us, an ammonia gas sensor and a hydrogen sulfide gas sensor with regard to the indicator component of the odor emitted from decomposing fish. These sensors showed characteristic responses for dimethylamine (DMA) and trimethylamine (TMA) that are assumed to be the products of fish decomposition. When applied to the measurement of the odor of salmon and sardine, the system was able to detect the characteristic signals from individual sensors with regard to the fish degradation. The output patterns of these sensors were evaluated by the sensory test and the colony count method. The deterioration of fish quality with time could be identified by a method of principal component analysis using the signals from the three sensors. Therefore, it was indicated that fish freshness can be evaluated using three potentiometric gas sensors.

1. Introduction

Recent incidents of foodborne illness pose important social problems from the view point of food safety. Since there is no applicable device (simple, handy, low cost and accurate) for quality control from the viewpoint of chemical measurements, it appears that the foodborne illness is caused by unsatisfactory quality control and quality testing during the manufacturing process. Therefore, appropriate devices which can examine the food cost effectively and in a simple manner are desired.

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In order to evaluate freshness, the odor emitted from fish is measured using gas sensors. To date, several studies of aromatic substances using quartz-resonator sensors and oxide semiconductor sensors have been reported,[1–7] but the measurement circuit of quartz-resonator sensors is complicated and the oxide semiconductor sensors are operated in the high-temperature range of 300°C to 400°C. Also, the response of these sensors is easily affected by humidity. A potentiometric gas electrode is operated at room temperature and the measurement circuit is simple. Moreover, it has the advantage of being unaffected by humidity, enabling measurement of the equilibrium potential between the gaseous sample component dissolved in the inner liquid and the component of the inner liquid. We have previously reported that the aroma of soup could be discriminated using a potentiometric gas sensor.[8] In this study, we examined the practical possibilities of a sensor system based on potentiometric gas sensors for use in sensing the odor from putrefied fish.

Generally, fish freshness quickly degrades by enzymatic decomposition of adenosine triphosphate after death.[9] Gradually, reductive gaseous species such as volatile sulfur compounds, volatile acidic compounds and volatile basic nitrogen compounds are emitted during the decomposition of fish.[10] In this study, an oxidation-reduction gas electrode was developed for the emitted gas measurement by combining a permeable membrane, gold plate and oxidation-reduction buffer solution. A multiple sensor system that was composed of an oxidation-reduction gas sensor, a hydrogen sulfide gas electrode and an ammonia gas electrode was used to measure the gaseous species emitted from decomposing fish. DMA and TMA, which are produced during fish decomposition were selected and the response of the sensor system to them was measured.[3–4] The response of the sensor system to the odors of salmon and sardine was also measured by letting these fish decay. A comparison was made between a sensory evaluation and the response of the sensor system. The signals from the sensors have been analyzed using principal component analysis (PCA). The possibility of evaluating fish freshness using the potentiometric sensor by PCA is discussed.

2. Experimental

2.1 *Reagent*

Dimethylamine (DMA) and trimethylamine (TMA) were purchased from Wako Pure Chemicals Industries, Ltd. Ultrapure water (conductivity, 2 μS/cm) was used for the preparation of sample solutions.

2.2 *Structure of sensor*

The gas sensor system consists of an oxidation-reduction (ORP) gas sensor, ammonia (NH_3) gas sensor and hydrogen sulfide (H_2S) gas sensor. The ORP-sensor was developed for our purpose, and the NH_3-sensor (DDK Co. 7300L, Tokyo, Japan) and H_2S-sensor (DDK Co. 7350L, Tokyo, JaBan) are commercially available instruments.

Figure 1 shows the structure of the ORP-sensor used in this study. It is a composite-type sensor, consisting of a gas-permeable membrane, a reference electrode of silver-silver chloride, a detection electrode and an inner solution. This structure enables the measurement of various potentials due to the reaction between the inner liquid and the gaseous

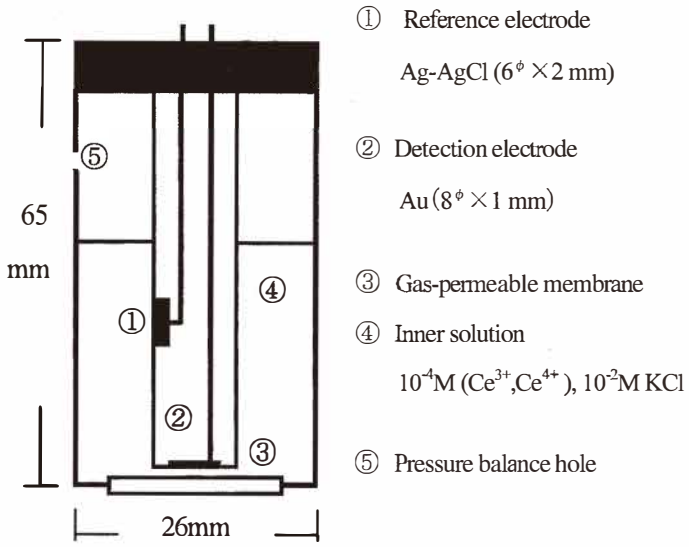
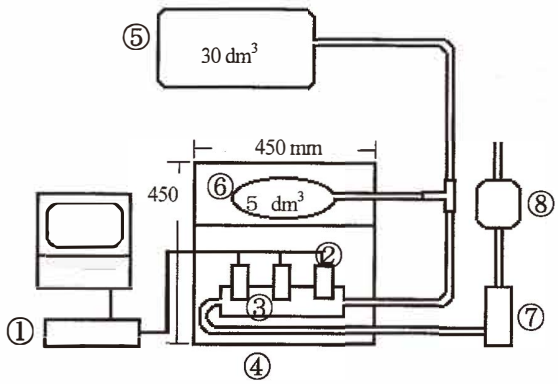


Fig. 1. Structure of the oxidation-reduction gas sensor.

sample that permeates into the electrode through the membrane. The gas-permeable membrane with a 0.22 μm pore diameter is made of Teflon and is not permeable by liquid, thus preserving the inner solution. The clearance between the surface of the detection electrode and the gas permeable membrane is 0.1 mm. The detection electrode and inner liquid of the ORP-sensor were gold and cerium (Ce^{3+}, Ce^{4+}), respectively. The NH_3-sensor and H_2S-sensor were also commercially available sensors, and their fundamental structures are the same that of the ORP-sensor. With respect to the inner solution of each sensor, for the NH_3-sensor, a 10^{-2} M NH_4Cl solution (pH 5.6) is used, and for the H_2S-sensor, a 10^{-2} M KCl/tris-buffer solution (pH 10) is used. Each solution contains 70% ethyl glycol in order to prevent their evaporation.

2. 3 *Experimental apparatus*

The structure of the apparatus is illustrated in Fig. 2. A sensor cell made of Teflon is attached inside a thermostat together with a receptacle for the gas sample to be tested. The air and gas samples are introduced via a tube (i.d. 2 mm) into the sensor cell containing the sensors. The experimental temperature was kept constant at 30°C. Dry air was first passed into the sensor cell and once the potential value reached a constant at every sensor, the gas sample was introduced. The values measured from each sensor are the potential differences within a definite time period subsequent to the introduction of the sample, and were measured by a four-channel potentiometer (DKK Co. LIO-40, Tokyo, Japan). Both the dry air and the gas sample were made to flow through the sensor cell at a constant rate of 40 ml/min. The measurements were repeated three times under the same conditions. The average of these measurements was used at the final result.



① Potentiometer ② Sensor ③ Sensor cell
④ Thermostat ⑤ Air ⑥ Sample
⑦ Flow meter ⑧ Pump

Fig. 2. Experimental apparatus

2. 4 *Preparation of odor samples*

2. 4. 1 *Chemical materials*

DMA (50%) solution or TMA (28%) solution (3 μl) was placed in a polyethylene bag and sealed with 5000 ml of dry air, the bag was left for one day at 35°C, then cooled to 30°C before being ready for use in the experiment. These concentrations were confirmed by means of gas chromatography.

2. 4. 2 *Fish samples*

Commercially available salmon (approximately 80 g) and sardine (approximately 40 g) were used. Each fish sample was placed in a polyethylene bag and sealed with 5000 ml of dry air, the bag was left at 25°C until the measurement. The three or four odor samples for each measurement were prepared at the same time. The 0 day sample was immediately placed in a polyethylene bag after its purchase, left for 2 h, and then prepared. In order to shorten the test duration, the experiments were carried out by maintaining the sample storage temperature at 25°C.

2. 5 *Sensory test*

Ten healthy participants (men in their twenties) judged the odors from every sample in the experiments. There were two indexes, "putrefaction" and "no putrefaction", which were selected by the participants. Even if one participant judged the sample as undergoing putrefaction, it was interpreted as a putrefied sample.

3. Results and Discussion

3.1 *Measurement of odor component substances of fish*

The main components of the odor resulting from fish decay are volatile sulfur compounds, volatile acidic compounds, volatile carbonyl compounds and volatile basic nitrogen compounds.[9] In this study, the responses of all sensors to DMA and TMA, which are representative of decay odor, were investigated. In order to examine the effect of the odor concentration on sensor output, the responses of the sensors to the samples diluted to half concentration by air were also measured. The relation between the odor concentration and the potential change of every sensor is shown in Fig. 3. It was revealed that the H_2S-sensor does not respond to DMA and TMA. On the other hand, for the ORP-sensor and NH_3-sensor, the potential change corresponds to the difference in the odor concentration. The responses of the ORP-sensor and NH_3-sensor to these amine compounds were studied. The example of the amine compound was considered to DMA. The reaction between DMA and the inner liquid in the NH_3-sensor is:

$$(CH_3)_2NH + H_2O \rightleftarrows (CH_3)_2NH_2^+ + OH^-. \quad (1)$$

DMA is hydrolyzed and hydroxyl group ion is generated. Thus, it is considered that the NH_3-sensor responds to DMA. For the ORP-sensor, DMA is reductive with the result that

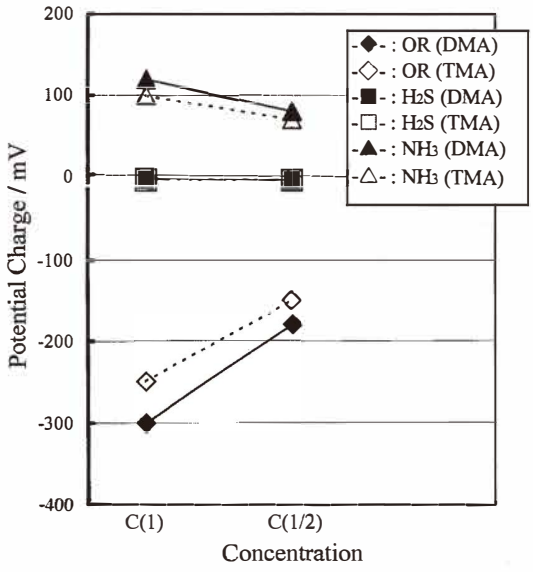


Fig. 3. Relationship between changes in potential and concentrations of aroma components. Concentrations of DMA: C(1) 140 ppm, C(1/2) 70 ppm; Concentrations of TMA: C(1) 90 ppm, C(1/2) 45 ppm.

the reaction between DMA and cerium ion in the inner liquid is:

$$(CH_3)_2NH + Ce^{4+} \rightleftarrows (CH_3)_2NH^+ + Ce^{3+}. \quad (2)$$

The positive ions of DMA and Ce^{3+} are generated by the redox reaction of DMA and Ce^{4+}. As a result, the ORP-sensor shows a response to DMA. It is thought that the mechanism of the response to TMA is similar to that of the response to DMA.

Therefore, the OR-sensor and NH_3-sensor are effective for the detection of volatile basic nitrogen compounds, which constitute one of the odor materials emitted during fish decomposition.

3.2 *Change in the sensor potential with time for sample*

The potential changes measured by each electrode with time are shown in Fig. 4 and Fig. 5. The ordinate shows the electric potential response by each sensor. The abscissa shows the sample changes with time in days, with 0 day as the day of obtaining samples and putting them in polyethylene bags. For 0 day, very small responses were obtained from every sensor in the case of both samples. The following results for each sample were obtained; concerning salmon, the potential change increased for the NH_3-sensor for the 1-

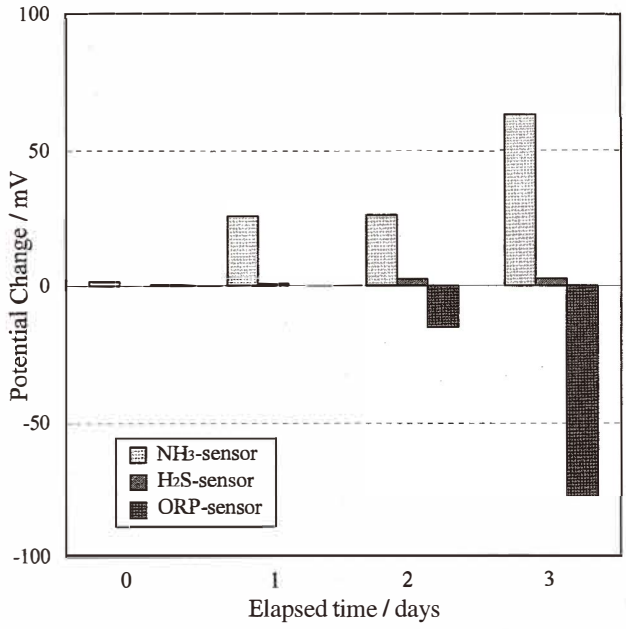


Fig. 4. Changes in potential measured by each sensor vs. time for salmon.

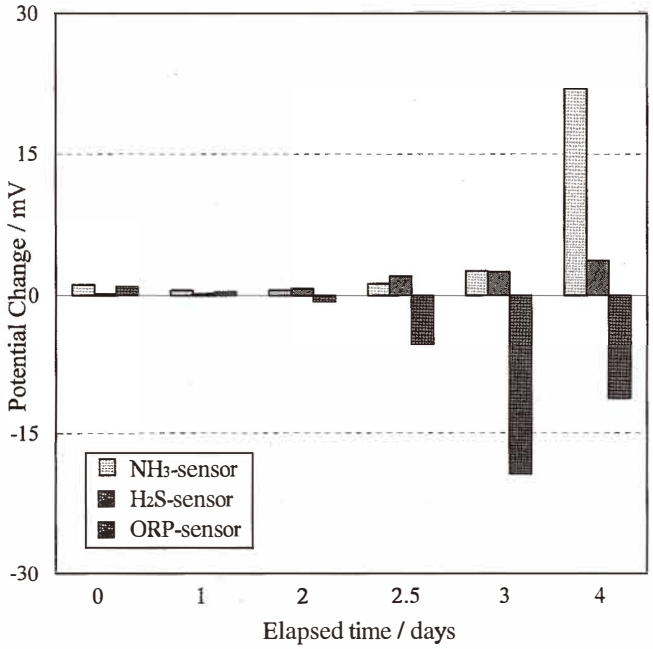


Fig. 5. Changes in potential measured by each sensor vs. time for sardine.

day sample. The potential change increased for the ORP-sensor for the 2-day sample. For the NH_3-sensor and ORP-sensor, the potential change increased with time. Concerning sardine, a very small potential change was observed for all sensors up to 1-day. For the 2, 2.5 and 3 day samples, the potential change increased negatively for the ORP-sensor. The potential change sharply increased for the NH_3-sensor using the 4-day sample. From these results, it was considered that the emission of reductive volatile compounds increases with the degradation of fish freshness.

3.3 *Comparison between sensor potential and sensory test*

In order to examine the reliability of the potential changes obtained from the sensors, the changes in the sensor potential were compared with the results of the sensory tests for the samples. From Table 1, it is seen that the samples of both salmon and sardine at 0 day were judged as under going no putrefaction by all participants, which showed that both samples were fresh. For salmon, eight participants judged the 1-day sample as having undergone putrefaction. At the same time, a response was obtained by the NH_3-sensor. This indicates that the NH_3-sensor can effectively determine the salmon condition. Concerning the 2-day salmon sample, all participants pointed out "putrefaction". A response by the ORP-sensor was also observed clearly. A small change in the electric potential of the H_2S-sensor was also observed. After 3 days, the change in the electric potential increased for the NH_3-sensor and ORP-sensor. Concerning sardine, all ten subjects judged

Table 1
Sensory test for fish samples.

sample	Judgment	Elapsed time / days					
		0	1	2	2.5	3	4
salmon	no putrefaction	10	2	0	-	0	-
	putrefaction	0	8	10	-	10	-
sardine	no putrefaction	10	10	7	0	0	0
	putrefaction	0	0	3	10	10	10

the 1-day sample as exhibiting no putrefaction. After 2 days, the sample was judged as exhibiting no putrefaction by seven participants. Furthermore, all participants judged the 2.5-day sample as exhibiting putrefaction. Against these changes of freshness, small electric potential changes were recorded by all sensors up to day 2. For the ORP-sensor, the electric potential change increased for the sample on day 2.5. After day 3, the responses gradually increased for all sensors.

Furthermore, using the colony count method[11] which is usually used for discrimination of fish freshness, the viable bacterial counts (N) of the samples were measured. Fish meat, for which N becomes 10^7 cells/g, is generally defined as being in the stage of putrefaction.[9] The N of the samples, for which the judgement was "no putrefaction" by the sensory test, were approximately 10^4 cells/g. The N of the samples, which were judged as putrefied by the sensory test, were approximately 10^5–10^7 cells/g. Based on the results of the colony count method, it was concluded that the validity of the sensory test was objectively evaluated.

Although the response time of the electric potential change differs based on the type of fish and sensor, it was revealed that characteristic responses were obtained from the fish decompostion with time by each sensor.

3.4 *Discrimination of fish freshness by principal component analysis*

The obtained data carry multidimensional information, because they were measured by three different devices. Therefore, we performed a principal component analysis, in order to interpret the data obtained by measurement of the volatile gases from the decomposed salmon and sardine. The results of these analyses are summarized in Figs. 6 and 7. The figure is plotted with the first principal component as the abscissa and the second principal component as the ordinate. Based on Fig. 6, the samples were classified into groups, from which the diurnal degradation of the salmon was discerned. Although the 1-day sample was judged as exhibiting putrefaction from the results of the sensory test, the 0 and 1-day samples were discerned by plotting. Based on Fig. 7, all samples except the 0 and 1-day samples were classified into groups. The 1-day samples (no putrefaction) and 2-day samples (putrefaction) were discerned by plotting. It was suggested that the diurnal degradation of fish could be identified by the method of principal component analysis using the signal from the sensors.

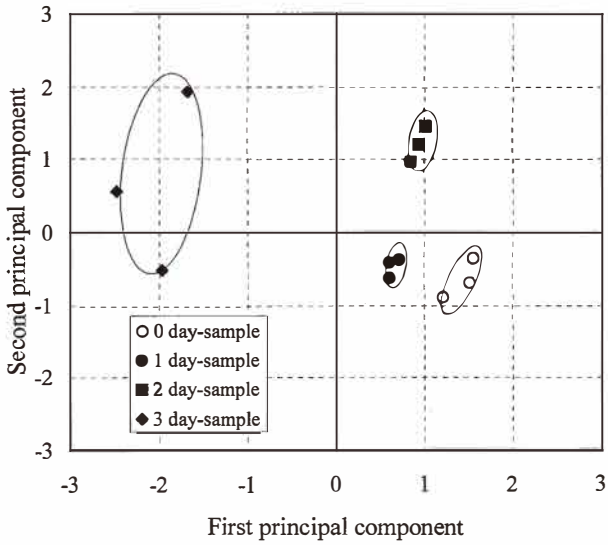


Fig. 6. Principal component analysis of responses to salmon.

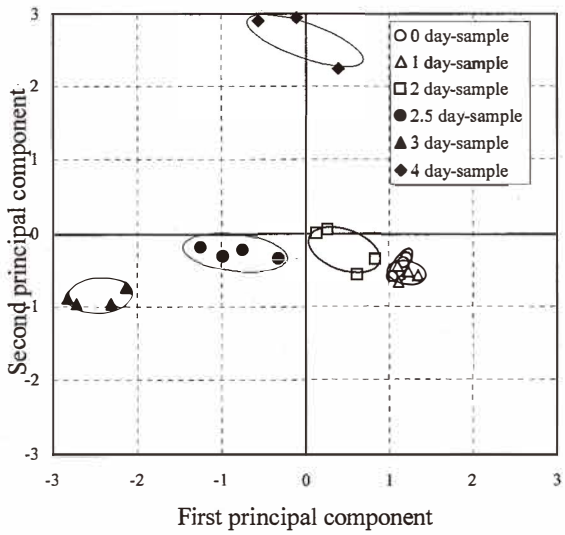


Fig. 7. Principal component analysis of responses to sardine.

4. Conclusion

In this study, we examined the practical possibilities of a sensor system based on a potentiometric gas electrode for sensing fish freshness. The multiple sensor system that was composed of an oxidation-reduction gas sensor, a hydrogen sulfide gas sensor and an ammonia gas sensor was used to measure the volatile gases emitted from decomposing fish. These sensors showed characteristic responses for dimethylamine (DMA) and trimethylamine (TMA) that are assumed to be fish degradation products. When used for the measurement of the odor of salmon and sardine, the system was able to detect characteristic signals from individual sensors with regard to the fish degradation. Also, the freshness of the fish was evaluated by a sensory test and the potential changes of the sensors were roughly related to the sensory test. The deterioration of fish quality with time could be identified by the method of principal component analysis using the signals from the three sensors. Thus, it can be postulated that the potentiometric gas sensor system is effective for fish freshness estimation in a convenient manner.

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