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Measurement of pork freshness using potentiometric sensor

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Abstract

This study evaluated pork freshness using potentiometric solid-state electrodes in order to detect chemical indices such as reduced compounds, organic compounds and sulfides, which are produced during the initial stage of putrefaction in meat. Pt, CuS and Ag_2S electrodes selected as solid-state electrodes have, respectively, been used to detect the organic compounds (regarded as chemical indices of deterioration in meat freshness). The outputs of these electrodes have been analyzed by principal component analysis (PCA) and multiple regression analysis (MRA) in order to find the correlation with the results of viable bacterial counts. By using the potentiometric sensor, the pork freshness was evaluated and the PCA and MRA corresponded to the degree of bacterial increases more simply and rapidly than other methods such as viable bacterial counts or a biosensor.

Keywords: Potentiometric sensor; Pork freshness; Multiple regression analysis; Principal component analysis

1. Introduction

Recent incidents about foodborne illness are sometimes important social problems from the point of view food safety. Since there is no useful instrument (simple, handy type, low cost and correct) for quality control from the viewpoint of chemical measurement, it seems that the foodborne illness is caused by unsatisfactory quality control and quality testing during the manufacturing process.

At present, quality control in the meat industry is mostly tested by two methods in order to evaluate meat freshness. One is the measurement of viable bacterial counts and the other is a sensory test by experts [1]. The former is a very objective method, but it takes 2–5 days to obtain results. This means that the method cannot simultaneously evaluate correct meat freshness when the meat is sold. The latter is a very rapid method. However, it is very difficult for this method to evaluate slight differences in the meat freshness before the initial stage of putrefaction. Therefore, the quality control in the meat industry demands the development of a freshness sensor, which can measure the meat freshness in situ, rapidly, simply and correctly. Yano and co-workers have proposed that some diamines (cadaverine (Cad), putrescine (Put)) produced by putrefaction in the meat have been used as a freshness index during the initial stage of putrefaction [2]. These substances have been detected using high performance liquid chromatography (HPLC) or a biosensor, however, they lack simplicity, rapidity and cost-effective performance [1–4]. Therefore, the meat freshness sensor should be fabricated using simpler principles. Since potentiometry is one of a simple method and it can measure only the electric potential of electrodes versus a reference electrode, a freshness sensor which is a handy type, has a lower cost and rapid measurement time may be fabricated by using potentiometry.

This study has been based on the concept ‘simple is best’, so that pork freshness can be measured by using

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the potentiometric sensor. Since the electrodes as the sensor need to be stable for long term, Pt, CuS and Ag_2S electrodes were selected as the solid-state electrodes in order to correspond to the chemical indices such as organic compounds which are produced during the initial stage of putrefaction. These signals regarded as chemical indices have been analyzed using principal component analysis (PCA) and multiple regression analysis (MRA) in order to find the correlation with the results of viable bacterial counts. The possibility of evaluating meat freshness using the potentiometric sensor by PCA and MRA is discussed.

2. Experimental

2.1. Measurement of putrescine and dimethyl sulfide

Each solid-state electrode of Pt, CuS and Ag_2S is commercially available apparatus (EL7974L, EL7144L, EL7104L, TOA-DKK Co., Tokyo, Japan). Exposed part of each electrode is 8 mm diameter. Since putrescine and dimethyl sulfide (DMS) are known as some substances produced by putrefaction in meat [1,2,5–8], each solution (10^{-1} to 10^{-5} mol l^{-1}) was prepared. Each electric potential (E), versus a reference electrode (Ag/AgCl, 3 M KCl), was measured in each of the solutions using a potentiometer (IOL-40, TOA-DKK Co.). E was measured in the solution kept at 30 °C and stirred using a magnetic stirrer.

2.2. Measurement of viable bacterial counts and electric potential during putrefaction in pork

The samples consisted of fresh pork (part of thigh), which is generally sold at a meat store. Each of the 20–30 samples was prepared to have a total weight of 30.0 ± 0.1 g by cutting the pork into blocks. They were then put into sterile Petri dishes, and the dishes were sealed by putting them in airtight polyethylene bags. At this time, we paid attention to the influence of bacteria in the air. We then made the samples lose their freshness in a refrigerator set at 10 °C for up to 6 days. For the extraction of the sample, the sample was placed in distilled water (65 ml), that had been sterilized at 121 °C for 20 min beforehand, and the extracted solution was prepared by shaking the distilled water for 3 min. The pork freshness was evaluated by measuring the viable bacterial counts (N) in the extract solution. E (versus Ag/AgCl) of the electrodes (Pt, CuS and Ag_2S) was measuring as the chemical indices.

Table 1 shows the composition of a culture medium for measuring the viable bacterial counts (N). The culture mediums were prepared by sterilizing (121 °C, 20 min) the mixed solution shown in Table 1. N was then measured using the extract solution (5 ml), which was removed from the extract solution (65 ml). The viable bacteria on the culture medium were cultured at 37 °C for 24 h in the incubator. During the measurement of E, the electrodes (Pt, CuS and Ag_2S) and the reference electrode were placed in the remaining extract solution (60 ml), and each of the electric potentials (E_{Pt}, E_{CuS} and E_{Ag_2S} versus Ag/AgCl) was measured. The experimental conditions during this measurement were the same as that in Section 2.1.

Table 1
Composition of a culture medium

Reagent	Concentration (%)
Tryptone	1.0
Yeast extract	0.5
NaCl	1.0
Agar (powder)	2.0

3. Results and discussion

3.1. Measurement of putrescine and dimethyl sulfide

Fig. 1 shows a typical response curve of the Pt electrode in each Put concentration (10^{-1} to 10^{-5} mol l^{-1}), wherein the ordinate and abscissa denote the electric potential (E versus Ag/AgCl) and response time, respectively. Since, the response curve for each concentration was saturated at about 2000 s, E_{Pt} was regarded as the electric potential of the Pt electrode at that time. E_{Pt} decreased with the increasing Put concentration. Therefore, E_{Pt} may detect changes in the Put concentration, which increases with the progressing putrefaction in the meat [1–4], because E_{Pt} showed a dependence on the Put concentration. Similarly, the electric potential of the CuS electrode decreased with the increasing Put concentration. Fig. 2 shows the changes in E_{Pt} and E_{CuS} (E at 2000 s) as a function of the Put concentration. Each of them has shown a deferent E for each Put concentration, and decreased with the increasing Put concentration from about 10^{-5} mol l^{-1}. Fig. 3 shows the changes in the electric

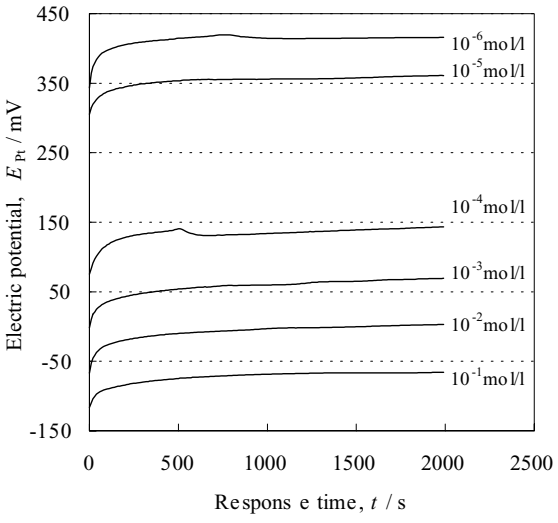

Fig. 1. Response curves of Pt dependent on Put concentration.

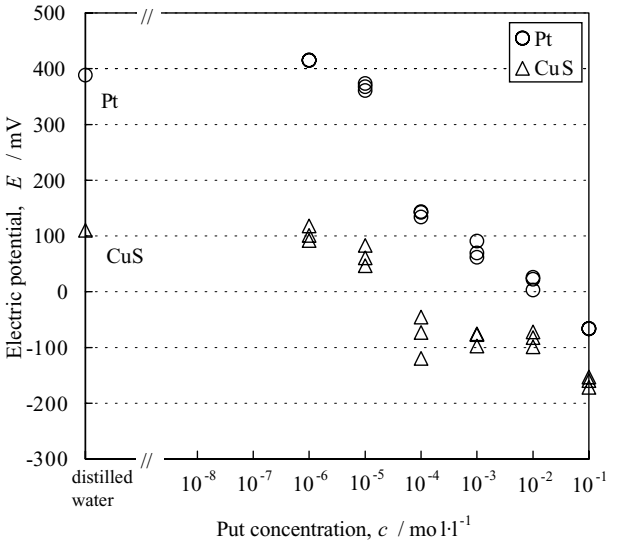

Fig. 2. Changes in electric potentials (E vs. Ag/AgCl) of Pt and CuS as a function of Put concentration.

potential of the Ag_2S electrode as a function of the DMS concentration. DMS is known as one of sulfides, which are produced by putrefaction in meat [8]. E_{Ag_2S} (E at 2000 s) decreased with the increasing DMS concentration from about 10^{-4} mol l^{-1}.

3.2. Measurement of viable bacterial counts and electric potential during putrefaction in pork

3.2.1. Measurement of viable bacterial counts (N)

Fig. 4 shows the changes in N (cells g^{-1}) as a function of the days of storage at 10 °C. N at 0 day was three orders of magnitude in most samples. This shows that the samples at 0 day are fresh, because the orders of magnitude are equal to that of N generally present in fresh meat [5]. N then becomes

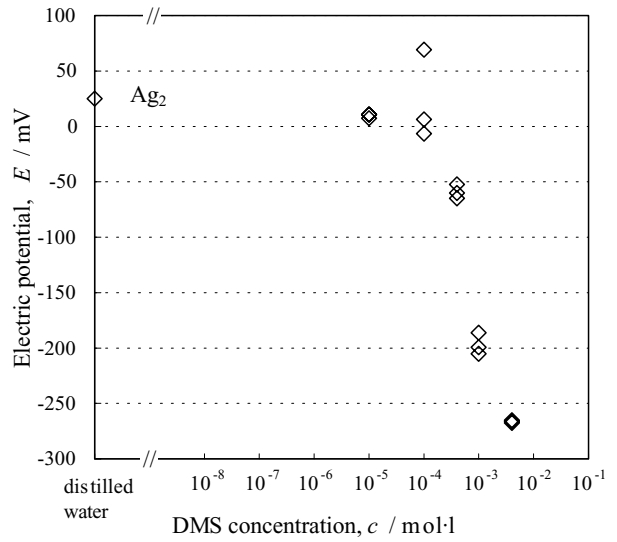

Fig. 3. Changes in electric potentials (E vs. Ag/AgCl) of Ag_2S as a function of DMS concentration.

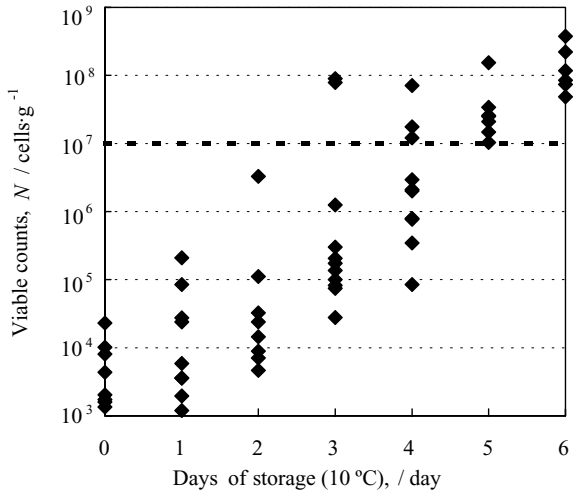

Fig. 4. Changes in viable counts (N) as a function of days of storage (10 °C) in real sample.

scattered, but N typically increased with the increasing days of storage. On some samples, N increased up to seven orders of magnitude that appeared when time of storage were 3 days. When the storage time was 5 days, N on all the samples was seven orders of magnitude. Since meat, on which N becomes 10^7 cells g^{-1}, generates a putrefaction odor and such meat is generally defined as being in the initial stage of putrefaction [5,9], all samples on and after 5 days of storage were completely putrefied. In this way, an increase in the days of storage at 10 °C means an increase in the viable bacterial counts and a decrease in the pork freshness.

3.2.2. Measurement of electric potential (E)

Fig. 5 shows the changes in the electric potential (E versus Ag/AgCl) measured on each electrode (Pt, CuS and Ag_2S) as a function of storage. In the figure, each E is the

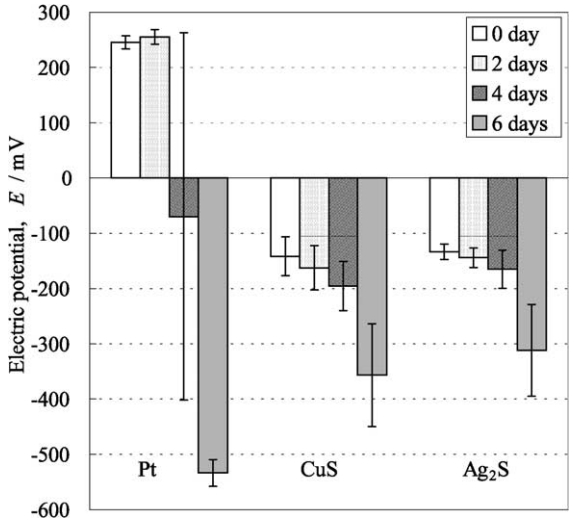

Fig. 5. Changes in electric potential (E vs. Ag/AgCl) measured on each electrode (Pt, CuS and Ag_2S) with days of storage.

average value of the measured values (sample number = 7) with storage time, and each error bar is their standard deviation. When the measured value is the value of the response curve at 2000 s similar to that in Section 3.1. E_{Pt} showed no changes from 0 to 2 days, but it decreased with the increasing storage time after 4 days. Especially, at 6 days, it significantly decreased from the plus value (0 day) to a minus value. Similarly, E_{CuS} and E_{Ag_2S} also showed a tendency to decrease with the increasing storage time. Both at 6 days were quite different values from that on day 0. Since the samples at 6 days have been ($N > 7$) completely putrefied (shown in Fig. 4), it seems that these response patterns depend on N on the pork. Therefore, measurement of E_{Pt}, E_{CuS} and E_{Ag_2S} (chemical indexes) may be roughly possible for that corresponding to N (freshness index) since E_{Pt}, E_{CuS} and E_{Ag_2S} decreased with an increase in N (the changes in E_{Pt} have been especially remarkable).

3.3. Discrimination of pork freshness by principal component analysis and multiple regression analysis

From the above-mentioned results, the measurement of E_{Pt}, E_{CuS} and E_{Ag_2S} (chemical indexes) may be useful as freshness indices corresponding to N. Therefore, we discussed whether the chemical indices measured by potentiometry are useful for evaluating pork freshness by using principal component analysis and multiple regression analysis.

3.3.1. Qualitative evaluation of pork freshness by PCA

PCA [10] was performed to obtain qualitative results for the dataset (E_{Pt}, E_{CuS} and E_{Ag_2S}). PCA is a linear feature extraction method that consists of projecting the m-dimensional dataset, m being the number of sensors, in a dimension smaller than m [11]. The uncorrelated and orthogonal coordinates of this reduced space are the eigenvectors (principal components) of the covariance matrix of the dataset [11]. These new variables are more descriptive because they are chosen to describe the maximum amount of variance in a data matrix [11]. The eigenvalue of a principal component is directly related to the percentage of "information" contained in the corresponding component, so that only the most relevant components can be preserved [11]. Therefore, PCA is a very useful classification or evaluation technique for food-sensing [11–21].

Fig. 6 shows the plot of scores as a function of the first and second principal components (PC1 and PC2) for E_{Pt}, E_{CuS} and E_{Ag_2S} (E at 2000 s). Since the contribution rate of the PC1 was about 96%, the PC1 was able to explain the information of most datasets. In this figure, since the PC1 for $\log(N) = 7$ was <-1, the value (PC1 = -1) shows a threshold line about putrefaction in the pork such as fresh or putrid pork. In this way, measurement (potentiometory) and analysis (PCA) of E_{Pt}, E_{CuS} and E_{Ag_2S} will be useful for qualitatively showing the degree of the pork freshness.

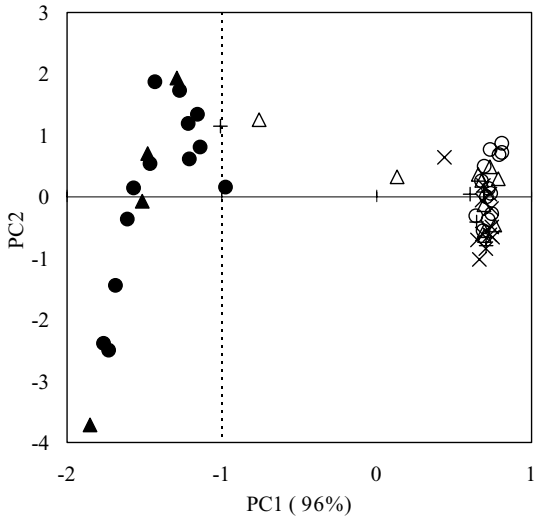

Fig. 6. Results of principal component analysis of the plural electrodes responses to viable bacterial counts of the pork, bacterial counts: (○) 10^3 orders; (×) 10^4 orders; (△) 10^5 orders; (+) 10^6 orders; (●) 10^7 orders; (▲) 10^8 orders.

3.3.2. Quantitative evaluation of pork freshness by MRA

MRA was performed to obtain quantitative results for the dataset. MRA is a very useful method to obtain the experimental predicting or controlling criterion variables by determining a linear expression explaining the relationship between the criterion and explanation variables [22]. The correlation coefficient between the experimental Y_{exp} and predicted Y obtained from the computed multiple regression models is designated as the multiple correlation (R) [23]. R^2, a multiple determination coefficient, expresses the explained ratio of variation in Y from the multiple regression model [23].

Eq. (1) is a multiple regression equation obtained by MRA:

$$\log(Y) = 5.239 - 3.82 \times 10^{-3} E_{Pt} - 3.72 \times 10^{-3} E_{CuS} + 2.49 \times 10^{-3} E_{Ag_2S} \quad (1)$$

Since the coefficient of determination (R^2) for Eq. (1) was 0.762, Y (predicted value) determined by Eq. (1) explained N (experimental value) very well. Fig. 7 shows the $\log(Y)$ obtained from Eq. (1) is plotted as a function of $\log(N)$. In this figure, since a dotted line is a line corresponding to $\log(Y)$:$\log(N)$ = 1:1, the line shows that if $\log(Y)$ fit the line better, both fit themselves better. $\log(Y)$ for $\log(N)$ = 3–7 has a difference from the line, but $\log(Y)$ for $\log(N) = 7$ is on the line. Therefore, the measurement and analysis of E_{Pt}, E_{CuS} and E_{Ag_2S} by using potentiometry and MRA will be useful for quantitatively showing the degree of the pork freshness.

Measurement of the pork freshness by using potentiometry will be able to be widely applied in the meat industry, because a potentiometric sensor for measuring can be a handy type and low cost and it can measure the pork freshness in

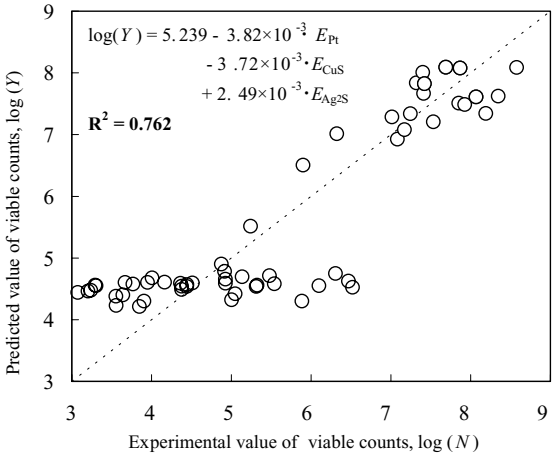

Fig. 7. Relationship between experimental values (*N*) and predicted values (*Y*) of viable bacterial counts using multiple regression analysis of the plural electrodes responses.

situ, simply and rapidly than other meat freshness sensors such as a biosensor.

4. Conclusions

We have summarized the following conclusions from measuring E_{Pt}, E_{CuS} and E_{Ag_2S} in putrescine, dimethyl sulfide and the extract solution using a potentiometric sensor by PCA and MRA:

1. E_{Pt}, E_{CuS} and E_{Ag_2S} decreased depending on putrescine and dimethyl sulfide, which are produced by the putrefaction of meat, the concentration being regarded as the chemical indices.
2. *N* on the pork stored at 10 °C increased with the increasing storage time in days. On the other hand, E_{Pt}, E_{CuS} and E_{Ag_2S} in the extract solution, which was obtained from the pork, decreased with the increasing storage days.
3. *N* on the pork in cold storage was predicted using potentiometry, PCA and MRA, both qualitatively and quantitatively. Therefore, E_{Pt}, E_{CuS} and E_{Ag_2S}, which correspond to the chemical indices, obtained by potentiometry may be able to evaluate the pork freshness (the meat freshness index) by using PCA and MRA.

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