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# Improved sensitivity of acetaldehyde biosensor by detecting ADH reverse reaction-mediated NADH fluoro-quenching for wine evaluation

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**Abstract**

Acetaldehyde (AcH) is found in ambient air, foods and living body. This toxic substance is also contained in wine and known as an important ingredient affecting the quality of wine. Herein, we constructed and evaluated two different fiber-optic biosensors for measurement of AcH in the liquid phase (AcH biosensor) using aldehyde dehydrogenase (ALDH) or alcohol dehydrogenase (ADH). The AcH biosensor measured a concentration of AcH using fluorescence intensity of reduced form of nicotinamide adenine dinucleotide (NADH) that was produced or consumed via catalytic reaction of the respective enzyme. In the AcH measurement system, an ultraviolet light emitting diode (UV-LED) and photomultiplier tube (PMT) were connected to a bifurcated optical fiber and were used to excite and detect NADH. A sensing region was developed using an optical fiber probe and an enzyme-immobilized membrane, buffer pH and concentrations of a coenzyme in buffer solution for ALDH forward reaction and ADH reverse reaction were optimized, and the dynamic ranges were compared. ADH-mediated AcH biosensor showed higher sensitivity, wider dynamic range (1–500 μM) and capability of rapid measurement (less than 3 min) than ALDH-mediated AcH biosensor (5–200 μM) were observed. ADH biosensor also presented a high selectivity and allowed measurement of AcH in 9 different wine samples (5 red and 4 white wines). The determined concentrations were comparable to those measured by NADH absorbance method, which validated the accuracy of the ADH biosensor in AcH measurement.

Acetaldehyde (AcH, $CH_3CHO$) is one of the volatile chemical compounds (VOCs) that generally presents in the environment. This toxic substance is also produced during the process of ethanol metabolism in human body. An animal experiment with long-term administration of AcH to rats conducted in 2002 reported a significant increase in malignant tumors and carcinogenicity of AcH.[1] Also, AcH has been pointed out to DNA toxicity, which can cause various diseases.[2] The International Agency for Research on Cancer (IARC) reported that AcH is highly likely to be carcinogenic to humans.[3,4] AcH was found in rainwater,[5] river water,[6] lake,[7] seawater,[8] plants,[9] human saliva[10] and blood.[11] AcH is also naturally contained in foods such as fruits and vegetables,[12] dairy products,[13] various beverages,[14] and sometimes it is used as a food additive since it has a fruit-like flavor.[15] Particularly, the wine using yeast fermentation during a production process, researchers have found that about 90% if aldehydes in wine was AcH, and its concentration affect color, taste and aroma of wine.[16,17] In general, a high concentration of AcH shows unpleasant irritating odor and impairs the aroma of wine. Furthermore, a high concentration of it in wine during brew process causes suppression of yeast function and decreasing of alcohol fermentation rate.[18] Therefore, it is necessary to add sulfur dioxide ($SO_2$) to control unnecessary yeast function and to remove the odor of AcH in wine.[19] In quality control for wine making, monitoring of AcH concentration plays an important role because its concentration is fluctuated with temperature,[20] pH,[21] concentrations of $O_2$[22] and $SO_2$.[23]

Generally, chromatographic methods including gas chromatography (GC), GC with flame ionization method (GC-FID),[24] high performance liquid chromatography (HPLC)[25] and reverse phase-HPLC (RP-HPLC),[26] have been used for determination of AcH in liquid samples. These analytical instruments usually have a high sensitivity and selectivity to enable detecting trace concentration of AcH in mixture, but it is not practical to use them at a winery for quality control since they are expensive, require expertise, need pretreatment of samples

and long-time for the measurement. Also, since a wine contains a great variety of chemical substances compared with other alcoholic beverages, high selectivity is essential for the measurement.

An enzymatic biosensor that has high selectivity and sensitivity is a good candidate for determination of AcH in mixture solution. Nicotinamide adenine dinucleotide (NAD)-dependent enzyme is useful for developing a biosensor. Avramescu et al. have been reported that amperometric biosensor using lactate dehydrogenase and aldehyde dehydrogenase (ALDH) for control of wine quality.[27] In this biosensor, AcH is detected as an electric current that originates in electron transfer caused by a redox reaction of NAD when AcH is catalyzed by ALDH. Also, Zhang et al. developed self-powered AcH sensor based on biofuel cell using alcohol dehydrogenase (ADH) as an amperometric biosensor.[28] These biosensors have a high selectivity based on substrate specificity of enzyme, but selectivity of amperometric biosensor could be affected by electroactive species such as ascorbic acid and uric acid caused by electrochemical oxidization.[29]

In our previous studies, we have developed an optical biosensor that allows selective detection of ethanol and isopropanol in mixture solution.[30,31] This sensor used the catalytic reaction of NAD-dependent dehydrogenase that was immobilized on an insoluble carrier membrane and was used to determine the concentration of targets through the fluorescence intensity of reduced NAD (NADH). Optical measurement of NADH is not interfered by electroactive species, thus higher selectivity could be possible to obtain. In this study, based on this technology, we developed a biosensor for AcH in the liquid phase (AcH biosensor). First, we developed two different AcH biosensors employing ALDH or ADH for detection of AcH via fluorescence intensity of NADH. Then, after conditions, including buffer pH and concentration of coenzyme, was optimized, performances of the sensor were compared and the better one was chosen. The selectivity of AcH biosensor was investigated by the wine,

which with various compounds. Finally, the sensor was applied for measurement of AcH in 9 different wine samples (5 red and 4 white wines).

## Experimental Section

### Materials and reagents

Aldehyde dehydrogenase (ALDH, EC 1.2.1.5 from *yeast*, 20 unit/mg solid, 10171832001) and alcohol dehydrogenase (ADH, EC 1.1.1.1, from *Saccharomyces cerevisiae*, 369 unit/mg solid, A7011) were purchased from Roche diagnostics (Germany) and Sigma-Aldrich (USA), respectively. Hydrophilic polytetrafluoroethylene (H-PTFE, porosity of 80 %, pore size of 0.2 μm, thickness of 80 μm, ommnipore membrane filters, HGWP14425) for a substrate of enzyme immobilization was from Millipore (USA). A polymer immobilizing ADH or ALDH in the H-PTFE membrane, poly[2-methacryloyloxyethyl phosphorylcholine (MPC)-co-2-ethylhexyl methacrylate (EHMA)] (PMEH), was synthesized in house by free radical polymerization method.[32] Reduced (NADH, No. 44327000) and oxidized form ($NAD^+$ No. 44057000) β-nicotinamide adenine dinucleotide were from Oriental Yeast (Japan). Buffer chemicals including acetic acid (99.7%, 017-00256), sodium acetate (98.5%, 192-01075), potassium dihydrogen phosphate (99.5%, 169-04245), sodium hydrogen phosphate (99.0%, 197-02865), 2-amino-2-hydroxymethyl-1,3-propanediol, (99.9%, 013-16385), hydrochloric acid (35%, 083-03485), sodium hydrogen carbonate (99.5%, 191-01305), sodium carbonate (99.8%, 199-01585) and standard AcH solution (90%, 015-09576) were purchased from Wako (Japan). All of the buffer solutions were prepared with ultrapure water obtained by Mill-Q purification system from Millipore (USA). Chemical substances used for investigating a selectivity of the biosensor were malic acid (99.05%, 135-00562, Wako, Japan), tartaric acid (99.5%, 03045, Yoneyama

yakuhin kogyo, Japan), succinic acid (99.5%, 190-04332, Wako, Japan), citric acid (99.5%, 251275, Sigma-Aldrich, USA), glycerol (87%, Kenei, Japan), glucose (98.0%, 049-31165, Wako, Japan) and ethanol (99.5%, 14033-00, Kanto Chemical, Japan). All of the wines were purchased at a local store in Japan.

**Construction of acetaldehyde measurement system**

Figure 1 shows detection principles of AcH for the (a) ALDH-mediated and (b) ADH-mediated AcH biosensors. In the reaction of ALDH, $NAD^+$ is reduced to NADH as an electron acceptor when AcH is oxidized to acetic acid. On the other hand, NADH is oxidized to $NAD^+$ when AcH is reduced to ethanol in the reaction of ADH. The most well-known catalytic reaction of ADH is to oxidize ethanol to produce AcH; this reaction was defined as a forward reaction of ADH. Vice versa, a reaction reducing AcH to produce ethanol was defined as a reverse reaction of ADH in this paper. Incidentally, ALDH is not capable of catalyzing to reduce acetic acid to AcH as a reverse reaction according to Black's research.[33] It is known that NADH used in both enzymatic reactions show auto-fluorescence at a wavelength of 490 nm by excitation with ultraviolet (UV) light at a wavelength of 340 nm. In contrast, $NAD^+$ does not exhibit such a fluorescence property. Therefore, detecting NADH selectively by measuring the fluorescence intensity at 490 nm is feasible. Since the fluorescence intensity of NADH depends on its concentration that is correlated with AcH concentration, it is possible to quantify AcH by measuring the fluorescence intensity of NADH in both enzymatic reactions.

**(Figure 1 comes here)**

AcH biosensor was constructed with an UV light emitting diode (UV-LED, λ of 335 nm, Sensor Electronic, USA) with a stabilized DC power source (Yokogawa, Japan) as a NADH excitation light source, a photomultiplier tube (PMT, Hamamatsu photonics, Japan) as a fluorescence detector and a bifurcated optical fiber (Ocean Optics, USA) to connect UV-LED and PMT. Two band pass filters were used on the UV-LED ($BPF_{ex}$, 340 ± 10 nm, MX0340, Asahi spectra, Japan) and PMT side ($BPF_{fl}$, 490 ± 10 nm, MX0490, Asahi spectra, Japan) for getting rid of unnecessary light, which improved the signal to noise ratio for photodetection of NADH. An optical fiber probe (F1000-900, Ocean optics, USA) with the enzyme-immobilized membrane was connected to the common end of the bifurcated fiber (Figure 2). The detail of enzyme-immobilized membrane was prepared as described in detail in our previous article.[30] Briefly, ADH (2.1 units/$cm^2$) or ALDH (2.1 units/$cm^2$) was dissolved in phosphate buffer solution (PB, pH 7.0, 0.1 M), and this enzyme solution was mixed with PMEH solution (10 % w/w in ethanol, 10 μL/$cm^2$). Then, the mixture was spread onto a cleaned H-PTFE membrane surface uniformly, followed by being cured in a refrigerator at 4°C for 3 hours. Afterward, the superfluous enzyme was rinsed by PB. Finally, the enzyme-immobilized membrane was cut out into 0.5 × 0.5 $cm^2$ and fixed onto the fiber probe by silicone tube (9-689092, outer diameter of 2 mm, inner diameter of 1 mm).

**(Figure 2 comes here)**

**Measurement of standard acetaldehyde solution by ALDH and ADH biosensors**

At first, ALDH and ADH biosensors were investigated to confirm whether AcH could be detected by each enzyme reaction. In the experiment, the biosensor was dipped in middle of a black cuvette (acryl disposal cell, semi-micro type, BRA759116,

As One, Japan) painted in black by acryl paint (blue-black, TURNER, Japan) and filled with coenzyme solution: 300 μM of $NAD^+$ in PB (300 μL, pH 8.0, 0.1 M) for the ALDH and 300 μM of NADH in PB (300 μL, pH 6.0, 0.1 M) for the ADH, respectively. Change in fluorescence intensity that emitted from NADH at around the enzyme-immobilized membrane before and after adding 1, 10, 100 and 1000 μM of AcH solution was measured.

**Optimization of reaction condition of ALDH and ADH**

Enzyme activity is influenced by pH value and coenzyme concentration of solutions, and each enzyme has different optimal conditions. Therefore, optimizing these conditions for ALDH and ADH is important in the development of AcH biosensor. At first, 100 μM of AcH was measured in various pH to decide an optimum pH at each enzyme. A coenzyme solution of 300 μM $NAD^+$ was prepared with different pH buffer solutions, including acetate buffer (AB, 0.1 M, pH 3.0, 4.0, 5.0 and 5.5), phosphate buffer (PB, 0.1 M, pH 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0) and carbonate-bicarbonate buffer (CB, 0.1 M, pH9.2, 9.5, 10.0 and 11.0) for ALDH forward reaction. Likewise, a coenzyme solution of 300 μM NADH in acetate, PB, CB was used for ADH reverse reaction. In ADH-mediated detection, it is necessary to consider an influence of the forward reaction because the reaction is reversible depending on solution pH. Thus, the effect of the solution pH for ADH forward reaction was investigated by measuring the fluorescence intensity when adding 100 μM ethanol into a coenzyme solution of 300 μM $NAD^+$ in acetate, PB, Tris-HCl buffer (TB, 0.1 M, pH 8.5, 9.0 and 9.5) and CB. Finally, influence of ethanol in ADH reverse reaction was investigated by adding 100 μM ethanol into an optimum coenzyme solution of 300 μM NADH.

Influence of a coenzyme concentration in coenzyme solution was also investigated. Various concentration of $NAD^+$ (0.1, 0.5 and 1 mM in 0.1 M of PB at pH 8.0) and NADH

(0.1, 0.5 and 1 mM in 0.1 M of PB at pH 6.5) were used to evaluate calibration curves of ALDH and ADH biosensor for various concentration (100 nM to 1 mM) of standard AcH solution.

**Evaluation of selectivity to the chemicals in wine**

In order to measure AcH in wine, a selectivity of the ADH biosensor was evaluated with main chemical substances contained in wine. Very diverse ingredients have been found in wine, but substances which account for about 90% of them are glycerol (76.0 mM), glucose (43.1 mM), malic acid (29.8 mM), tartaric acid (16.3 mM), succinic acid (10.5 mM), AcH (1.6 mM) and citric acid (1.3 mM).[34–36] Therefore, selectivity was examined using the above listed components and concentrations. In addition, taking into consideration that dilution is practically necessary for the measurement of wine samples, measurement was made at 10-fold dilution of each substance. In the experiment, the end of the ADH biosensor was immersed in a black cuvette filled with 270 μL of NADH solution (500 μM in 0.1 M PB, pH6.5), and then fluorescence intensity was measured when a sample solution prepared with PB was added into the cuvette.

**Measurement of AcH in wine**

The concentration of AcH contained in wine was determined using developed ADH biosensor. The NADH absorbance method[37] was used as a comparison, in order to confirm reliability of the measured concentration by the ADH biosensor. Characteristics of each sample are shown in Table 1. In preparation of the sample, it has been reported that it is necessary to decolorize a red wine sample if it has an optical density of more than 0.5 at 340 nm.[38] However, when the sample prepared this time was diluted 10-fold, no pretreatment was needed because OD was much less than 0.5 in all samples (Figure S-2). In this experiment,

AcH sensor tip was immersed in 270 μL of NADH coenzyme solution (500 μM in 0.1 M PB, pH 6.5), and then 30 μL of wine was injected after the background was stabilized. Change in fluorescence intensity from the baseline after the sample injection was defined as $\Delta I$, and the concentration of AcH in the wine sample was determined from the calibration curve.

In NADH absorbance method, firstly 135 μL of a NADH coenzyme solution (1 mM in 0.1 M PB, pH 6.5) was dispensed into a micro tube, and then 30 μL of wine was added to the tube. Three minutes after vortexing this mixed solution (NADH-wine solution), absorbance at a wavelength of 340 nm (*A1*) was measured by a spectrophotometer (nanodrop 2000, Thermo Fisher Scientific, USA). Afterward, 135 μL of ADH solution (4.2 units in 0.1 M PB, pH 6.5) was added to the NADH-wine solution and the mixture (NADH-wine-ADH solution) was vortexed and let the solution stand for 10 minutes. Then the absorbance of NADH-wine-ADH solution ($A_2$) was measured. Finally, AcH concentration of the wine was determined by substituting the value of $A_1$ - $A_2$ into a calibration curve prepared using a standard AcH solution. In addition, in order to confirm whether it was properly quantified, NADH-wine solution spiked with 100 μM and 500 μM of AcH were prepared, and the absorbance was measured.

**(Table 1 comes here)**

## Results and discussion

### Sensor responses using ALDH forward reaction and ADH reverse reaction

At first, it was investigated whether AcH biosensor using ADH or ALDH shows a response to AcH in the liquid phase. Figure 3 shows the responses to AcH, and it was observed that the sensor output before injecting AcH was stable in the both biosensors. In ALDH biosensor, increase in the fluorescence intensity accompanied by dropping of AcH

solution. In contrast, in the ADH biosensor, decrease in the fluorescence intensity was observed. These were attributed to NADH production in ALDH-mediated reaction and consumption in the ADH-mediated, respectively. The output signal $\Delta I$ in both sensors were defined as the difference between the average intensity from 2.5 to 3 minutes after the injection and the baseline. $\Delta I$ showed dependence on the concentration of AcH in both sensors, which suggested the possibility of quantifying the AcH concentration based on the fluorescence intensity of NADH produced or consumed by the forward reaction of ALDH and the reverse reaction of ADH.

**(Figure 3 comes here)**

**Optimization of solution conditions and selection of a suitable enzyme for AcH biosensor**

Figure 4a shows normalized intensities of ALDH biosensor to 100 μM of AcH in coenzyme solution adjusted to pH range of 3–11. The intensity peaked when using pH 8.0 and drastically decreased at the other pH; thus, it was decided to carry out subsequent experiments at pH 8.0 for ALDH sensor. Similarly, Figure 4b shows the results for the ADH sensor. In the graph, the filled markers show the outputs in the reverse reaction of ADH, and indicated that the reduction of AcH by ADH reached a maximum at pH 6.5. Also, when 100 μM of ethanol was injected to the optimum solution (500 μM NADH in 0.1 M PB, pH 6.5), no output was observed, which suggested that forward reaction of ADH did not occur under this optimum condition of the solution. Although this result was obtained without $NAD^+$ in the buffer solution, even in the measurement of AcH via ADH reverse reaction that produces ethanol and $NAD^+$ ADH forward reaction would neither occur nor interfere the sensor outputs from following reasons: First, according to Dickenson *et al.*,[39] a product inhibition probability of ADH reverse reaction is 1/7000 of that of ADH forward reaction, which indicates the reverse

reaction is unlikely to be affected by ethanol; second, Figure 3b shows that fluorescence intensity was stable after it reached plateau even when applying high concentration of AcH (1 mM). Suppose that the forward reaction remarkably occurred, the fluorescence intensity would increase because of producing NADH.

The outputs of the ADH biosensor were kept close to the maximum at a slightly acidic condition (pH 6.0–7.0), while the ALDH was decreasing about 20% of the signal at pH 6.0. This result implies that the ADH biosensor is suitable for measurement of AcH in wine because wine is usually slightly acidic (pH of 2.9–3.9).

**(Figure 4 comes here)**

Here we investigated the influence of coenzyme concentration. In ALDH biosensor, when the concentration of $NAD^+$ was 100 μM, AcH solution could be measured from 1 μM and the output reached saturation at the concentration above 20 μM due to lack of $NAD^+$. In the cases of using 500, 1000 μM of $NAD^+$, there was no difference in the dynamic range (5–200 μM), thus we decided to select 500 μM of $NAD^+$ in the ALDH biosensor to reduce the consumption of the coenzyme (Figure S-1a). In ADH biosensor, dynamic range can be adjusted by the concentration of NADH in coenzyme solution (Figure S-1b): dynamic range of 0.5–20 μM (100 μM NADH), 1–500 μM (500 μM NADH) and 20–1000 μM (1000 μM NADH). The lowest concentration of AcH (0.5 μM) was detected by using 100 μM of NADH. The broadest dynamic range was achieved by using 500 μM of NADH, thus we selected 500 μM of NADH in following experiment since reported concentrations of AcH in 10-fold diluted wines were 9–481 μM.[35]

Figure 5 shows calibration curves of ALDH and ADH biosensors for AcH solution obtained under the optimum conditions. A dynamic range of each sensor was determined as

the range where the correlation coefficient of a fitting curve is over 0.999. ADH biosensor showed a wider dynamic range of 1–500 μM with respect to that of ALDH biosensor (5–200 μM).

**(Figure 5 comes here)**

**AcH concentration in wine**

Figure 6a describes the relative sensor outputs to the 10-fold diluted substances, which exist in approximately 90% of red wine. The output of 160 μM AcH was defined as 100%, and the others were divided by the AcH value. The ADH-mediated AcH biosensor showed an obvious output from the AcH solution, while it was rarely observed from the other substances. It validated a high selectivity of the ADH biosensor to AcH that was based on the substrate specificity of the enzyme. Although, in general, ADH shows an activity against various chemical substances that with aldehyde groups, it was reported that about 90% of aldehydes contained in wine was AcH[16]. Therefore, other aldehydes were not considered in this investigation.

Figure 6b shows the results of quantitative determination of the AcH concentrations in nine different wine samples (5 red and 4 white wines) by the ADH-mediated AcH biosensor. Note that the concentrations in Figure 6b represent those of undiluted wine samples which were obtained by multiplying the experimental data by ten. For comparison, AcH concentrations in the wines samples were also measured by a standard NADH absorbance method (Figure S-3a). As displayed in Figure 6b, determined AcH concentrations by both methods were consistent, which suggested that ethanol in the wine samples did not influence on AcH biosensor and the measurement was accurate. AcH concentrations in the red wines were slightly lower than those in the white, which were in good agreement with the previous

reports.[40] Reasons of difference in AcH concentration between red and white wines are mainly related to malolactic fermentation during the production process, which can degrade AcH in wine.[41] Malolactic fermentation is rarely applied to white wine for which sour taste is preferred since the fermentation converts malic acid with strong sourness to lactic acid, and adds a complexity in taste. On the other hand, malolactic fermentation is routinely used to impart rich flavor to almost all red wines and to improve storage stability. Therefore, red wines tend to show lower AcH concentration than that in white wines.

To further confirm the accuracy of the measurement, AcH concentrations of spiked samples prepared by adding a standard AcH solution of 100 μM and 500 μM to the wine were also measured. The recovery rates were 92 and 94%, and it proved the accuracy of the quantified AcH concentrations by the ADH biosensor. Additionally, the high correlation coefficient ($R = 0.999$) and probability ($p < 0.001$) between results of ADH biosensor and NADH absorbance method were observed (Figure S-4).

These results demonstrated some notable advantages of AcH biosensor with respect to conventional NADH absorbance method: AcH biosensor was 100-fold more sensitive than NADH absorbance method as the limit of quantification of conventional NADH absorbance method for AcH was 100 μM (Figure S-3b); the sensing part of AcH biosensor can be miniaturized and is reusable for several measurements with a single enzyme immobilized membrane, which allows saving enzyme consumption; AcH biosensor holds potential for continuous measurement of AcH, which is useful, e.g., for monitoring of AcH in wine through the production.

**(Figure 6 comes here)**

## Conclusions

In this study, a fiber-optic biosensor was developed for measurement of AcH in solution by combination of the NADH fluorescence detection system and the enzyme-immobilized membrane that exploited ALDH forward reaction or ADH reverse reaction. It showed a wider dynamic range (1–500 μM) of the ADH-mediated biosensor than that of the ALDH (5–200 μM). thus, the reverse reaction of ADH was selected to the AcH biosensor. High selectivity of the ADH-mediated AcH biosensor to AcH was validated by main substances contained in wine. Measurement of AcH in 9 different wines (5 red and 4 white wines) was also carried out, and accurate quantification by the ADH biosensor was demonstrated. The developed ADH biosensor provides a simpler and more rapid method to quantify AcH concentration in solution than conventional analytical instrument. Furthermore, it is capable of real-time measurement. This novel sensor not only can apply to analyze the concentration of AcH in wine but also can use for determination of AcH in body fluid for medical and health care related applications.[42]

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Supporting Information Available: The following files are available free of charge.

170609-AcH-sol-SI.docx

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**Graphics and caption**

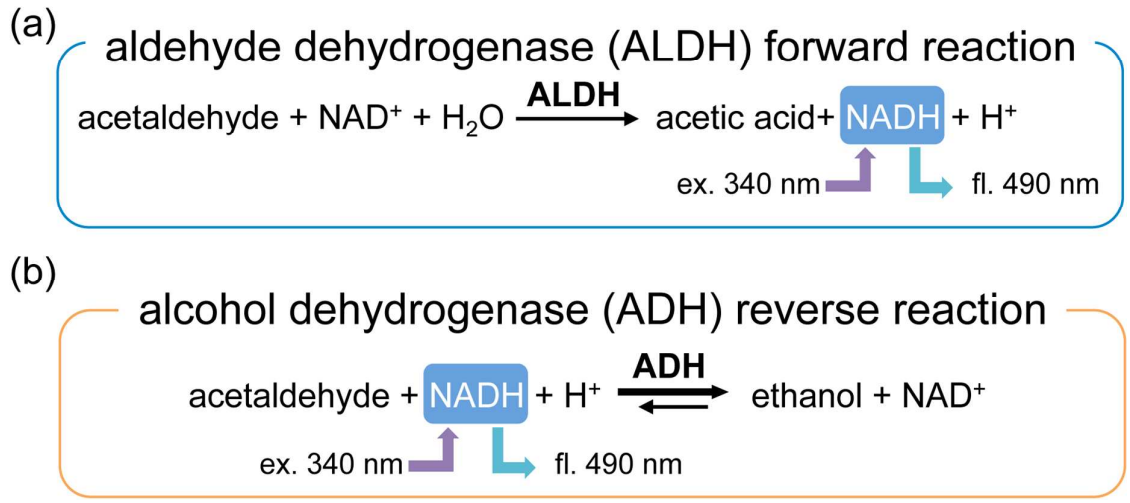



**Figure 1.** Detection principles of two different AcH biosensors. (a) ALDH forward reaction and (b) ADH reverse reaction. NADH absorbs UV light at the wavelength of 340 nm and emits visible fluorescence light at the wavelength of 490 nm.

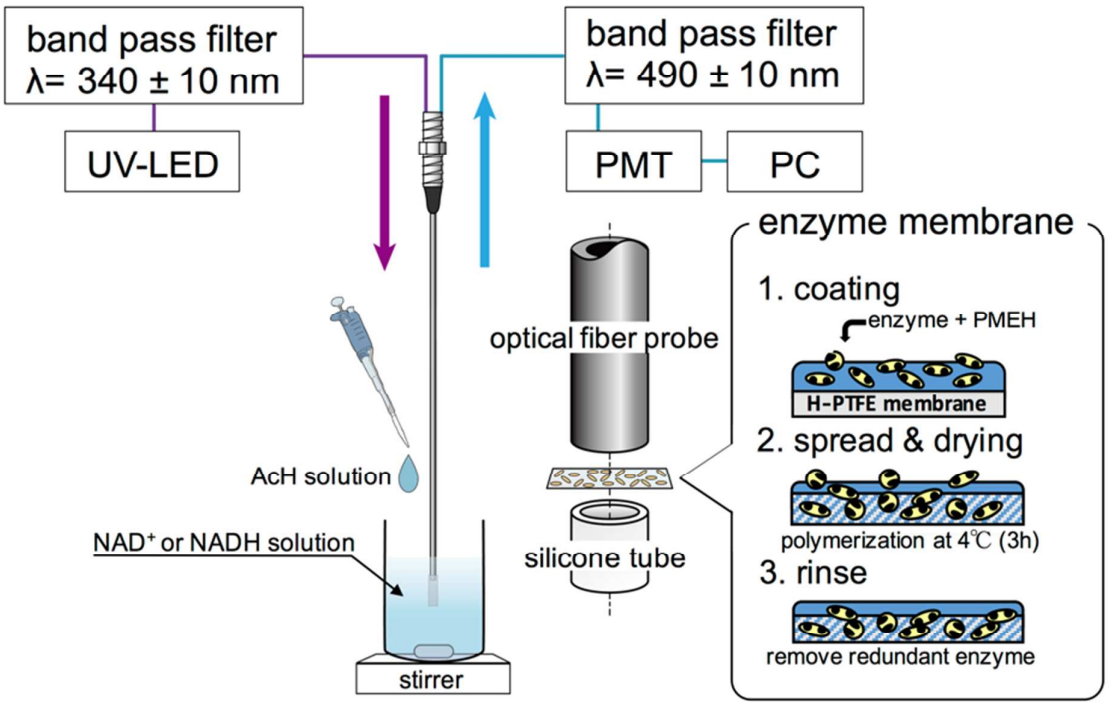



**Figure 2.** Schematic illustration of AcH biosensor including a UV-LED, PMT and optical fiber probe with an enzyme immobilized membrane. Enzyme immobilization for both ALDH and ADH were performed by physical entrapment with PMEH.

**Table 1.** Information of measured wine samples.

| ID | Type | Ethanol conc. (%) | Ethanol conc. (M) | Additives | pH | OD | |
|---|---|---|---|---|---|---|---|
| | | | | | | Original | 10-fold diluted |
| 1 | Red | 12 | 2.06 | $SO_2$ | 3.4 | 1.537 | 0.130 |
| 2 | Red | 13.5 | 2.32 | $SO_2$ | 3.6 | 1.515 | 0.186 |
| 3 | Red | 13 | 2.23 | $SO_2$ | 3.5 | 1.704 | 0.186 |
| 4 | Red | 13.5 | 2.32 | $SO_2$ | 3.5 | 1.453 | 0.165 |
| 5 | Red | 13 | 2.23 | $SO_2$ | 3.5 | 1.535 | 0.157 |
| 6 | White | 13 | 2.23 | $SO_2$ | 3.1 | 0.313 | 0.027 |
| 7 | White | 13 | 2.23 | $SO_2$ | 3.1 | 0.240 | 0.026 |
| 8 | White | 12 | 2.06 | $SO_2$ | 3.0 | 0.210 | 0.025 |
| 9 | White | 12.5 | 2.15 | $SO_2$ | 3.3 | 0.321 | 0.038 |

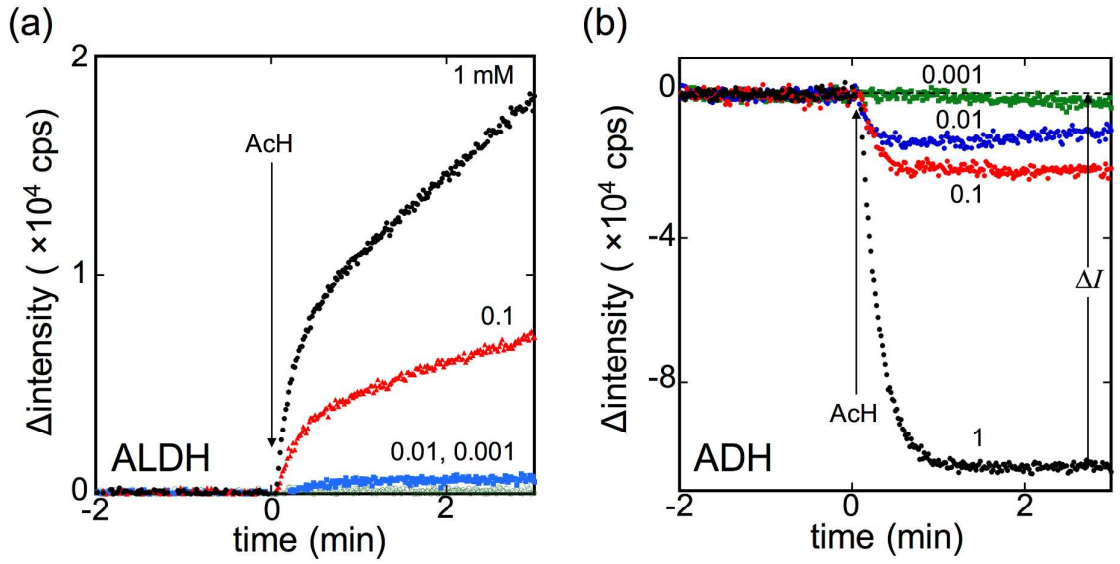



**Figure 3.** Responses of (a) ALDH-mediated and (b) ADH-mediated AcH biosensors to injection of AcH solution at different concentrations (0.001, 0.01, 0.1 and 1 mM). The sensor output $\Delta I$ was determined by averaging the fluorescence intensity from 2.5 to 3.0 min.

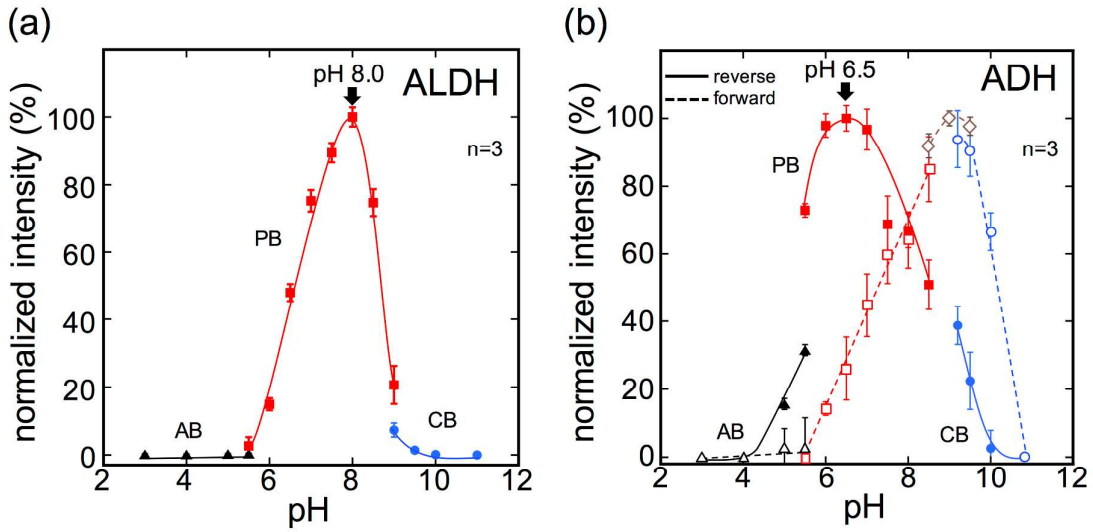



**Figure 4.** Dependence of (a) ALDH and (b) ADH activities to AcH on the buffer pH. (▲) acetate buffer, AB; (■) phosphate buffer, PB; (●) carbonate-bicarbonate buffer, CB; (◊)Tris-HCl buffer, TB. Solid and dashed lines in (b) indicate the reverse and forward reactions of ADH, respectively. The forward reaction was examined by injection of ethanol.

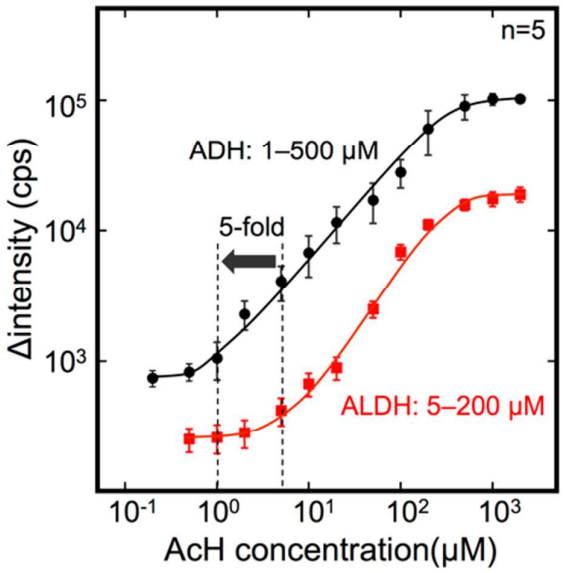



**Figure 5.** Dynamic ranges of the ALDH (■) and ADH biosensors (●) to AcH solution.

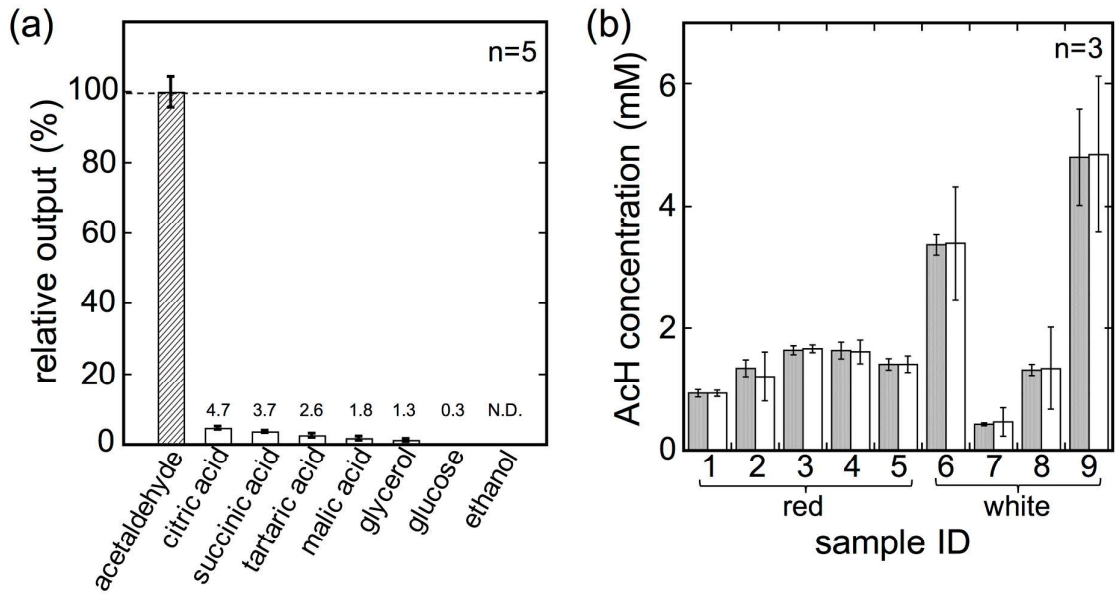
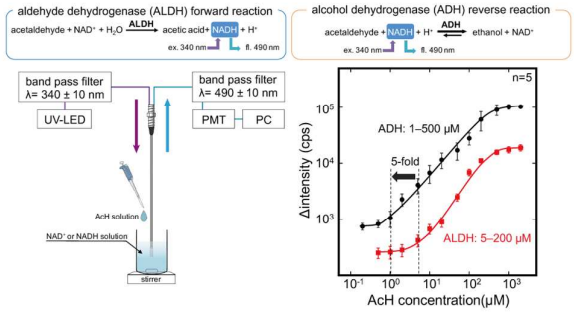



**Figure 6.** (a) Selectivity of the ADH biosensor against main chemical substances in red wine. Sensor output to each substance was normalized by that from AcH. (b) Measurement results of AcH in red (ID 1–5) and white wines (ID 6–9) using the ADH biosensor (filled) and NADH absorbance method (blank). The concentrations represent those of undiluted wine samples. The correlation between the concentrations determined by the ADH biosensor and those by NADH absorbance method is presented in Figure S-4.

for TOC only


aldehyde dehydrogenase (ALDH) forward reaction
acetaldehyde + NAD+ + H2O
ALDH
acetic acid + NADH + H+
ex. 340 nm
fl. 490 nm
alcohol dehydrogenase (ADH) reverse reaction
acetaldehyde + NADH + H+
ADH
ethanol + NAD+
ex. 340 nm
fl. 490 nm
band pass filter λ= 340 ± 10 nm
UV-LED
band pass filter λ= 490 ± 10 nm
PMT
PC
AcH solution
NAD+ or NADH solution
stirrer
n=5
ADH: 1–500 μM
5-fold
ALDH: 5–200 μM
Δintensity (cps)
AcH concentration(μM)