

Detection of acetone in milk through odor towards monitoring of ketosis in dairy cows

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Abstract—Determining subclinical ketosis of dairy cows is of great importance in terms of economic losses because ketotic cows even in subclinical ketosis reduce milk production. When dairy cows are affected by ketosis, they produce high level of ketone bodies including acetone, therefore, the detection of such ketone bodies is useful for diagnosis of ketosis. In this study, we demonstrated the detection of acetone in milk samples in the range from 1 to 10 ppm in the vapor phase, which corresponds to the acetone level of subclinical ketosis in a milk sample, using a nanomechanical Membrane-type Surface stress Sensor (MSS). It was proved that the signal output clearly correlates to the vapor concentration of acetone with ± 0.40 ppm root-mean-square deviation (RMSD). The present study provides a potential sensor for non-invasive monitoring of ketosis through milk odor.

Index Terms—milk, acetone, nanomechanical sensors, Membrane-type Surface stress Sensor (MSS), dairy cows, ketosis

I. INTRODUCTION

Dairy cows often suffer from ketosis due to dramatic changes in energy balance before and after calving. Ketosis in dairy cows reduces milk production [1]–[3]. In addition, subclinical ketosis, which is the elevated level of blood ketone bodies (i.e., β -hydroxybutyric acid (BHB), acetoacetic acid, and acetone) in the absence of clinical signs of ketosis, has become a problem in recent farm practice [4] because dairy cows in subclinical ketosis have been reported to produce less milk than healthy cows [5].

To diagnose ketosis, blood BHB levels have been used because measurements of BHB are stable and reproducible compared to other ketone bodies [3], [6]–[8]. However, blood tests require veterinary investigation and are expensive and time-consuming, making it difficult to quickly link the diagnosis of subclinical ketosis to treatment. Moreover, the blood tests are invasive and difficult to perform daily from an animal welfare perspective. For rapid on-site diagnosis, colorimetric methods such as test papers have been developed, but they are still difficult to use for daily monitoring of ketosis on the dairy farm because of cost and milk sampling issues.

In this study, we demonstrate that a nanomechanical sensor can be utilized for detecting acetone in the range of subclinical ketosis through milk odor. Since BHB and acetoacetic acid are less volatile, we focus on acetone as an indicator of

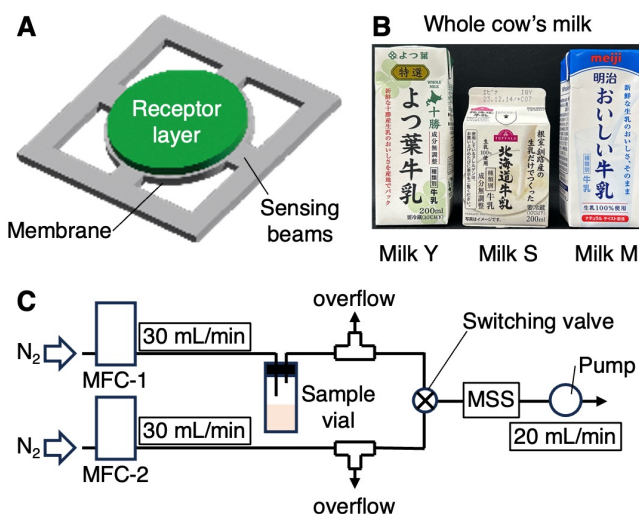


Fig. 1. Schematic illustrations of MSS and its sensing system. (A) Structure of MSS. (B) Photo of whole cow's milk samples. (C) Sensing system.

ketosis for monitoring ketotic cows. We used a nanomechanical Membrane-type Surface stress Sensor (MSS) as a sensing platform (Fig. 1A) [9]. By focusing on the purging process, the acetone can be detected within a couple of minutes at the concentrations ranging from 1 to 10 ppm, which corresponds to the level of subclinical ketosis milk samples. Since the nanomechanical sensors including MSS have various advantages on rapid and on-site measurements [10], this study provides a promising platform towards daily monitoring of clinical and subclinical ketosis in dairy cows.

II. EXPERIMENTAL

A. Preparation of MSS

In this study, we used an MSS as a sensing unit because of the high robustness and sensitivity (Fig. 1A) [9]. The construction of the MSS chips and its working principle have been previously reported [9]–[11]. Briefly, MSS consists of a silicon-based membrane suspended by four piezoresistive beams, composing a full Wheatstone bridge. The membrane is coated with a receptor material, which generates the surface stress caused by the sorption-induced expansion. The surface

stress on the membrane is transduced to the four sensing beams as amplified uniaxial stresses, resulting in the changes in the electrical resistance of the piezoresistors embedded in the beams. The output signal (V_{out}) can be expressed as [9]

$$V_{out} = \frac{V_B}{4} \left(\frac{\Delta R_1}{R_1} - \frac{\Delta R_2}{R_2} + \frac{\Delta R_3}{R_3} - \frac{\Delta R_4}{R_4} \right), \quad (1)$$

where V_B is the bridge voltage applied to the Wheatstone bridge circuit and $\Delta R_i/R_i$ ($i = 1-4$) is the relative resistance change in each sensing beam.

The MSS chip was provided from Asahi Kasei Corporation. AK02-04, which was provided by Asahi Kasei Corporation, and Tenax [12] were used as receptor layers. Each receptor material was coated directly onto the MSS membrane using an inkjet spotter according to the previous reports [12]–[14].

B. Sample preparation

To investigate the different concentrations of acetone in water and commercial whole cow's milk, aliquot of acetone was added to MilliQ water (Merck Millipore) or whole cow's milk (Milk Y, Yotsuba Milk Products Co., Ltd., Hokkaido, Japan; Milk S, Megmilk Snow Bland Co., Ltd., Kanagawa, Japan; Milk M, Meiji Co., Ltd., Tochigi, Japan) (Fig. 1B) at the concentrations of 0.1, 0.2, 0.3, 0.4, and 0.5 mM. The commercial whole milk samples have been pasteurized at least 120 °C for 2 s. Averaged nutritional compositions are listed in Table I.

To determine the acetone concentration in the vapor phase at 25 °C, proton transfer reaction-time of flight-mass spectrometer (PTR-TOF-MS; PTR-TOF 6000 X2, Ionicon Analytik GmbH) equipped with Static Headspace Autosampler was used. The detailed measurement conditions are described in the previous report [12]. The mass spectrum was recorded in the mass range of $m/z = 9-400$, and mass calibration was performed using two ion peaks of known exact masses, i.e., hydronium ion isotope ($H_3^{18}O^+$; $m/z = 21.022$) and diiodobenzene fragment ($C_6H_4I_2H^+$; $m/z = 203.943$). The measured concentrations are summarized in Table II.

TABLE I
NUTRITIONAL COMPOSITIONS OF WHOLE COW'S MILK SAMPLES
USED IN THIS STUDY.

Composition per 100 mL ^a	Whole cow's milk		
	Milk Y ^b	Milk S ^b	Milk M ^b
Non-fat solids	≥8.5%	≥8.4%	≥8.3%
Fat	≥3.7%	≥3.7%	≥3.5%
Energy (kJ)	293	287	287
Protein (g)	3.50	3.40	3.40
Fat (g)	4.05	3.90	3.90
Carbohydrate (g)	4.85	4.95	4.95
Dietary fibre (g)	—	0	—
Salt (mg)	105	100	110
Calcium (mg)	117	114	114

^a Average value through a year provided by each company.

^b Milk Y, Yotsuba; Milk S, Megmilk Snow Bland; Milk M, Meiji.

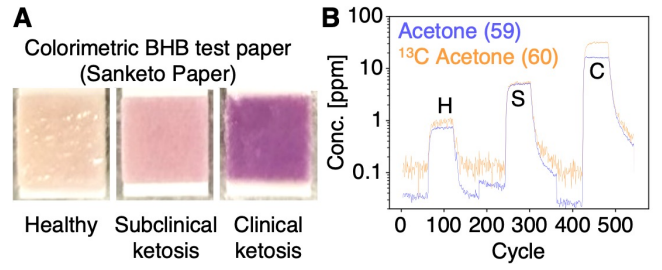


Fig. 2. Evaluation of the acetone concentration range in a subclinical ketotic cow. (A) Photos of colorimetric BHB test papers (Sanketo paper) of healthy, subclinical, and clinical ketosis cows. (B) Results of PTR-TOF-MS. Vapor concentration of acetone of milk samples from healthy, subclinical ketosis, and clinical ketosis cows. H, healthy; S, subclinical; C, clinical.

C. Sensing

The MSS chip was placed in an MSS standard measurement module produced by the industry-academia-government framework called “MSS Alliance” [15]–[17]. The MSS module consists of a Teflon chamber, an aspiration pump, and a switching valve with sampling and purging lines (Fig. 1C). All measurements by the module were performed in an incubator kept at 25.00 ± 0.02 °C. The headspace vapor in a 20 mL sample vial, in which 10 mL of each sample was placed, was flowed by mass flow controller 1 (MFC-1) with flow rate at 30 mL min^{-1} . The flow rate of the aspiration pump was set at 20 mL min^{-1} . Before measuring MSS signals, pure nitrogen gas was introduced into the module for at least 2 min to recover the original baseline. Subsequently, the valve was switched to the sampling line for 1 min and then switched back to the purging line for 4 min. Sensing signals of MSS were measured at the bridge voltage (V_B) of -3.0 V and recorded at a sampling rate of 10 Hz.

III. RESULTS AND DISCUSSION

To evaluate the range of vapor phase concentration of acetone in milk of subclinical ketosis cows, we measured the milk samples collected from healthy, subclinical ketosis, and clinical ketosis cows. Dairy cows were examined for ketosis based on BHB as an indicator using a test paper (Fig. 2A). The concentrations of acetone in the vapor phase

TABLE II
CONCENTRATIONS OF ACETONE IN WATER AND WHOLE MILK SAMPLES
DETERMINED BY PTR-TOF-MS.

Concentration in liquid [mM] ^a	Concentration in vapor phase [ppm]			
	water	Milk Y ^b	Milk S ^b	Milk M ^b
0.00	0.05	0.58	0.60	0.49
0.10	1.13	1.72	1.86	1.70
0.20	2.22	2.85	3.18	2.98
0.30	3.38	4.04	4.47	4.22
0.40	4.48	5.17	5.80	5.59
0.50	5.59	6.34	7.08	6.95

^a Concentration of acetone added to water or milk samples.

^b Milk Y, Yotsuba; Milk S, Megmilk Snow Bland; Milk M, Meiji.

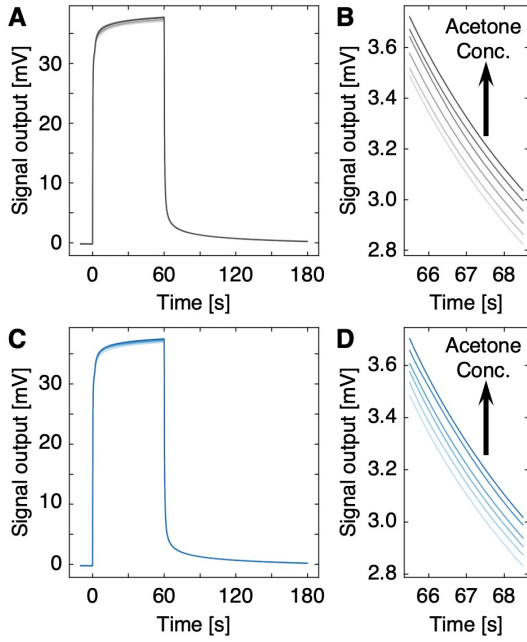


Fig. 3. MSS signal outputs. (A) Signal responses of MSS to aqueous solutions of acetone. (B) Magnified signal responses to aqueous solutions of acetone with different concentrations in the purging process at around 67 s. (C) Signal responses to acetone-Milk M mixtures. (D) Magnified signal responses to acetone-Milk M mixtures with different concentrations of acetone in the purging process at around 67 s.

of milk samples were determined by PTR-TOF-MS. The ions of acetone ($\text{C}_3\text{H}_6\text{OH}^+$, $m/z = 59.0491$; $\text{C}_2^{13}\text{CH}_6\text{OH}^+$, $m/z = 60.0525$) were measured (Fig. 2B). Milk samples from healthy dairy cows contained approx. 1 ppm of acetone in the vapor phase, while one from ketotic cows contained more than 10 ppm of acetone. In contrast, milk samples from subclinical ketosis dairy cows contained approx. 5 ppm of acetone in the vapor phase. Therefore, acetone should be monitored in the range of 1 to 5 ppm to prevent the development of ketosis.

To demonstrate the detection of acetone through odor of milk samples, we prepared milk samples with varying concentration ranging from 0.10 to 0.50 mM by adding aliquots of acetone into whole cow's milk as well as into water as a reference. The commercial whole milk samples contain some amounts of acetone (approx. 0.5 ppm as shown in Table II). In addition to various other components that may affect the volatility of acetone, we purchased three milk samples from three different companies (Fig. 1B and Table I; see also Experimental section). The acetone concentration in the vapor phase was measured by PTR-TOF-MS and the results are summarized in Table II. The same samples were then measured using MSS.

Fig. 3 shows the signal responses to the aqueous solutions of acetone and the milk samples. When gas molecules are absorbed into a receptor layer, the receptor layer generates surface stress caused by the sorption-induced expansion [9], [18]–[20]. Since all samples, including milk samples, are aqueous solutions, sample vapors contain a large amount of

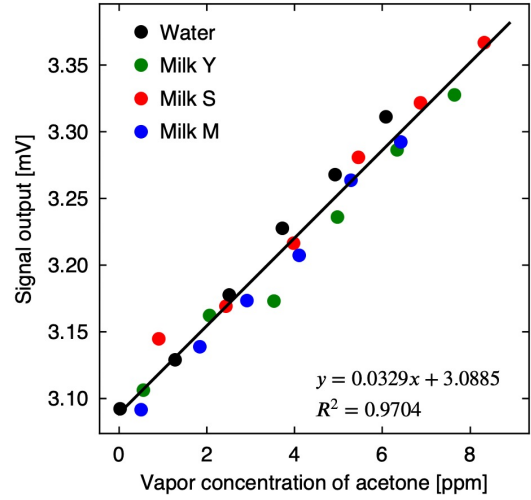


Fig. 4. Plots of signal outputs at 67 s obtained from the whole milk samples and the aqueous solutions with different concentrations of acetone added as a function of vapor concentration of acetone measured by PTR-TOF-MS.

water, resulting that the signal responses are dominated by the response to water (Figs. 3A and C). As previously reported [12], water molecules tend to desorb more quickly than other components including acetone, and hence a clear concentration dependence of a target analyte can often be observed in the purging process. As shown in Figs. 3B and D, the increase in signal output was observed in the purging process as the acetone concentration increased.

To evaluate the concentration dependence, we then extracted the signal outputs at 7 s after starting the purging process (i.e., 67 s) and plotted them as a function of the vapor phase concentrations of acetone determined by PTR-TOF-MS (Table II). Although the receptor materials have cross-reactivity to various odorous molecules, major volatile organic compounds in the whole cow's milk are water and acetone determined by PTR-TOF-MS. Therefore, as shown in Fig. 4, the signal outputs show a linear correlation with acetone concentrations. The root-mean-square deviation (RMSD) of acetone concentration is ± 0.40 ppm and the limit of detection, which is defined as the concentration with a signal-to-noise ratio of 3 [21], is 0.46 ppm, demonstrating the possibility to predict the acetone concentration level of subclinical ketosis cows through the odor of milk samples by nanomechanical sensors.

IV. CONCLUSION

We have demonstrated that a nanomechanical sensor, MSS, can detect acetone through odor of milk at the level of subclinical ketosis in dairy cows. Focusing on the desorption rates between water and acetone molecules from the receptor layer, the concentration-dependent measurement of acetone in the range from 1 to 10 ppm was achieved with the RMSD of ± 0.40 ppm. This study presents a great potential for non-invasive monitoring of subclinical ketosis, leading to daily monitoring using automated milking systems.

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