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Research paper

Attenuated total reflectance terahertz spectroscopy for quantitative measurement of somatic cell count in bovine milk

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ABSTRACT

Quantitative somatic cell count (SCC) measurement for detection of mastitis using an attenuated total reflectance (ATR) terahertz (THz) spectroscopy was investigated. Using samples with controlled SCC derived from raw milk, we measured their ATR signal in the THz region 30–440 cm^{-1} with a Fourier-Transform THz spectrometer. We found ATR signals decreased as SCC increased. Furthermore, we found the correlation between the ATR signal and SCC from 8.0×10^4 to 1.57×10^6 cells/mL was optimal at 139 cm^{-1} ($R^2 = 098$). These findings demonstrate a potential to detect mastitis with a monochromatic THz wave.

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1. Introduction

Mastitis in cows is a serious disease in dairy farming, resulting in total annual losses estimated to be $2 billion per year in the United States (Viguier et al., 2009). This is approximately 10% of farm milk sales, and about two-thirds of this loss is due to reduced milk yield from cows with subclinical mastitis levels. Mastitis is an inflammation of the udder that results from bacterial infection, a variety of micro-organisms, including algae and yeasts, and can also be the result of trauma and chemical burns. It induces decreases in lactase, and increases in somatic cell count (SCC) due to mammary cell destruction and release of white blood cells (Emanuelson and Funke, 1991). Thus, the extent of mastitis can be determined by the concentration of SCC (Blood and Radostitis, 1989). However, this diagnostic procedure is limited to periodic inspection in large milk supply chains. What is needed is a measurement procedure that dairy farmers can carry out on-site, on a day to day basis, that can provide early detection of subclinical mastitis to facilitate treatment and prevent development of chronic mastitis.

While on-site tests, such as the California Mastitis Test (CMT) (Sargeant et al., 2001; Green et al., 2004), and electrical conductivity (Milner et al., 1996; Nielen et al., 1995) to measure SCC do exist, they are, however, not sensitive enough to measure subclinical mastitis levels for early detection of pathogenesis. Other methods have tried to address this sensitivity issue, such as the application of electromagnetic (EM) wave (Frank and Birth, 1997; Tsenkova et al., 2001). Such techniques that use near infrared (NIR) are, however, prone to scattering effects from fat globules, which are of a comparable size to NIR waves. To minimize such effects, preprocessing of spectra, hyperspectral imaging or integrating sphere techniques are needed to discriminate absorption information from that of scattering (Qin and Lu, 2007) (Silje et al., 2010).

Another solution to resolve this issue of scattering, use of a longer wavelength, can be applied since scattering effects decrease with the fourth power of wavelength according to the theory of Rayleigh scattering. However, transmission techniques utilizing longer wavelengths over 2.5 μm cannot be used since the extinction coefficient of water is more than 10^{-1} (10^8 times bigger than in the visible region) (Querry et al., 1991). To overcome this high absorbance of water in aqueous samples, such as milk, an attenuation total reflection (ATR) method has been applied in the middle infrared (MIR) region (Fahrenfort, 1961). The ATR method generates an evanescent wave at the interface between the ATR prism and the sample placed on the prism. It is the absorbance in this evanescent wave that is measured in the reflected EM wave. Miyamoto et al. (2007) showed that an ATR-MIR method could be used to observe cells on an ATR prism. However, in this case, the depth of

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penetration of the evanescent field is about 500 nm, and thus the measured absorption of cells will be almost entirely derived from cell membranes. With this set-up, it would be difficult to discriminate between cells and fat globules, since the membranes of both are composed of phospholipids. Since the penetration depth of the evanescent wave is proportional to the wavelength of the light used, the penetration depth can be tailored to measure the SCC by shifting to a longer wavelength. Terahertz (THz) spectroscopy, a subset of EM waves longer than MIR (30–3000 μm), which has advanced rapidly since the 1990s (Matthew et al., 2002), may be able to provide a sensitive and selective means for measuring SCC content. That is because the penetration depth of the evanescent wave is approximately ten times larger when the ATR method is used with THz waves. Recently, the ATR method has been also applied in the THz region (Hirori et al., 2004), as well as MIR spectroscopy. According to Jepsen et al. (2011), such a procedure in measuring absorption of water in the THz region is more sensitive than a combined transmittance and thin sample chamber set up. Moreover, absorption bands are derived from intermolecular slow vibrations in the THz region, which depend on the polar characteristics of molecules (90% of the milk). On the other hand, there is little absorption by low polar proteins and fat that are found in the cell cytoplasm. Thus, it is considered that absorption in the THz region will be selectively influenced by the cells in the milk. Research in a parallel field has shown that this technique can selectively measure cells. (Liu, 2007).

The objective in this study is to confirm the potential for quantitative measurement of somatic cells derived from milk using ATR-THz spectroscopy. To facilitate initial determination of SCC content in raw milk samples, in the absence of any potential interference, the cell content of milk samples were separated from other components of the milk using a centrifuge procedure. The ATR spectra of these samples were then measured with a Fourier transform THz (FT-THz) spectrometer.

2. Materials and methods

2.1. Samples

The SCC content of milk samples was manually manipulated using a sampler (TRU-TEST.Ltd, New Zealand) of raw cow's milk with high SCC content (Hokkaido Research Organization). First, the high SCC content milk was centrifuged at 1780 G, 20 degree Celsius for 10 min. The resulting samples were then separated into an upper cream layer, a middle low SCC content layer, and a bottom high SCC content layer. By remixing the middle and bottom samples at different ratios (middle: bottom = 1:0, 4:1, 3:2, 1:4, 0:1) samples with varying SCC content were obtained. We then measured the milk fat, lactose, milk protein, and SCC components of these samples using MIR spectroscopy and a flow cytometry (Milk-O-Scan FT120 and Fossomatic90, Foss Analytical A/S, Hillerød). As a result it was confirmed that milk samples with SCC contents between 8.0×10^4 and 1.57×10^6 cells mL^{-1} were obtained with a minimized dispersion of fat: 0.14 wt%, protein: 0.16 wt%, and lactose: 0.02 wt% (standard deviation (SD)). The milk samples were stored in a refrigerator below 10 degree Celsius for one day after milking in order to stabilize milk quality. They were taken from the refrigerator 60 min before measurement in the THz spectrometer, and stirred in a 40 degree Celsius water bath to ensure the size of fat globules remained uniform.

2.2. Terahertz spectra and data analysis

All spectra of the milk samples in the THz region were obtained using an FT-THz spectrometer (FARIS-1S, JASCO Co. Ltd, Japan). This device had a silicon broadband beam splitter in the interferometer to obtain low frequency THz waves, and a deuterated L-alanine-doped triglycine sulfate (DLATGS) detector set at room-temperature. For sample measurements, we used the ATR method. A high resistance silicon whose refractive index (n) was higher than water, and whose extinction coefficient (κ) in the THz region is minimal, was used as an ATR prism ($n = 3.4$ at 33.3 cm^{-1}, $\kappa = 9.4 \times 10^{-5}$–$1.4 \times 10^{-3}$ from 40 to 440 cm^{-1}) (Palik, 1998). The diameter of the sample area and the focal point of the light on the ATR prism was designed to be 10 mm. When the light from the light source entered the ATR prism with a high refractive index and hits a sample, it reflects above a critical angle from the top of the prism, in the process generating an evanescent wave on the ATR prism that penetrates into the sample. Absorption spectra of the sample were obtained by measuring the attenuated reflectance signal that includes sample absorption in the evanescent wave.

The intensity of the evanescent electric field (E) generated on the ATR prism decays exponentially with distance into the sample, $E = E_0 e^{-z/dp}$, where z is distance, dp is depth of penetration, where the intensity of the evanescent wave attenuates 1/e compared with that on the surface of the ATR prism. The dp of the evanescent field can be derived using the following Formula (1). (Muller et al., 1981).

$$dp = \frac{\sqrt{2}\lambda}{2\pi n_1 \sqrt{-\nu + \sqrt{\nu^2 + \mu^2}}}$$
$$\nu = \sin^2\theta - \frac{{n_2}^2}{{n_1}^2} + \frac{{n_2}^2{\kappa_2}^2}{{n_1}^2} \quad (1)$$
$$\mu = \frac{2{n_2}^2{\kappa_2}^2}{{n_1}^2}$$

where λ is the wavelength of the incident light, n_1 is the refractive index of the ATR prism, n_2 is the refractive index of the sample, κ_2 is the extinction coefficient of the sample, and θ is the incident angle (45°); and dp is a function of wavelength. Thus, the penetration depth of the ATR-THz method is larger than that of the ATR-MIR method. The optical pass of the ATR sample measurement procedure is shown in Fig. 1.

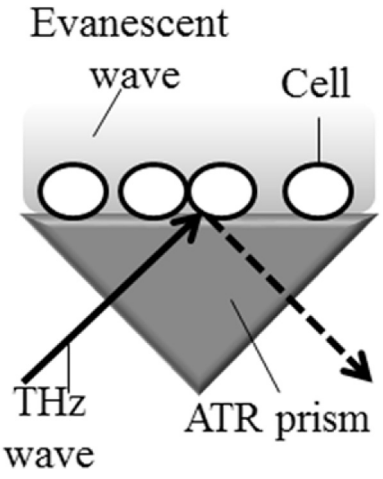

Fig. 1. Schematic model of the ATR-THz spectroscopy of the cells. The penetration depth of evanescent wave (dp) was calculated by Formula (1). The dp was approximately 27.5 μm at 1 THz (33.3 cm^{-1}) in our system. The mean size of the somatic cells was confirmed by microscope to be 9.8 μm (Min: 4.2 μm, Max: 35 μm). From this, it can be assumed the above dp will encompasses the entire cells on the ATR prism.

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