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Portable Raman Spectroscopy Based Prototype for the Clostridia Detection in Milk

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Summary:
In the dairy industry the detection of Clostridia is a remarkable issue. These bacteria are known for their hydrogen and carbon dioxide production, which compromises the shape and flavor of the cheese in which they reside. Early *Clostridia* detection allows infected milk to be redirected to less-aged products without any loss of quality, thus avoiding economic losses. Current methods are time-consuming and not specific for *Clostridia*, this paper presents a portable instrument based on Raman gas spectroscopy tailored to the detection of Clostridia and a test routine developed to simplify the experimental setup.

Keywords: Raman spectroscopy, *Clostridium*, Food Quality, Gas analysis, Hydrogen

Motivation

Clostridium detection in milk is crucial because these bacteria can survive pasteurization and then return to their active form. Their metabolism produces large amounts of hydrogen and carbon dioxide which leads to late blowing defects like cracks and slits in cheese. This not only changes the taste and appearance of the cheese but also contributes to food waste. Milk contaminated with *Clostridium* can still be used in making less aged cheeses. Thus, it is important to identify its presence as quickly as possible. Rapid microbiological methods are available [1], but they are expensive and require skilled operators, while the standard method [1], though inexpensive, lacks in specificity for *Clostridium*. The proposed solution is represented by a portable instrument based on Raman spectroscopy adapted to detect *Clostridium* through the hydrogen they release in the headspace of culture vials, as they are the only hydrogen-producing bacteria commonly found in milk. The developed instrument is specific for detecting *Clostridia*; unlike the traditional method, it has also proven to be faster [1,2,3]. Significant effort has been made in making it portable, allowing the direct use in its field of application.

Experimental setup

For the Raman scattering excitation, a 532 nm Nd:YAG DPSS laser with an power output of 1 W was employed. The laser beam is focused into the headspace of the vial under exam. After the interaction with the sample, the beam is directed towards a light trap and absorbed. The scattered radiation is collected by a custom made spectrometer positioned orthogonally to the laser path. The Raman signal is imaged onto the spectrometer focal plane with a pair of Hastings achromatic triplets. In the collimated light region between the two triplets, a 550 nm long-pass interferometric filter is placed in order to reject the strong Rayleigh scattering. The compact f/2.8 spectrometer setup includes an entrance objective lens, an additional long-pass filter, a diffraction grating, and a CMOS camera equipped with its objective lens. The instrument is also equipped with a linear translator useful to move a batch of vials and analyze them one at a time. Additionally, a pulley is placed in order to rotate the vials during the measurements. The entire instrument is enclosed in a controlled temperature box (53 cm x 38 cm x 40 cm) which is internally stabilized at 37 degrees Celsius by a heater. For this specific test the pulley could be

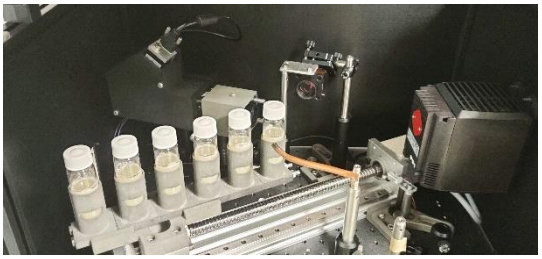

Figure 1: Experimental setup

disabled so during the measurements it is possible to rotate or just translate the vials.

Spectra generation

Raman spectra are generated by averaging several rows of the acquired frames. An example spectrum is shown in Figure (2). This technique is sensitive to most gas molecules, allowing the simultaneous detection of hydrogen, water vapor, nitrogen, oxygen, and carbon dioxide.

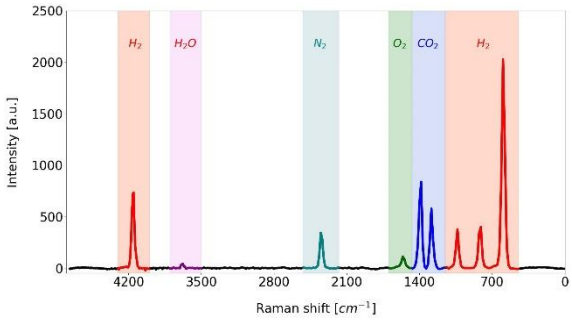

Figure 2: Example of Raman spectrum

The proposed method operates on a threshold based algorithm to determine the *Clostridium* contamination of a vial. A vial is considered contaminated if the hydrogen peak integral (587 cm^{-1}) exceeds the background mean plus 5 times its standard deviations in the same spectral region. The main limit to the measurement is represented by scattering and fluorescence caused by dirt, condensation, or milk droplets on the walls of the vials since those phenomena can lead to partial or complete saturation of the detector. For this reason, the idea is to rotate or translate the vial until an acceptable measurement condition is achieved. In any case, as long as the contribution of fluorescence does not lead to saturation of the detector, it can be subtracted by fitting with a 3rd degree polynomial equation.

Testing

To compare the effects of the two movements, rotation and translation, 24 vials containing a concentration expected to yield 19 positive results were prepared. The vials were measured by testing both rotations and translations. The sample preparation and image processing procedures followed standard methods used in previous measurements [1]. For each vial, 5 camera frames were acquired while rotating the vial around its vertical axis, subsequently 5 images were acquired after translating the vial 1 millimeter in both directions around the center (defined as the position when the laser passes through the vial's diameter). A measurement of 24 vials takes approximately 23 minutes. After choosing the most effective measurement strategy, the analysis time can possibly be reduced. To validate the experiment, a control test was conducted with the standard method on samples containing the same concentration of spores per volume unit.

Results

The method based on Raman spectroscopy revealed 7 positive vials out of 24 after 48 hours; while the standard method detected only 1 positive vial after the same incubation time. After 3 days, the number of positive vials detected by Raman spectroscopy increased to 20, while the standard method identified 16 positive vials.

Conclusions

The developed prototype was able to detect 35% (7/20) of the positive vials within 48 hours, whereas the standard method detected only 6% (1/16) as positive, showing the potential for a faster and more specific testing method. Measurement through successive translations did not present particular issues compared to rotation, leading to a possible simplification of the hardware setup. Future tests will be conducted to validate the proposed method and for further hardware and software improvements.

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