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An identification of raw milk samples obtained from estrus and non-estrus dairy cows using Micro-NIR Spectrometer

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Abstract

This project is a feasibility study of an identification of raw milk obtained from estrous and non-estrous dairy cows using near-infrared spectroscopy combing with principal component analysis techniques. The samples were measured by a micro-NIR spectrometer(950-1650nm). The use of traditional tools that need to be installed on cow and chemical methods can be challenging. However, using near-infrared spectroscopy had more advantage such as no effect on cows and fast. The raw milk samples used in the study came from four Holstein dairy cows that were sampled in the morning and evening for 40 days. The samples were scanned for collecting NIR spectra and then the principal component analysis (PCA) was used to analyze milk spectra across the MATLAB program. The results showed that the spectral pretreatment by the Moving Average Smoothing + Standard Normal Variate method was clearly classified into two groups: 1) Proestrus and 2) Estrus and Metestrus. The study shows that it was possible to use NIR spectroscopy to classify milk samples from estrus and non-estrus cows. However, future studies should investigate additional factors may affecting the classification model, such as study more algorithms and create model using important individual. Wavelength, in order to get a more accurate.

Keywords: Principal component analysis, Progesterone, Artificial insemination, Golden time

1. Introduction

From 2017 to 2021, the total number of dairy cows in Thailand increased by 3.73% per year. In 2021, the number was 745,871, up from 724,901 in 2020, an increase of 2.89%. Milk production during the years 2017-2021 increased by 3.06% per year, with the production in 2021 being 1,374,491 tons, up from 1,342,181 tons in 2020, an increase of 2.41%. However, in the year 2020/2021, the expansion in the export value decreased by 0.95% [1]. One reason for not achieving the desired milk production volume is due to unsuccessful artificial insemination, caused by farmers missing the optimal timing for insemination, resulting in decreased milk production.

On average, dairy cows begin their estrous cycle at about 11 months old, depending on the breed. After the onset of the cycle, there is a regular interval typically between 18-24 days, or an average of 21 days. Optimal breeding should occur within an 8–16 hours window during the estrous cycle [2]. Therefore, if artificial insemination is missed, one must wait another 21 days. Accurate detection of estrus is crucial to reduce the costs associated with unsuccessful insemination attempts.

To detect estrus without technology, one method is observation, where cows display behavioral changes or physical signs on the day they are in heat, such as standing still (allowing mating), being excitable, swelling and reddening of the genitalia, resistance to manual uterine examination, and uterine enlargement. However, observing behaviors can be challenging, especially with a large number of dairy cows, and accuracy in identifying the estrus period decreases [3].

Various technologies have been employed to detect estrus, such as pedometers to track activity indicative of estrus and pressure sensors on the hips. These technologies generally offer greater accuracy than visual observation of behavior. Despite their higher accuracy, there are limitations, as some cows in estrus may not exhibit typical behaviors [4]. One key hormone related to ovulation and behavior during estrus in dairy cows is progesterone, but chemical analysis to monitor it can be time-consuming [3]. Progesterone has been tested for tracking estrus and reproductive status [5].

Near-infrared spectroscopy (NIRs) is a technology applied in the food industry, including milk production, to check quality from raw milk at the farm or processed into products like cheese, yogurt, and others. Numerous studies have shown that NIRs provides accurate results in predicting various milk parameters such as fat, protein, urea, lactose, fatty acids, etc. [6]. Experiments using NIRs on cows in estrus have shown significant changes in pH and fat levels, which were analyzed using machine learning techniques [7].

The aim of this research is to explore the possibility of differentiating raw milk from cows in estrus and not in estrus using near-infrared spectroscopy (NIRs) and PCA techniques, using MATLAB to create data distribution diagrams and improve spectra with

seven different methods to find the optimal way to classify samples of dairy cows in and out of estrus. This study used Holstein cows, the most commonly raised breed in Thailand.

2. Materials and methods

2.1 Instruments and Equipment

- Micro-NIR Spectroscopy instrument, Onsite – W model, by VIAVI Solutions, USA (Figure 1). The details of the instrument are as described in Table 1.
- The appropriate measurement range is from 0 – 3 mm from the front of the light port.
- Uses linear variable filter (LVF) technology as the main component in its operation.
- The light detector has a resolution of 128 pixels.

Figure 1 Micro-NIR spectroscopy, Onsite – W model, VIAVI solutions, USA.

Table 1 Details of the Micro-NIR settings

Type, model	Micro-NIR spectroscopy รุ่น Onsite-W ยี่ห้อ VIAVI Solution, USA
Measurement method	Reflectance mode
Wavelength	950 – 1650 nm
Sample scan time	10 scans
Background scan time	10 scans

2.2 Samples preparation

Sample cows were obtained from the Dairy Farm, Faculty of Agriculture, Khon Kaen University. The breed used was Holstein, which is the most commonly raised in Thailand. These cows were black and white (Figure 2) and produced a high volume of milk.

Figure 2 Holstein sample cows from the Faculty of Agriculture, Khon Kaen University, and a milking machine.

2.3 Raw Milk Samples from Cows in Estrus and Not in Estrus

During the first 30 days, samples were collected from a single cow, number 6427, to capture periods of estrus and non-estrus. However, since cow number 6427 did not display estrus behavior, hormone injections were administered to induce estrus. Raw milk was then sampled from four cows, numbers 6318, 6427, 6316, and 6314 (Figure 3), over 10 days to obtain raw milk samples from cows in estrus. The samples were stored in 15.5 ml Vial bottles.

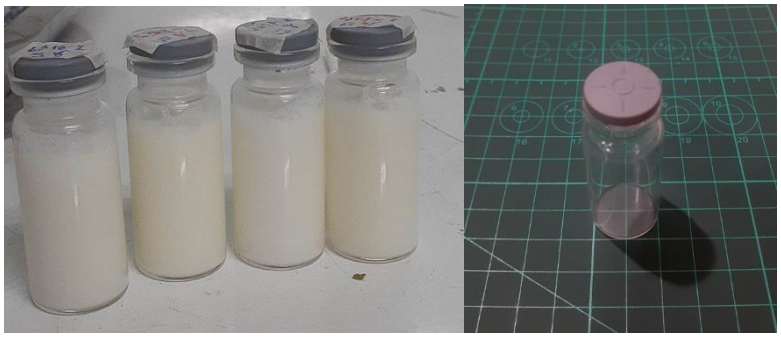

Figure 3 Raw milk samples contained in Vial bottles.

2.4 Methods for Measuring Spectrum Values

- Raw milk is collected from the Dairy Section, Faculty of Agriculture, Khon Kaen University between the hours of 08:30-10:00 AM and 4:00-5:30 PM. The milk is milked from the cows at 5:00 AM and 2:00 PM, after which it is stored in a refrigerator at a temperature of 13-17 degrees Celsius.
- The raw milk is then brought to the lab and left until it reaches room temperature (20±3 degrees Celsius).
- Spectral measurements of the samples are conducted before and after estrus. For one cow, the raw milk is divided into three bottles and tested five times. During estrus, the raw milk from one cow is divided into two bottles and tested five times.
- After each experiment, the raw milk samples are stored in a freezer at 0 degrees Celsius for further testing in subsequent rounds.

2.5 Spectral pre-processing

The spectral data obtained from the research was adjusted to correct deviations caused by various factors affecting near-infrared light, to increase the accuracy of the data. The improvement of the spectral data through mathematical methods derived from NIR machine measurements was used to reduce the influence of these deviations, as observed in previous research [8].

Samples designated for analysis were subjected to seven methods of spectral improvement, which include:

1. First derivative
2. Second derivative
3. Mean normalization (MNL)
4. Standard Normal Variate (SNV)
5. Moving Average Smoothing + Standard Normal Variate
6. Moving Average Smoothing + Baseline Offset
7. Baseline offset + Standard Normal Variate

2.6 Analysis of Samples Using Principal Component Analysis (PCA)

2.6.1 Selection of Samples for Analysis

Samples were divided equally among the periods before estrus, during estrus, and after estrus based on Micro-NIR inspection results, with totals of 199, 199, and 200 scans respectively for each period (Table 2).

2.6.2 Examination of Differences Between Raw Milk Samples in Estrus and Not in Estrus

The examination of differences in the spectrum values was conducted using Principal Component Analysis (PCA) to calculate the principal component scores [9] for all spectrum data obtained from scans of raw milk samples before, during, and after estrus. Codes were written in MATLAB to analyze the relationships between PC1-PC2 and PC3, which represent the components with variance values in the data. If the relationship showed clear group differentiation, it might indicate the possibility of classifying raw milk samples using near infrared.

Table 2 Number of samples selected for analysis from the Micro-NIR device inspection

Cow status	Cow no.	Day/Month/Year	Number of scanning	Data Collection Period
Proestrus	6314	11/09/23	20	Morning-Afternoon
	6316	11/09/23	20	Morning-Afternoon
	6318	11,12,13/09/23	69	Morning-Afternoon
	6427	18,22,24,27,25/08/23	90	Morning-Afternoon
Estrus	6427	11/09/23	20	Morning-Afternoon
	6314	13/09/23	20	Morning-Afternoon
	6316	14/09/23	40	Morning-Afternoon
	6318	18/09/23	50	Morning-Afternoon
	1789	18/09/23	70	Morning-Afternoon
Metestrus	6427	15,16,17/09/23	100	Morning-Afternoon
	6314	16,17/09/23	70	Morning-Afternoon
	6316	17/09/23	30	Morning-Afternoon

3. Results and Discussion

3.1 Spectral characteristics

The spectrum values obtained from the Micro-NIR inspection ranged between 950-1650 nm, showing several significant variations across the spectrum as shown in Figure 4. The peak of each spectrum range, which was prominent, indicated the absorption values of substances such as fats, proteins, lactose, or others with high absorption capabilities in the measured spectrum [6].

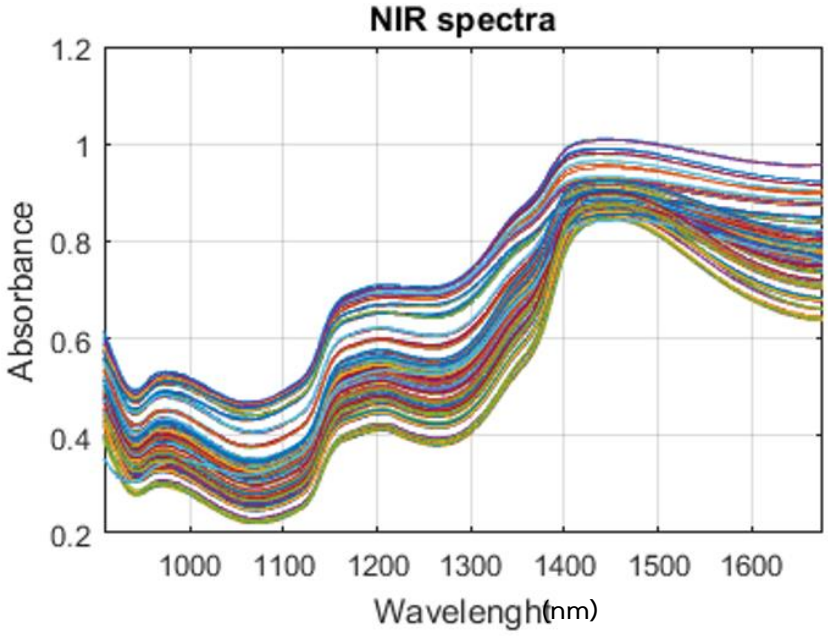

Figure 4 Spectrum of raw milk from all dairy cows.

3.2 Analysis of Original Spectrum Using Principal Component Analysis (PCA)

From using a sample group that showed significantly different absorption ranges in the spectrum, it was possible to distinguish the differences between sample groups using PCA. This method can classify a large number of samples based on related variables within the sample groups. The samples were divided as follows: 1) before estrus, 2) during estrus, and 3) after estrus. After performing PCA, it was possible to divide the samples into two groups: the group before estrus and the group during and after estrus (Figure 5).

3.3 Spectra After Pre-treatment

All seven spectral pre-treatment techniques were used to investigate the possibility of enhancing the clarity of estrus detection (Figure 6**).** The distribution chart of the sample groups from the spectrum pre-treated with the Moving Average Smoothing + Standard Normal Variate method proved to be the best pre-treatment technique compared to others, as observed from the clear division of the sample groups into two distinct groups. Spectrum pre-treatment thus aided in distinguishing the groups of samples before estrus and the groups during and after estrus. However, the pre-treatments still could not resolve the limitations in differentiating between the groups during and after estrus. Therefore, performing PCA demonstrated that using NIR could potentially predict the estrus in dairy cows.

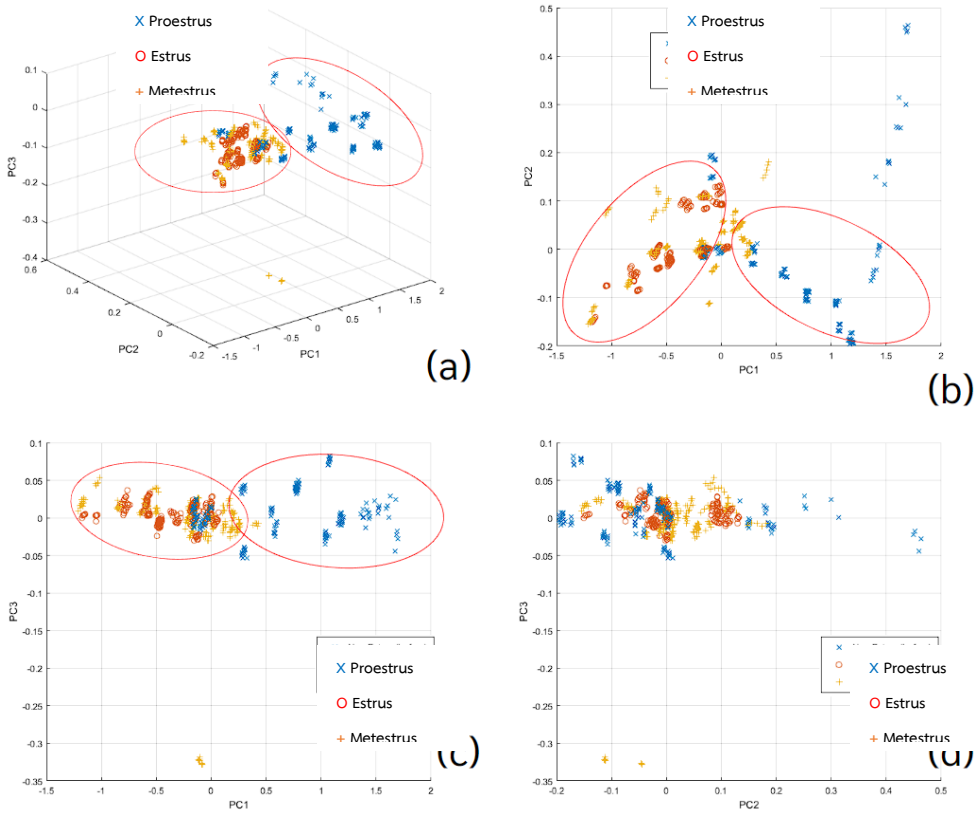

Figure 5 Distribution charts of the original spectrum sample groups (Raw Spectrum) from the Micro-NIR device: (a) Distribution chart of the samples PC**1**-PC**2**-PC**3**, (b) PC**1**-PC**2** on the x-y axis, (c) PC**2**-PC**3** on the y-z axis, (d) PC**1**-PC**3** on the x-z axis.

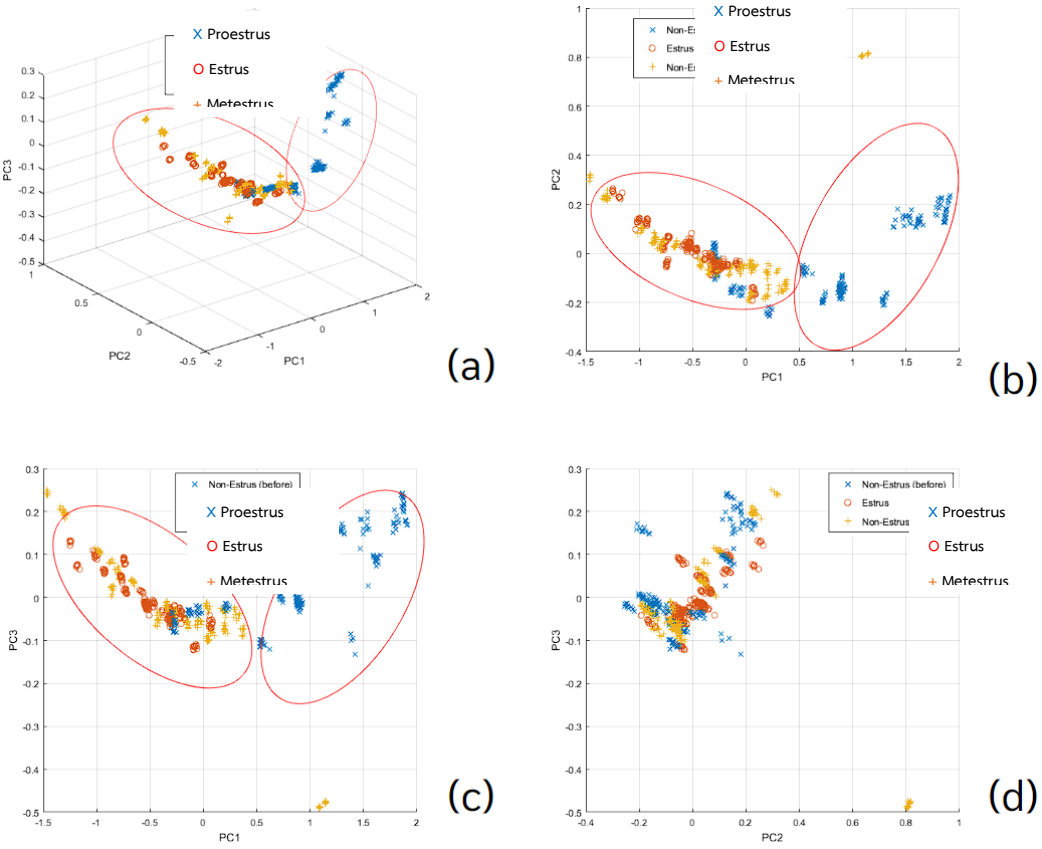

Figure 6 Distribution charts of samples with spectrum pre-treated by Moving Average Smoothing + Standard Normal Variate method: (a) Distribution chart of samples PC1, PC2, and PC3, (b) PC1-PC2 on the x-y axis, (c) PC2-PC3 on the y-z axis, (d) PC1-PC3 on the x-z axis, using the Micro-NIR device.

4. Conclusions

The study of NIR techniques using the Micro-NIR Spectrometer showed potential in distinguishing raw milk from dairy cows in estrus and not in estrus using PCA to differentiate spectrum variations across different wavelengths. The results indicated that it was possible to divide the sample groups from the PC1-PC2-PC3 diagrams into two groups: 1. Before estrus, 2. During estrus and after estrus. Since the spectra analyzed encompassed the entire range of wavelengths, it might have obscured the ability to clearly differentiate samples before estrus, during estrus, and after estrus. Employing model segmentation that selects specific wavelengths could potentially improve the accuracy of group classification. Using amber-colored bottles to store raw milk samples after milking might enhance the preservation of the milk's intrinsic properties. Additionally, there are several other more complex and accurate measurement techniques available beyond PCA. However, the study showed that PCA still has the potential to differentiate raw milk from cows in estrus and not in estrus.

5. Acknowledgements

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